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FOREWORD

The potential of radionuclides in therapy has been recognised for many decades. A number of radionuclides such as iodine-131, phosphorous-32, yttrium-90 and I-131 MIBG have been in use for the treatment of many benign and malignant disorders. Recently, however, there has been a significant growth of this branch of nuclear medicine with the introduction of a number of new radionuclides and radiopharmaceuticals for the treatment of metastatic bone pain, neuroendocrine and other tumours.

The prospect of localising or treating neoplastic diseases using specific antibodies labelled with radioactive isotopes capable of delivering large amounts of internally administered radiation may have the potential to fulfil the promise of Ehrlich's "magic bullet", which has tantalised investigators worldwide for the past sixty years. Recent success in this area has been largely due to genetic and molecular techniques that now permit production of a large number of suitable peptides and monoclonal antibodies directed against specific epitopes individually characteristic of specific tumours. The input of the radiochemist and the development of labelling techniques that do not destroy the immunological integrity of the monoclonal antibodies have also been essential ingredients of the success story.

Recent significant advances in monoclonal antibody techniques for pretargeting make it very likely that radiopharmaceuticals will become an important part of therapy for various cancers. It may also be possible that in addition to the use of beta particles, alpha particles may soon become a mainstay of therapeutic nuclear medicine. Cancer researchers, looking for an extremely potent and highly specific way to target cancer cells, are investigating the use of monoclonal antibodies and peptides attached to alpha emitting radionuclides in early clinical trials.

Today the field of radionuclide therapy is going through an extremely interesting and exciting phase and is poised for greater growth and development in the coming years. The IAEA organised an international seminar at Hyderabad, India, with the objective of bringing together in one place medical professionals and biomedical researchers from all over the world who are engaged in clinical research & development aspects of radionuclide therapy. The seminar addressed some of the current trends in therapeutic nuclear medicine, evaluated the established procedures and assessed the re-emergence of certain old procedures.

Ninety-nine official participants and 11 observers from 36 countries participated in the seminar. A total of 48 scientific papers and 13 invited lectures on a wide spectrum of basic and clinical aspects of radionuclide therapy were presented. The topics included research and development in the field of therapeutic radiopharmaceuticals, treatment of thyroid disorders with I-131 and metastatic bone pain with Sr-153, radionuclide therapy with monoclonal antibodies and peptides, radiation synovectomy, intravascular radionuclide therapy and treatment of neuroendocrine tumours. One of the novel applications of radionuclide therapy presented during the seminar was the use of I-131-CD-20 monoclonal antibodies for the treatment of non-Hodgkin's lymphoma. The seminar also highlighted the importance of dosimetry in radionuclide therapy.

This publication contains papers and invited lectures presented at the seminar. The IAEA officers responsible for the publication were A.K. Padhy of the Division of Human Health and H. Vera Ruiz of the Division of Physical and Chemical Sciences.

EDITORIAL NOTE

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SUMMARY

Therapy using unsealed sources of radioactivity has the advantages of both systemic administration (as in chemotherapy), as well as selective irradiation (like brachytherapy and external beam radiation) of tissues. For a long time, radionuclide therapy was mainly limited to radioiodine for thyrotoxicosis or thyroid cancer, and P-32 for polycythemia vera. However, considerable advances have taken place in the field of radionuclide therapy during the past one or two decades.

A variety of radiopharmaceuticals are being developed which have different targeting mechanisms, routes and forms of administration. Some are given in the simple salt form, or attached to more complex molecules. For instance, regulatory peptides are natural substances having a wide range of receptor-mediated functions. Many neoplasms express high affinity receptors for these peptides. Therefore, peptides labelled with suitable radionuclides have considerable potential for diagnosis and therapy of such tumours. Among the commercially available peptides is In-111-labelled Octreotide for neuroendocrine tumours.

For some years, monoclonal antibodies to tumour antigenic sites have been labelled with radionuclides that emit gamma rays, permitting detection of disease. Often, this is the only means for detecting recurrence or metastases from tumours that are generally difficult to demonstrate with other imaging modalities. Labelling with beta-emitting radionuclides for therapy is the logical extension, but this application has been limited by the development of antibodies to the monoclonal antibodies (usually of murine origin). Prior administration of the monoclonal antibodies for diagnostic imaging usually precludes subsequent therapeutic use. Bone marrow toxicity also limits the amount that can be safely administered. These limitations are being addressed by current research studies.

Significant advances in monoclonal antibody techniques for pretargeting make it likely that radiopharmaceuticals will become an important part of therapy for various cancers. It may also be possible that in addition to the use of beta particles, alpha particles may soon become a mainstay of therapeutic nuclear medicine. With advances in molecular medicine and genetic engineering, new antibodies are being synthesised with greater ease. In the next few years a host of useful substances is expected to become available. It will also be seen if the current crop of agents available will continue to be used clinically.

A major problem encountered in oncology practise is the patient with intractable pain secondary to bone metastases. This is most often seen in cancers of the prostate, breast, and lung. When the pain has become unresponsive to all available analgesics, a multimodal approach involving radiation, hormonal, and even surgical therapy, becomes necessary. The presence of multiple, scattered bone lesions is ideal for radiopharmaceutical therapy since the lesions can be targeted selectively through a single systemic administration of the radiopharmaceutical. The available list of radionuclides has been growing, and now includes phosphorous-32, iodine-131, strontium-89, yttrium-90, rhenium-186/188 and samarium-153.

The papers presented during the seminar represent the spectrum of current therapeutic applications of radiopharmaceuticals. These include the well-established role of I-131 in thyrotoxicosis and thyroid cancer, monoclonal antibodies, treatment of bone pain, radiation synovectomy, and novel radiopharmaceuticals. The importance of dosimetry was also highlighted. The Seminar provides an important means of assessing the levels and extent of current radionuclide therapy practise in the different countries represented.

This book of proceedings contains most of the papers and invited lectures presented at the seminar.

The first section contains ten articles by seven eminent experts from various fields of nuclear medicine. In his paper on Dosimetry in Radionuclide Therapy, G. Riccabona highlighted the inter-relationship between therapeutic effects of radionuclides, absorbed radiation dose and radio-

sensitivity of the tissues or organs being irradiated; as well as the importance of internal dosimetry for optimization of administered doses of radioactivity.

In his review paper on New Aspects of Radionuclide Therapy of Bone and Joint Diseases, M. Fischer gave a detailed account of the current trends in radionuclide therapy in the palliation of metastatic bone pain. The treatment offers significant pain relief in about 70–80% of cases when radioactivity (Sr-89, P-32, Sm-153 EDTMP and Re186/188 HEDP) is administered systemically. The greatest advantage of radionuclide therapy according to the author is its ease of administration, cost-effectiveness and possibility to treat bone pain at multiple sites due to disseminated metastases. The author also reviewed the effectiveness of radiosynovectomy using Y-90, Sm-153 EDTMP and Re-186 in patients suffering from arthritis.

The promising role of labelled peptides for targeted therapy of cancer was presented by M. Chinol who reviewed the labelling of DOTATOC, a new bifunctional chelate of octreotide with In-111 and Y-90. Y-90-DOTATOC has been prepared and evaluated as a potential candidate for treatment of tumours containing somatostatin receptors. In another invited paper Chinol also reviewed the problems in radioimmunotherapy using monoclonal antibodies labelled with beta emitting isotopes. He also enumerated the potential advantages of an alternate three-step approach based on avidin-biotin systems where the tumour is pre-targeted with avidin labelled MoAb and after allowing the circulating MoAb to clear, the radiolabelled biotin is injected. This method allows administration of high activities with acceptable toxicity. Pilot studies using Y-90-MoAbs in advanced stage tumours have shown that this approach produces significant tumour regression.

Radioiodine treatment of hyperthyroidism is probably the oldest and most commonly practiced radionuclide therapy. However, even after more than 55 years of its first introduction in clinical practice many questions pertaining to this application still remain unanswered. M. Poshyachinda in her paper on Management of Hyperthyroidism reported the incidence of various forms of hyperthyroidism and the therapeutic approaches to treat the disease. Currently radioiodine therapy is the most common and cost-effective therapy for Graves' disease. It is being increasingly used as the first-line therapy in elderly patients and is the treatment of choice for patients with recurrent hyperthyroidism after antithyroid drug or surgical treatment. Radioiodine is also the preferred treatment for autonomous toxic nodules.

Cancer is a series of somatic mutations leading from the normal cell to the cancer cell. In his paper on Current trends in Radionuclide Therapy K.E. Britton reviewed the current concepts in cancer treatment including the role of mutations in apoptosis phenomenon and the concepts of radiobiology and radiation physics in radionuclide therapy. In another paper Britton outlined his experience with radioimmunotherapy and radiopeptide therapy. The expression of antigens and peptides on cell surface of target cells has provided the use of monoclonal antibodies and peptides for experimental tumour therapy. Britton reported the results of several studies using monoclonal antibodies such as the anti-ovarian cancer monoclonal antibody Y-90 HMCFI, the anti-CEA monoclonal antibody PR1A3 (ICRF), the anti-CD20 monoclonal antibody and the Y-90 labelled lanreotide peptide.

C. Divgi described the utility of mathematical models in radionuclide therapy and its potential application in fractionated therapy. In another paper Divgi outlines the targeting characteristics of radiolabelled monoclonal antibodies for the selective delivery of potentially cytotoxic radioactivity to tumour. This review highlights milestones and pitfalls, suggests guidelines for future development and outlines potential clinical utility for radioimmunotherapy in developing countries.

Rhenium-188 has been considered to be one of the most exciting radionuclides of future. It can be conjugated with particles, phosphonates, peptides and monoclonal antibodies for use in therapy. F.F. Knapp and co-workers described the development of W-188/Re-188 generators and various Rhenium labelled radiopharmaceuticals for therapy. Availability of rhenium-188 from the generators and its multipurpose utility in the treatment of metastatic bone pain, arthritis, arterial restenosis and

liver cancer, makes it one of the highly attractive radionuclides in nuclear medicine, especially for use in the developing countries.

The section on Radiopharmaceuticals contains twelve papers on various aspects of radiopharmaceutical production, quality control and dosimetry. The paper by Malja et al. describes a new method of producing Y-90 generator by adsorbing parent Sr-90 on Aminex A-5 ion exchange resin, eluting Y-90 with 0.7M α-hydoxy isobutyrate and later converting it to chloride form. The group also has developed the methods for determining the purity of Y-90 and standardized its labelling procedures with a few radiophaceuticals. In another related paper, Castillo and co-workers reported a method of purifying generator derived, Y-90 from Sr-90 and other metallic chemical impurities. This method permits the use of the Sr-90/Y-90 generator for longer periods. It also permits use of purified Y-90 for efficient labelling of MoAbs and peptides without any interference from metallic impurities.

M. Venkatesh and co-workers reported the results of their studies in the preparation of Y-90 and Rh-105 labelled therapeutic radiopharmaceuticals. Y-90 labelling conditions of polycarboxylate, DTPA and DOTA and the phosphonate, EDTMP have been optimized to get high yields and purity. Also, preparation conditions for particulate agents like Y-90 ferric hydroxide macro aggregates (FHMA) and Rh-105 sulfur colloid have been standardized and their stability in buffer and human serum evaluated.

In the paper entitled Radiochemical Processing of Radionuclides (^{105}Rh, ^{166}Ho, ^{153}Sm, ^{186}Re and ^{188}Re) for Targeted Radiotherapy, P.R. Unni and co-workers reported the standardization of the production and processing procedures for some of the important therapeutic radionuclides like Rh-105, Ho-166, Sm-153, Re-186 and Re-188 using the Dhruva research reactor in Mumbai, India. Ru-105 was separated from Rh-105 by a solvent extraction process, while Ho-166, Sm-153 and Re-186 were produced by (n, γ) reactions on natural targets. However the authors reported a very small yield of Re-188 by irradiating natural tungsten (W).

G. Ferro-Flores and co-workers reported a general method of Re-188 labelling of several biomolecules using EHDP as a weak transchelating agent. The technique has been successfully applied to Re-188 labelling of MoAb (biotinylated MoAb), polyclonal IgG, octreotide analogue and MoAb fragments. The section contains two more papers on similar lines. S. Sostak and co-workers reported development of Re-186 bleomycine for possible use in cancer therapy, while V. Lungu and co-workers reported the results of Re-186 labelling of HIgG, a model compound for MoAbs, using direct reduction method. The disulfide reduction was investigated using different reducing agents. Re-186 labelling with >90% yields was achieved using ascorbic acid or active hydrogen for reduction of Re-186.

The results of trials to optimise dosimetry for Sm-153 EDTMP-therapy to improve therapeutic effects were presented by G. Riccabona and co-workers who carried out dosimetry studies in patients with disseminated bone metastases. Whole body retention (WBR) of various Tc-99m labbelled compounds was compared with WBR of Sm-153 EDTMP. Volume of metastases and regional Tc-99m phosphonate uptakes were assessed by SPECT and conjugated whole body scan data after phantom studies. The results of the trial showed that the dosimetric approach to Sm-153 EDTMP therapy could necessitate the application of higher amounts of Sm-153 EDTMP to reach adequate radiation doses in lesions without necessarily increasing risk of myelodepression and with even better clinical results.

M. Rahman and co-workers presented the preparation of Ho-166 complexes of DTPA, DMSA and EDTA using Ho-166 produced by the n-γ method including conditions which had enabled them to obtain very high yields, procedures for determining radiochemical purity and biodistribution studies in rats.

Radionuclide therapy plays an important role in arthritis refractory to conventional therapy. Currently there is a search for a cost-effective and easily affordable radiopharmaceutical suitable for use in developing countries. G. Prabhakar and co-workers in their paper reported the preparation of colloidal chromic phosphate P-32 suspension for use in radio-synovectomy. The size of the particles reported was in the range of 0.6–2.5 micrometer in vitro, while significant lung uptake was detected in animal studies. The authors have proposed further studies to optimize this preparation.

M.A. Majali and co-workers reported the preparation of Sm-153 complexes of two α-amino methylene phosphonic acid ligands and their evaluation for potential use in bone pain therapy. While the butylene diamine tetramethylene phosphonate (BDTMP) exhibited poor complexation with Sm-153, better quantitative complexation could be obtained with the propylene diamine analog (PDTMP). Sm-153-PDTMP showed significant bone uptake and retention but a high level of liver uptake.

S.H. Ahn and co-workers reported a method of Re-188 labelling of biocytin for potential use in pre-targetted molecular antibody therapy. Biocytin was conjugated to MAG2GABA (Merceptoacetyl diglycine coupled to g-amino butyric acid) to enable conjugation with Re-188. The Re-188 biocytin was found to have high binding avidity for streptavidin.

The section on Thyroid Cancer contains four presentations from four centres, mostly on clinical experience. A.S. Hossain et al., Asghar et al. and Kucuk et al. reported the results of their overall experience with radioiodine treatment of differentiated thyroid cancer. Y.E Demidchik and co-workers reported their experience of radioiodine therapy of childhood thyroid cancer in 753 children below the age of sixteen years treated during the period 1986–1998. All four papers reported excellent results of I-131 therapy in differentiated thyroid cancer. Although these papers come from different parts of the world, there appears to be a general consensus on the management of differentiated thyroid cancer with I-131.

Section 4, Hyperthyroidism, contains three papers, two of them on the treatment of hyperthyroidism and one on regulations to be followed for such therapies. H. Amaral reviewed various regulatory aspects in the I-131 treatment of benign and malignant disorders of thyroid. Amaral recommended adoption of the criteria proposed by the United States Nuclear Regulatory Commission (NRC) published as 10 CFR 35.75 and the Regulatory Guide 8.39 for this purpose. E.A. Barrenechea reported her ten years of experience on radioiodine therapy for hyperthyroidism wherein she analyzed the treatment and follow-up data of 162 patients of hyperthyroidism treated with I-131. Phan Sy An summarised his experience of more than 20 years in the radioiodine treatment of 723 patients treated with I-131 in his hospital in Hanoi. Both authors reported acceptable limits of post therapy hypothyroidism in their series.

Section 5, Treatment of Bone Pain, contains eight papers on palliative treatment of bone pain in patients with metastatic bone disease. The paper by Fettich et al. is based on the results of an IAEA sponsored multi-centre prospective randomized clinical trial comparing the efficacy and toxicity of Strontium-89 and Phosphorous-32 in the treatment of metastatic bone pain. The study has shown almost identical efficacy of Sr-89 and P-32 ($p = 0.122$). Further, there was no significant difference in the toxicity of the two compounds. P-32 has therefore been recommended as an effective alternate to Sr-89 due to its comparable efficacy, safety, general availability and low cost.

K. Kothari and co-workers have investigated the preparation and biodistribution of Sm-153 complexes of a series of α-aminoethyl phosphonic acid analogues of the well-known ligand EDTMP. The conditions for preparing the Sm-153 complexes in good yield and purity with adequate stability were standardized. The preparations showed significant but varying degrees of bone uptake in rats.

N.Ö. Küçük and co-authors reported the clinical results of palliative treatment of metastatic bone pain using Re-186 HEDP in 31 patients suffering from cancers of prostate, breast, rectum, lungs and nasopharynx. The overall pain response rate was observed to be 68% with best results in patients of

breast and prostate cancer and worst in lung cancer. The authors reported a mean palliation period of 8 weeks in the study.

In another study, J. Gaudiano and co-workers reported the results of a phase-I and phase-II study of Re-188 HEDP in the palliative treatment of metastatic bone pain. Results obtained in 12 patients with multiple metastases from carcinomas, with pain surpassing other analgesic options revealed more than 50% pain relief in 91% of the patients and total relief for a variable period in 41% of patients. The authors have suggested further studies of this option in order to determine higher dose protocols without toxic bone marrow reaction.

Another paper by Saichi et al., based on the results of an IAEA sponsored co-ordinated research project describes the production of Sm-153 from the Algerian research reactor and its complexation with EDTMP.

The report by Olea et al. describes the results of graded administered doses of (0.5 mCi/1 mCi per kg body weight) in the treatment of metastatic bone pain. Overall response to treatment was found to be 66%. No significant difference in efficacy was observed in the two groups. However the authors reported a slightly increased myelotoxicity in the higher dose group.

A similar study conducted by Pan et al. by administering graded doses of (1 and 1.5 mCi per kg body weight) Sm-153 EDTMP to two groups of patients (Group-1: 33 patients and Group-2: 34 patients) also has shown almost identical results to those described by Olea et al.

In the last paper of this section, P. Saraswathy and co-workers reported the methodology for the preparation of Sm-153-EDTMP using Sm-153 produced by (n, γ) reaction on natural Samarium targets. The radio Europium contamination which could be expected, was estimated and considered acceptably low. Subsequent animal studies confirmed excellent bone uptake and retention of the compound. The authors concluded that natural Samarium targets could be used in the preparation of Sm-153-EDTMP for use in bone pain palliation.

Section 6, Radiation Synovectomy, consists of two papers related to radiosynovectomy. The paper by Unni et al. describes the procedure for the preparation of Ho-166 labelled hydroxy-apatite, wherein the authors reported high labelling yield and good stability of Ho-166 labelled particles. On the other hand, Pusuwan et al. reported the preliminary results of the clinical studies (radiosynovectomy) carried out by them using Samarium-153 particulate hydroxyapatite. The study carried out on 14 patients revealed good distribution of radioactivity in the joint with minimal extra-articular leakage.

There were very few presentations on monoclonal antibodies. Section 7 contains only two articles on monoclonal antibodies. Yang Zhi and co-workers presented the results of their study on the potential use of Re-188 labelled monoclonal antibody to prevent peritoneal micro-metastases from gastric cancer. Nude mice injected with BGC-823 gastric cancer cells intra-peritoneally were used as models. The effectiveness of Re-188-MoAb in preventing the survival, growth and dissemination of the cancer cells were compared with Re-188-HIgG and saline. Re-188-MoAb injected mice were found to survive longer.

In the second paper of this section, G. Ferro-Flores and co-workers reported a new approach for labelling Sm-153 to MoAbs for potential use in radio-immunotherapy using 1,5,9.13-tetra-azacyclohexadecane N,N',N",N"' tetra-acetic acid (H_4ETA) as a bifunctional chelate. The authors reported a very high Sm-153-H_1ETA labelled MoAb yield with a specific activity up to 1.14 GBq/mg (30.7 mCi/mg).

Section 8, entitled, General, contains nine general and miscellaneous articles including a few on the treatment of neuroendocrine tumours, alpha particle therapy and internal dosimetry. The paper by Zubillaga and colleagues describes a study on Pirocarbotrat™, a new radiopharmaceutical labeled

with P-32 for the treatment of solid tumours. They evaluated its efficacy as a therapeutic agent in the treatment of solid tumours in an experimental model. The authors have also conducted preliminary dosimetric studies following intra-tumoural single dose administration of the radiopharmaceutical in the same experimental model. It has been concluded that, Pirocarbotrat™, a non-sealed beta radiation source, behaves very closely to a sealed beta radiation source when it is intratumourally injected into solid tumours.

Somatostatin-analogue scintigraphy using In-111 labelled ligands have demonstrated a high density of somatostatin receptors in a variety of cancer types. G. Riccabona et al. presented the results of a therapeutic trial in 15 patients to control metastatic cancer using Y-90-DOTA-Lanreotide. The radiopharmaceutical was administered for therapy in 15 patients with rapidly progressing metastatic disease in whom no other therapy was found to be effective. The study has shown variable results including complete/ partial remissions in 2/15, stable disease in 6/15 and no effect in 6/15 patients. The study had also shown that even with low doses of Y-90-Lanreotide some improvement in the management of patients with cancer types expressing somatostatin receptors could be achieved when rapid progression of metastases occurs. The authors concluded that modification of the therapy protocol could perhaps further improve their preliminary results

A. Laznickova et al. in their report described the labelling procedure for Ga-67 Octreotide and its pharmacokinetic studies carried out in rats.

G. Vaidyanathan and M.R. Zalutsky reported the results of their study using a number of labelled MIBG analogues like carrier free I-131-MIBG, MABG (meta At-211 astatobenzyl guanidine) and I-131-FIBG (4 fluoro 3 iodo benzyl guanidine) for possible enhanced effectiveness in targeted therapy. The authors concluded that these analogues have better therapeutic potential than the currently used I-131-MIBG produced by exchange reaction.

B.U. Petelenz reported experimental results and theoretical considerations on using P-32 for intravascular therapy in different types of sources. They have investigated ion implantation of P-31 followed by neutron activation or depositing P-32 chemically on metal surfaces or using liquid P-32 filled balloons. The relative advantages and disadvantages are enumerated.

S. Palm and L. Jacobsson reported on the dosimetric considerations in Astatine-211 radioimmunotherapy. Astatine-211 is an alpha-emitter and to evaluate the expected biological effects, a Monte Carlo program was set to register the single-event distribution of both specific energy and alpha-particle track length to a cell nucleus. The theoretical survival curves presented could, combined with experiments using “bound” and “non-bound” At-211 in a single-cell suspension, reveal which dosimetrical quantity is most suitable for At-211 radioimmunotherapy.

The short range and high LET radiations emitted from alpha particles and Auger electrons have high radiotoxic effects on living tissue. T. Ünak reports on the results of a microdosimetry study on the alpha and Auger electron emitter Astatine-211 and its comparison with that of Iodine-125, an effective Auger electron emitter. The author has concluded that the radiotoxicity of I-125 when the labelled agent is bound to DNA or lodged very close to it, could be considerably higher than that of Astatin-211.

In another paper on Astatin-211, G. Vaidyanathan and M.R. Zalutsky provided the details about the methods for cyclotron production of Astatin-211, its purification and labelling with monoclonal antibodies for potential use in targeted radionuclide therapy of micrometastases, lymphomas and other tumours.

In the final paper of this section, A. Freud describes the production and QA practices adapted in Israel for the production of I-131 diagnostic and therapeutic capsules. Various components of GMP implemented in I-131 capsule production and QC procedures are explained.

1. INVITED PAPERS

DOSIMETRY IN RADIONUCLIDE THERAPY

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Abstract. While it is known that therapeutic effects of radionuclides are due to absorbed radiation dose and to radiosensitivity, individual dosimetry in "Gy" is practiced rarely in clinical Nuclear Medicine but "doses" are described in "mCi" or "MBq", which is only indirectly related to "Gy" in the target. To estimate "Gy", the volume of the target, maximum concentration of the radiopharmaceutical in it and residence time should be assessed individually. These parameters can be obtained usually only with difficulty, involving possibly also quantitative SPET or PET, modern imaging techniques (sonography, CT, MRT), substitution of y- or positron emitting radiotracers for ß⁻-emitting radiopharmaceuticals as well as whole-body distribution studies. Residence time can be estimated by obtaining data on biological half-life of a comparable tracer and transfer of these data in the physical characteristics of the therapeutic agent. With all these possibilities for gross dosimetry the establishment of a dose-response-relation should be possible. As distribution of the radiopharmaceutical in lesions is frequently inhomogenous and microdosimetric conditions are difficult to assess in vivo as yet, it could be observed since decades that empirically set, sometimes "fixed" doses (mCi or MBq) can also be successful in many diseases. Detailed dosimetric studies, however, are work- and cost-intensive. Nevertheless, one should be aware at a time when more sophisticated therapeutic possibilities in Nuclear Medicine arise, that we should try to estimate radiation dose (Gy) in our new methods even as differences in individual radiosensitivity cannot be assessed yet and studies to define individual radiosensitivity in lesions should be encouraged.

1. INTRODUCTION

The Radionuclide Therapy Committee of the European Association of Nuclear Medicine states correctly in the introduction to it's protocols that therapeutic effects of radionuclides in the management of disease are due to the amount of absorbed radiation energy and to the radiosensitivity of the irradiated tissue [1]. Absorbed radiation dose (=Gy), however, is frequently replaced in practical Nuclear Medicine by "mCi" or "MBq" as dose units, even as the amount of activity applied is certainly not the only factor in delivery of an absorbed radiation dose. Radiation dose to an organ or tumour is defined by the simple equation [2]:

$$\text{Gy} = \frac{\text{activity}}{\text{volume}} \times \text{residence time (t)} \times \text{S (mGy/MBq/sec)}$$

The specific S-value of a radionuclide refers to linear energy transfer of it's radiation including also relative biological weighting factors. It would seem logical to establish a clear dose response relationship for Nuclear Medicine therapy (Table I), so that adequate clinical results could be expected. Specific modalities especially of systemic radionuclide therapy, however, make dosimetry and, therefore, an estimate of the dose response relationship quite difficult.

In Nuclear Medicine there is only one therapeutic method which allows a dosimetric calculation as in other forms of radiotherapy: This is radioembolization of hepatoma with ^{90}Y-particles [3]: The tumour (=target) volume is known from CT-scans, 100% of the selectively intraarterially applied activity are in the tumour, no metabolic break-down of the labelled particles occurs for several physical half-lives of ^{90}Y so that "residence time" is derived from physical half-life only. Even intratumoural application of radioactive colloids which should stay in the tumour does not fit in this model, as intratumoural distribution is variable.

TABLE I. DOSE RESPONSE RELATIONS FOR ^{131}I-THERAPY OF THYROID DISEASE

Radiation dose	Radiation effect	Clinical effect
80–100 Gy	moderate atrophy	metabolic activity significantly reduced, growth potential impaired, moderate volume reduction of irradiated tissue
100–150 Gy	significant atrophy	metabolic activity severely reduced growth potential blocked significant volume reduction of irradiated tissue
200–300 Gy	severe atrophy	metabolic activity and growth potential blocked, volume of irradiated tissue: almost gone
500 Gy	necrosis	tissue dead and gone

In all other therapeutic methods (systemic, intracavitary therapy) using unsealed radionuclides dose estimates are much more difficult. In systemic therapy the amount of radioactivity accumulating in the target tissue can basically be measured for radionuclides which emit also y-radiation (e.g. ^{131}I, ^{153}Sm, ^{186}Re) by uptake measurements or quantitative SPET [4]. When therapeutic agents, however, emit only ß$^-$-radiation registration of maximum local concentration of the radionuclide is impossible. It is also not always easy to assess the volume of the target even with modern imaging techniques [5]. Finally "residence time" remains another essential parameter, which can be influenced by metabolic activity and can directly be measured only when y-emitting radiopharmaceuticals are used. When dosimetry should include also dose estimates to bone marrow or critical organs without consideration of the target the same parameters should be obtained to predict and possibly avoid side effects [6].

2. METHODS TO IMPROVE DOSIMETRY

2.1. Assessment of target volume

Without consideration of the specific volume of target tissue with accumulation of the radiopharmaceutical ("functional volume"), anatomic volume of target organs or lesions can today be estimated quite well with modern imaging methods [7] (Table II) in many diseases (e.g. thyroid volume, volume of metastases in lymph nodes, lungs, brain, liver).

TABLE II. ASSESSMENT OF TARGET VOLUME BY

Sonography
CT
MRI
SPECT
PET

In other disorders, however, even these techniques cannot define "target volume": This occurs in intracavitary therapy and in diseases with diffuse bone marrow involvement (Fig. 1). Moreover appropriate diagnostic radionuclide studies can show abnormal uptake in metastases, which could not be localised by other imaging techniques [8]. In such cases emission tomography (ET) can be helpful [9] as it can estimate volume of tissue with uptake of the tracer (or radiopharmaceutical) by now with

sufficient accuracy. Of course such ET-methods are impossible when the radiopharmaceutical emits only ß$^-$-radiation. As discussed later, the substitution of the ß$^-$-emitter by a gamma- or positron emitter can help, so that it might even be possible to estimate target volume in radiosynovectomy [10] by using identical colloids labelled with ^{86}Y for PET! One could also suggest that e.g. ^{124}I-MIBG could be used to define target volume by PET in patients with diffuse bone marrow metastases of neuroblastoma, ^{124}I for cases with negative conventional imaging and positive ^{131}I-scans in thyroid cancer [11] or ^{124}I labelled tumour antibodies. Essentially all ET-studies for volume estimates use basically the same approach:

$$\text{Volume (mL)} = \frac{\text{pixel vol (ml) x } \Sigma \text{ n pixels / ROI / slice x n(sl)}}{\text{CF}}$$

n	=	number of labelled pixels
n(sl)	=	number of slices with labelled pixels
CF	=	correction factor determined for system using appropriate phantom.

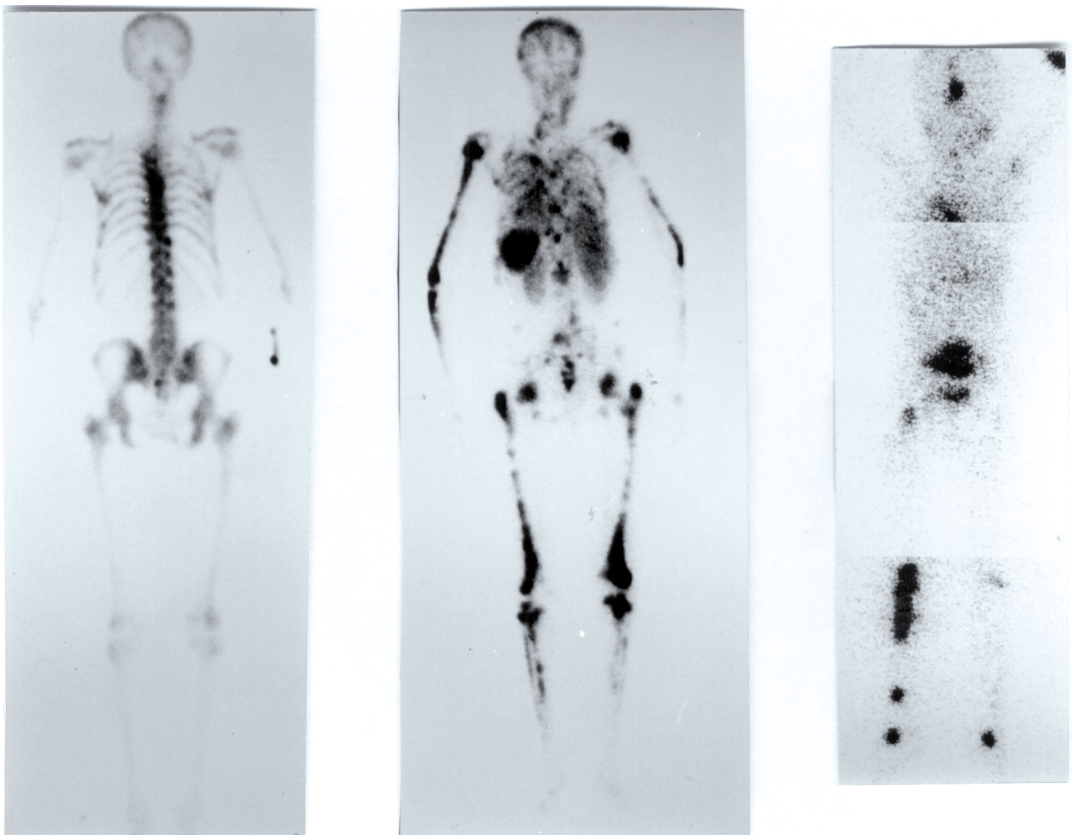

FIG. 1. ***Left****: Bone scan of patient after breast Ca.: diffuse ^{99m}Tc-DPD-uptake in skeleton, kidneys and bladder not visible, renal function normal: "Superscan", suggesting diffuse bone marrow metastases.* ***Middle****: Bone marrow scan of same patient after ^{99m}Tc-granulocyte antibody showing large "cold" areas in axial skeleton as evidence of metastases and displacement of red marrow to long bones in limbs.* ***Right****: Whole body scan after ^{131}I-MIBG in patient with diffuse bone marrow involvement in neuroblastoma.*

2.2. Assessment of maximum local amount of radioactivity in target (Table III)

This again is easy in intracavitary, intraarterial or intratumoural therapy as almost 100% of the applied radiopharmaceutical is localised in the target volume initially. It is more difficult in systemic therapy. But even here, the relative maximum uptake of the radiopharmaceutical (% of administered activity) can well be registered if there is also y-emission by external counting considering also geometry factors, attenuation, scatter and partial volume effects using simple y-counters or quantitative SPECT when appropriate phantoms are studied under comparable conditions.

TABLE III. ASSESSMENT OF UPTAKE OF RADIOPHARMACEUTICAL

(A) Local uptake

1) Radiopharmaceutical emits y- and ß$^-$-radiation
   (e.g. ^{131}I, ^{153}Sm, ^{186}Re)
2) Radiopharmaceutical emits only ß$^-$-radiation

ad 1) Use SPECT (perhaps ^{123}I instead of ^{131}I) or PET e.g. with ^{124}I

ad 2) a) Use radiotracer with identical chemical composition and y-emission
   (e.g. ^{111}In-DOTA-Octreotide, ^{111}In-DOTA-Lanreotide)
   b) Use PET-radiotracer (e.g. ^{86}Y-Octreotide)

(B) Whole body retention and whole body dose estimate

1) Urinary activity excretion over 48–72 hrs.
2) Blood clearance in serial blood samples
3) Whole body ROI when y-emitting radionuclide

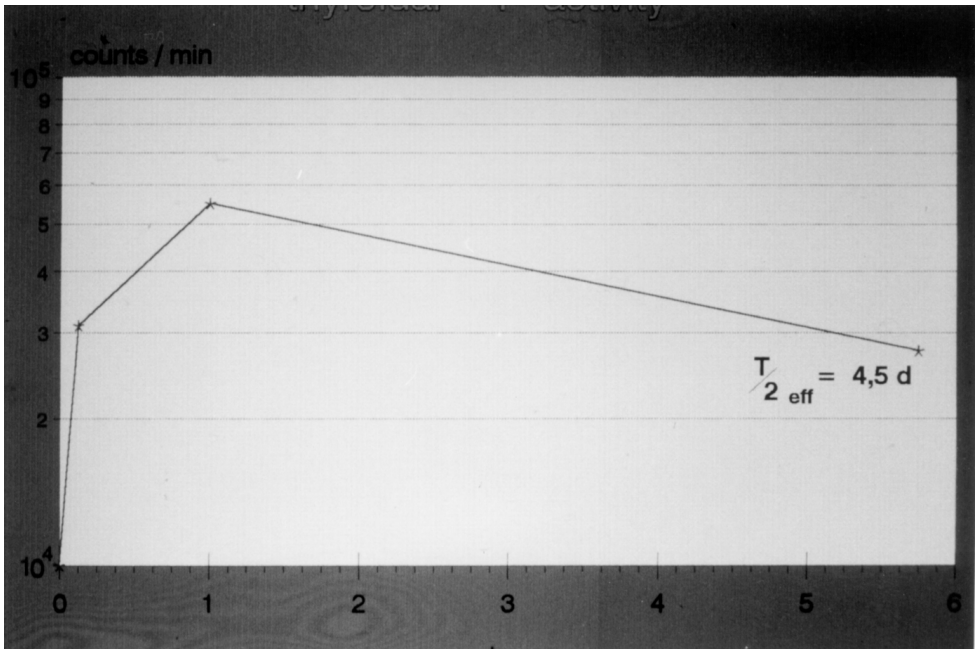

FIG. 2. Direct assessment of effective half-life before ^{131}I-therapy of hyperthyroidism by following thyroidal ^{131}I-activity over several days.

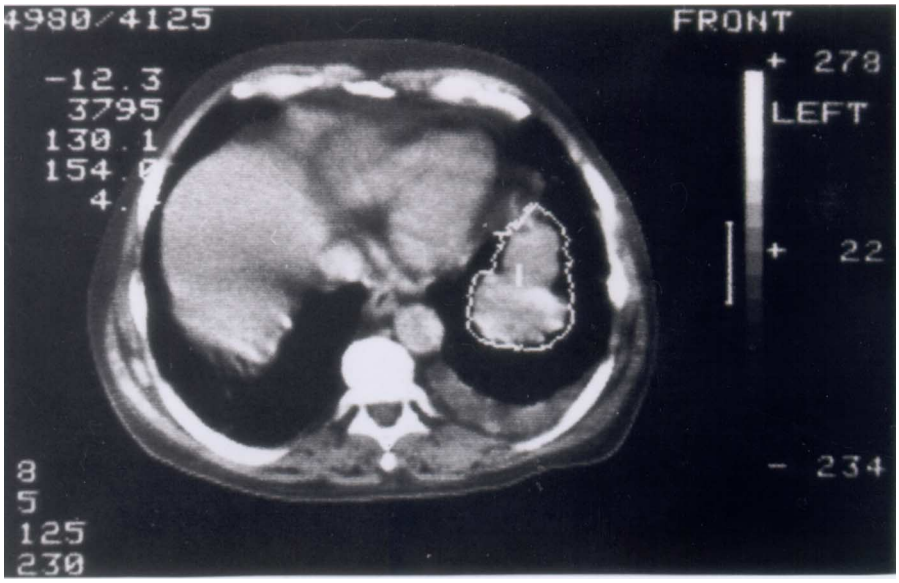

FIG. 3. CT-scan of lung metastases after thyroid cancer: ROI placed over tumour on one slice, similarly relevant ROI's in other slices give tumour volume, when pixel size is known.

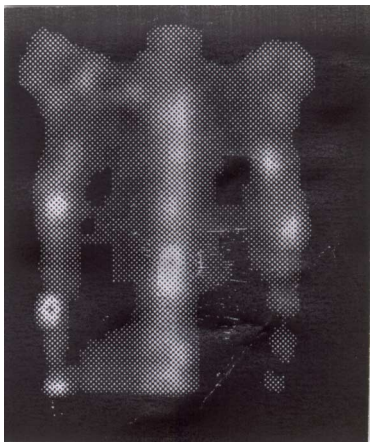

FIG. 4. SPECT of disseminated bone metastases with ^{99m}Tc-DPD allowing estimates of lesion volumes in similar manner as described for CT in Fig. 3.

In some applications, even an analogue ^{99m}Tc-tracer can be used for such proposes [12]. When the radiopharmaceutical, however, emits only ß$^-$-radiation these estimates become possible only, when chemically identical radiotracers with y-emission (e.g. ^{111}In-Octreotide for ^{90}Y-Octreotide, ^{85}Sr for ^{89}Sr) or positron emitters (e.g. ^{86}Y for ^{90}Y) are used [13]. When y-emission is present, specific uptake of the radiopharmaceutical in lesions can also be estimated by comparing count-rates in lesion and normal surrounding tissue on conjugate whole body scans [14].

2.3. Assessment of residence time

Serial blood and urine samples of tracer amounts of the radiopharmaceutical or it's substitute (see above) can help to evaluate radiation doses to blood, marrow, kidney and bladder. For residence time estimates in the target, however, similar considerations as for assessment of local radioactivity concentration become important (Fig. 2). There are certainly no problems, when y-emitters are used for therapy but there are considerable difficulties if pure ß$^-$-emitters are applied. Again, y-emitting substitutes can be used as mentioned above, data must be corrected for decay to biological half-life,

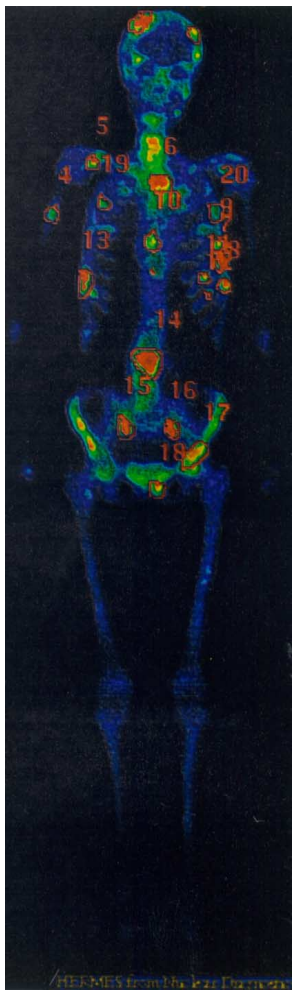

FIG. 5. Whole body bone scan after 555 MBq ^{99m}Tc-DPD. ROI over whole body: 4,134 kcts. = 100%, ROI's over metastases: 544 kcts. = 13,2%. Therefore, maximum uptake 13,2% of administered dose in lesions using ^{153}Sm-EDTMP.

which then gives appropriate information on residence time, as residence time can be derived from effective half-life using

$$\text{(Residence time) } \tau = \frac{T1/2\ \text{eff}}{\ln 2} \qquad (15).$$

2.4. Results of dosimetric radionuclide therapy

Fig. 3 shows an example of tumour volume assessment by CT in metastatic thyroid cancer, Table IV, the conventional formula to assess thyroid volume by sonography [16]. Fig. 4 shows, how volumes of bone metastases can be estimated by SPET. Estimates of local uptake in the target by conjugated view scans with ROI-technique are shown in Fig. 5. Fig. 6 shows an example for the substitution of the ß$^-$-emitter ^{90}Y by ^{111}In. Overall therapeutic strategies using a dosimetric approach seem justified (Table V) as — at least in some diseases — results seem to be better than without dosimetry [17] and dosimetry certainly helped to avoid complications of radionuclide therapy in many applications [18].

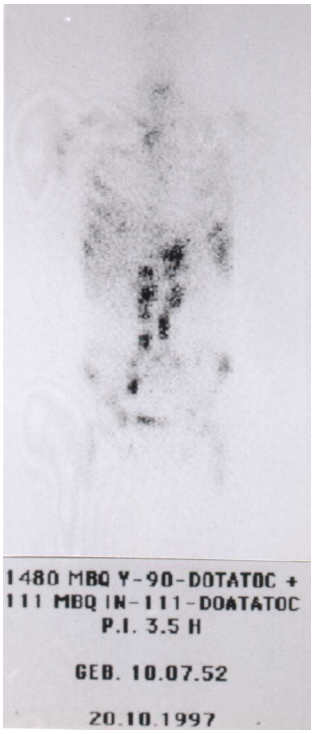

FIG. 6. Whole body scan after ^{111}In-DOTA-Octreotide applied together with therapeutic ^{90}Y-DOTA-Octreotide in patient with disseminated metastases of a carcinoid tumour.

TABLE IV. ESTIMATE OF THYROID VOLUME BY SONOGRAPHY

Lobe Volume (mL)	=	max. depth (cm)	×	breadth (cm)	×	length (cm)	× 0,479

TABLE V. FORMULA FOR DOSIMETRY IN ^{131}I-THERAPY OF THYROID DISEASE

Activity (Ci) $= \frac{\text{cGy} \times \text{W} \times 6{,}67}{\text{T/2 eff.} \times \% \text{ uptake 24 hrs.}}$

or

Activity (MBq) $= \frac{\text{cGy} \times \text{W} \times 25}{\text{T/2 eff.} \times \% \text{ uptake/24 hrs.}}$

W = thyroid weight (g)

3. DISCUSSION

It is obvious that a fairly precise macrodosimetry for radionuclide therapy has become possible today. Several problems in dosimetry for Nuclear Medicine therapy persist: one is the acknowledged fact, that frequently concentration of the radiopharmaceutical is not homogenous in a lesion [19], so that parts of the target will receive a higher dose (Gy/MBq) than others and the second is

microdosimetry, which has provided important insights in microscopic radiation biology in recent years, considering also the effects of Auger electrons and (-particles [20]. It is also obvious that the application of all the mentioned techniques necessary for individual dosimetry require intensive work and high costs as possibly also SPET or PET can be essential. On the other hand it is known, that even without such efforts for dosimetry radionuclide therapy is successful in many diseases [21, 22], even when only "fixed doses" (e.g. ^{131}I, ^{89}Sr, ^{32}P, ^{186}Re-HEDP, intracavitary therapy) are applied. This discrepancy between clinical outcome without dosimetry and scientifically predictable radiation effects in the target can partly be explained by the mentioned unsolved problems of in vivo dosimetry but also by differences in radiosensitivity within targets. This assessment of specific radiosensitivity of a lesion is still an unsolved problem. Studies using Palladium-islets or well plates were done [23] but results so far show, that it still is almost impossible to register e.g. radiosensitivity of a certain tumour in an individual patient. While the efforts to improve registration of specific radiosensitivity by ex-vivo assays should be encouraged in the future one should also try to overcome the old habit of using only amounts of radioactivity as "doses" especially as new and exciting therapeutic applications of radionuclides are being developed. In this situation one should try at least to estimate absorbed radiation dose (= Gy) in therapy, which could improve results of our therapeutic approaches significantly.

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NEW ASPECTS OF RADIONUCLIDE THERAPY OF BONE AND JOINT DISEASES

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Abstract. Whereas in developing countries P-32 is widely used for radionuclide therapy of painful bone metastases, in Europe three radionuclides or radiopharmaceutical agents are available for pain palliation: Sr-89, Sm-153-EDTMP, and Re-186-HEDP. Radionuclide therapy for pain palliation is indicated for bone pain due to metastatic malignancy that has involved multiple skeletal sites and has evoked an osteoblastic response on bone scintigraphy. Response rates of about 70–80% in patients with breast or prostate cancer is reported in the literature, less in metastatic lesions of other primary malignancies. Sm-153-EDTMP may also be used for curative treatment of primary bone tumours or their metastases. Radiosynovectomy as therapeutic procedure or rheumatoid arthritis, other inflammatory joint diseases, persistent synovial perfusion, and other joint diseases is widely used. Using Y-90 for the knee joint, Re-186 for middle sized joints, and Er-169 for small joints an improvement of symptoms may be observed in about 70–80%.

1. INTRODUCTION

A great German scientist and physician, Paul Ehrlich (1854–1915) first described "specific chemotherapy" as a search for a chemical substance capable to be taken up by and killing parasites without doing any harm to normal tissue or organism. To reach such an objective, Ehrlich as a student wanted to determine the microscopic and biological distribution of metals in the organism, because he thought metals to be effective therapeutic agents. He also stated that "particles must be attached to something to be effective". These are the fundamental principles of radionuclide therapy and if radionuclides were known at that time, Ehrlich could have been the father of radionuclide therapy.

In 1936, John Lawrence studied total body irradiation after intravenous administration of P-32 using an animal model of leukemic mice and various lymphomas in animals. Together with a 29-year-old student, who was diagnosed as having myelogenous leukemia Lawrence performed the first P-32 therapy. After 3 courses with a cumulative dose of 394 MBq (10.64 mCi) P-32 the student, symptomatically and clinically, was normal.

In 1940/41 a patient with prostate cancer and painful osteoblastic bone metastases was treated with 8 mCi of Sr-89 with positive effect concerning pain by C. Pecher. About ten years later Friedell reported P-32 therapy to breast cancer bone metastases.

In Europe, about 55 new patients/100 000/year with prostate cancer and 114 new patients/100 000/year with breast cancer are diagnosed. By autopsy in about 80% of patients with prostate cancer and 75% of patients with breast cancer bone metastases were observed. (Table 1). About 30% of patients with bone metastases develop severe pain syndrome which needs therapy.

TABLE I. INCIDENCE (%) OF SKELETAL METASTASES IN AUTOPSY STUDIES

Primary tumour	Mean	Range
breast	73	47–85
prostate	68	33–85
thyroid	42	28–85
kidney	35	33–40
lung	36	30–55
oesophagus	6	5–7
gastro-intestinal	5	3–11
rectum	11	8–13

2. RESULTS

2.1. Radionuclide therapy for pain palliation

For radionuclide therapy for pain palliation because of bone metastases in Europe 3 radionuclides are available: Sr-98, Sm-153, and 186-Re [1–8, see also Table II] Whereas Sr-89 exchange with calcium component of hydroxyapatite, the more recently available radiolabelled bisphosphonates (Sm-153-EDTMP and Re-186-HEDP) localize in bone by bridging the hydroxyapatite. The amount of uptake depends on metabolic activity of normal bone and tumour tissue. In Europe, P-32 no longer is used extensively for bone pain palliation because of possible myelotoxicity. Most of these patients underwent high-dose chemotherapy causing myelotoxicity prior to pain palliation therapy with radionuclides.

TABLE II. PHYSICAL CHARACTERISTICS

Radionuclide	Pharmaceutical	Half life(days)	Maximum ß energy MeV	Mean ß energy MeV	Maximum range in tissue (mm)	γ Photon keV (%)
Sr-89	chloride	50.5	1.46	0.583	6.7	--
Sm-153	EDTMP	1.95	0.8	0.224	3.4	103 (28)
Re-186	HEDP	3.8	1.07	0.349	4.7	137 (9)
P-32	orthophosphate	14.28	1.71	0.695	7.9	--
Sn-117m	DTPA	13.6	conversion	0.129	0.3	159
(in a phase III trial)			electrons	0.153		

Prior to the administration of the radiopharmaceutical agents increased osteoblastic activity in the metastases should be documented by bone scintigraphy.

Indications

Strontium-89-chloride, Sm-153 EDTMP and Re-186 HEDP (and the other unsealed beta-or conversion electron-emitting radiopharmaceuticals under development or available commercially, i.e. P-32-orthophosphate, 117m-Tn-DTPA, Re-188-bisphosphonate) are indicated for the treatment of bone pain due to a metastatic malignancy that has involved multiple skeletal sites and has evoked an osteoblastic response on bone scintigraphy.

Contraindications

Absolute

- pregnancy, continuing breast feeding.

Relative

- myelosuppression
- chronic renal failure or deterioration of renal function (urea >12mmol/l; creatinine >150mmol/l
- urinary incontinence
- acute or chronic spinal cord compression and/or metastases at the base of the skull

Where there is danger of either spinal cord compression from vertebral metastases or pathologic fracture in the extremities, radionuclide therapy for pain palliation should only be used in conjunction with other forms of management directed at these complications.

Simultaneous administration of cytotoxic agents, external wide field radiotherapy and radionuclides may cause significant myelosuppression.

In general, patients should not have received long-acting myelosuppressive chemotherapy for 6-8 weeks prior to administration of Sr-89 and for 6–12 weeks after Sr-89 administration because of the potential for severe leukopenia or thrombocytopenia. Caution should be used if Sr-89 is used in conjunction with myelosuppressive chemotherapy. In patients to be treated with Sm-153 or Re-186 the intervals could be shorter, depending on blood cell counts.

The patient should not have received external beam hemibody radiation within 2–3 months prior to administration of Sr-89, Sm-153 or Re-186 to reduce the probability of combined myelotoxicity from the external and internal radiation sources during this period.

Complete blood cell counts should usually be obtained within 7 days prior to administration of Sr-89, Sm-153 or 186-Re. The patient's platelet count should probably exceed 60,000 and preferably 100,000/mL; the leukocyte count should probably exceed 2,400 – 3,000 and preferably 5,000/mL; and the absolute granulocyte count should exceed 2,000/mL to receive Sr-89, Sm-153 or Re-186. Results below these blood cell levels are not absolute contraindications to treatment but raise the chance of infection or bleeding. Haematological toxicity should be monitored at 3–6 weekly intervals for up to 3 months post Sr-89 therapy and at 1–2 weekly intervals up to 6–8 weeks post Sm-153 or Re-186 therapy.

Other contraindications are renal failure, changing pharmacokinetics of the tracers, and active disseminated intravascular coagulation.

The usual administered activity of Sr-89 ranges from 1.5–2.2 MBq/kg (150 MBq in a single dose vial) (40–60 μCi/kg), of Sm-153-EDTMP 37 MBq/kg body weight and of Re-186-HEDP 1295 MBq. Radionuclides should be administered by slow intravenous injection via a peripheral vein using a butterfly cannula or an intravenous catheter. The cannula or catheter should be flushed thoroughly with normal saline (0.9% NaCl) post injection.

The mean absorbed dose by bone metastases is about 23cGy/MBq (range 6–61cGy) after Sr-89 administration, 1000–14000 cGy for a therapeutic dose of 1295 MBq Re-186-HEDP and 86,5Gy for a therapeutic dose of 2590 MBqSm-153-EDTMP.

The therapeutic procedure may be repeated 12 or more weeks, using Sr-89, 4–6 weeks using Sm-153 or Re-186 after the first injection if blood cell counts are at the suggested levels. The response rate after the first treatment is about 70–80%, after the second treatment about 50%. Only few patients may become really pain-free, but most patients treated with radionuclide therapy may reduce medication especially opioids.

Independent from the radionuclide used for pain palliation the onset of pain relief is more rapidly after Sm-153 or Re-186 administration than after Sr-89, but the mean duration of response after Sr-89 is longer (6 months vs. 3 months). That is why some centres prefer a "cocktail" treatment to optimize the effect of pain palliation without increasing the risk of primary adverse effects.

In animal experiments Sm-153 was used for curative treatment of primary bone tumours. Clinical dose escalation trials are running in some centres in the US and Europe treating metastases of primary bone tumours. Preliminary results are quite promising.

2.2. Radiosynovectomy

Radiosynovectomy is a well accepted therapeutic procedure in inflammatory joint diseases [9,10]. There are several radionuclides available for this treatment (see Tables III and IV).

TABLE III. PHYSICAL CHARACTERISTICS:

Nuclide	Y-90	Re-186	Er-169
phys. half-life	64 h	90,6 h	9,4 d
mean range (soft tissue)	3.6 mm	1.2 mm	0.3 mm
max. range	11.0 mm	3.6 mm	0.7 mm

Indications

- Rheumatoid arthritis
- other inflammatory joint diseases (except bacterial, tuberculous)
- persistent synovial effusion (knee prosthesis)
- pigmented villonodular synovitis
- haemophilic joint disease
- chronic pyrophosphate arthropathy

Relative indications

- persistent effusion after knee prosthesis
- Baker´s cyst
- activated arthropathy
- polyarthrosis of finger and toe joints

Contraindications

Absolute

- pregnancy and continuing breast feeding

Relative

- in children and young patients (< 45 years) the radionuclide should be administered only if it has been estimated that the benefits to be gained outweigh the potential hazards.

Before radionuclide administration for radiosynovectomy a three-(two-)phase scintigraphy and/or scintigraphy with ^{99m}Tc-HIG is recommended to study the degree of inflammation in the joint to be treated. By ultrasound or MRI the joint space, structure of the synovia and amount of effusion should be evaluated to ensure that homogeneous distribution of the tracer is possible.

The puncture of the joint has to follow precautions for asepsis. The needle should be flushed with 0.9% NaCl before being withdrawn. Simultaneous administration of corticosteroids may improve the results of radiosynovectomy of large or middle size joints. For the puncture of middle and smaller joints X-ray control is mandatory.

A particle size of the colloids used for radiosynovectomy of 2–10 nm is essential to avoid leakage from the joint.

TABLE IV. ACTIVITY, RECOMMENDED FOR THE JOINTS TO BE TREATED

	Nuclide (MBq)		
Joint	Y-90	Re-186	Er-169
Knee	185–222		
Hip		150	
Shoulder		110	
Elbow, ankle, wrist		75	
Metacarpo-phalangeal			20–40
Metatarso-phalangeal			30–40
prox. Interphalangeal			10–20

The volume administered to middle size joints should not exceed 1–3 mL, to small joints 1 mL, depending on the joint space.

After nuclide administration the treated joint has to be immobilized for at least 48 hours.

The results of treatment depend on the stage of the disease and bone destruction. The overall results in joints without severe destruction show an improvement in about 70–80%. Nearly same results were published for surgical synovectomy. First preliminary results of treatment with systemically administered Sm-153-EDTMP in patients suffering from rheumatoid arthritis are promising.

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RADIOLABELLED PEPTIDES: NEW RADIOPHARMACEUTICALS FOR TARGETED THERAPY

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Abstract. Radiolabelled peptides have been the focus of an increasing interest by the nuclear medicine community within the last few years. This has mainly been due to successful development of one of these peptides, somatostatin, as a tool to visualise various pathologic conditions known to express a high number of somatostatin receptors. Somatostatin receptors have been identified in different tumours such as neuroendocrine tumours, tumours of the central nervous system, breast, lung and lymphatic tissue. These observations served as the biomolecular basis for the clinical use of radiolabelled somatostatin analogs, which are at present of great interest for diagnostic and therapeutic applications. A promising somatostatin analogue, DOTA-D-Phe^{1}-Tyr^{3}-octreotide, named DOTATOC, has shown favourable biodistribution and high affinity binding to SSTR2 and SSTR5, high hydrophilicity and ease of labelling and stability with ^{111}In and ^{90}Y. A clinical trial aimed at evaluating the biodistribution and dosimetry of DOTATOC radiolabelled with ^{111}In, in anticipation of therapy trials with ^{90}Y-DOTATOC in patients was undertaken. ^{111}In-DOTATOC showed favourable pharmacokinetics (fast blood clearance and urinary excretion) and biodistribution, and high affinity to tumours expressing somatostatin receptors (thus, a high residence time in tumour). These results are promising for therapy trials with ^{90}Y-DOTAOC, for which radiation dosimetry appears acceptable for normal organs (including the red marrow). Moreover, labelling conditions of DOTATOC with ^{90}Y has been optimised in order to achieve labelling yields of more than 98% and specific activities of greater than 60 GBq (1.6 Ci)/umol.

1. INTRODUCTION

In recent years, peptide receptor specific radioligands have been investigated for in vivo applications in tumour patients and among them somatostatin analogues are certainly the most widely studied.

Somatostatin (SST) constitutes a multi gene peptide family with two principal bioactive products, SST-28 and SST-14. The latter, a natural neuropeptide compound formed by 14 amino acids, was discovered in hypothalamus 18 years ago, has an inhibitory effect on the secretion of the growth hormone [1–2]. Further studies have revealed the presence of this peptide in neurons and in the endocrine system cells, with greater density in the brain, pancreas and gastrointestinal section [3–4].

The presence of specific somatostatin receptors (SS-R) has also been observed in the tissues and the physiological activity of somatostatin has been proved to be directly mediated by these receptors (SS-TR). At present, five somatostatin receptors (SS-TR 1–5) are known and have been cloned [5]. They are overproduced in most tumours, among which are melanoma, lymphoma, breast tumours, small cell lung cancer and other tumours.

Animal experiments have shown that somatostatin has an inhibitory effect on the growth of different kinds of malignant tumours, such as osteosarcoma, breast and prostate carcinoma [6–7]. These pharmacological properties of somatostatin have generated great interest for the therapeutic potential application of the molecule. Natural somatostatin, however, cannot be used as a medicine, because of its short plasmatic half-life (about 2').

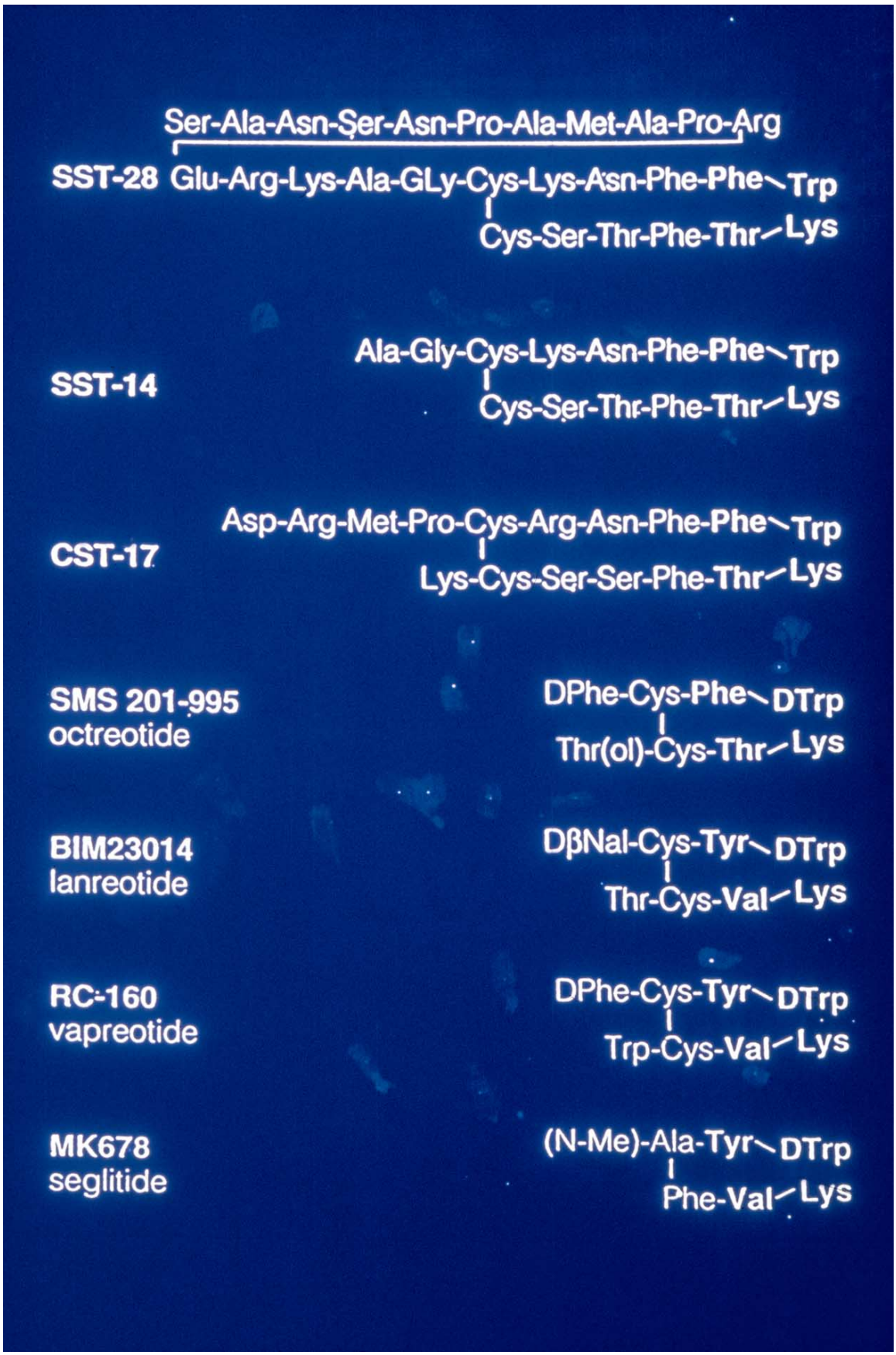

FIG. 1. SST-receptor agonists.

To overcome this obstacle, many different SST peptide analogs have been synthesized for investigational and clinical applications. Structural function studies, conducted on the natural peptides, evidenced that amino acid residues Phe^7, Trp^8, Lys^9 and Thr^{10} present in the loop are necessary for the biological activity with residues Trp^8 and Lys^9 being essential whereas Phe^7 and Thr^{10} can undergo minor substitutions like Phe replaced by Tyr and Thr replaced by Ser or Val. The general strategy for designing SST analogs has been to retain the crucial segment of the four amino acids and to incorporate a variety of cyclic and exocyclic restraints to stabilize the loop around the conserved residues.

Octreotide, the first SST analog introduced for clinical use, inhibits the release of growth hormone, glucagon and insulin more powerfully than SST-14. Its plasma half life, after subcutaneous administration, is two hours, and rebound hypersecretion of hormones does not occur. While the natural peptides SST-14 and SST-28 bind all five SS-TR receptor subtypes, octreotide binds with high affinity SS-TR2 and SS-TR5 and with moderate affinity the subtype 3 but does not bind to SS-TR1 and SS-TR4 [8].

Thanks to its long half-life, this peptide has proved to be effective in blocking hormone over-secretion in different neuroendocrine tumours. The effect of octreotide on these tumours depends on the presence of somatostatin receptors in bioptic samples analysed through autoradiographic techniques, which employ ^{125}I labelled octreotide [9].

This in-vitro demonstration has led other research teams to label octreotide with gamma isotopes for scintigraphic studies of tumours expressing these receptors [10–11]. For routine clinical use, the compound obtained by coupling octreotide with the bifunctional chelating agent DTPA can be effectively labelled with ^{111}In (physical half-life = 68 h). This radiopharmaceutical, commercially known as Octreoscan, has shown favourable biodistribution and pharmacokinetic properties [12]. After intravenous administration, the compound is rapidly cleared from circulation almost exclusively via the kidneys; one-third of the injected dose remains in the blood pool at 10 min. after administration.

The great success of this radiopharmaceutical inspired the researchers to postulate that an anti-tumoural therapy, based on the use of this compound can be tried, by simply substituting ^{111}In with a strong beta emitter radionuclide like yttrium-90 (^{90}Y) (E_{max} β = 2.2 MeV). In fact, the radiotherapeutic use of this radionuclide associated to the somatostatin analog will lead to a high and more evenly distributed radiation dose to the tumour because of the long particle range and tissue penetration (range 8–10 mm for soft tissue-solid tumours, respectively). Even tumours with an inhomogeneous distribution of receptors may respond favourably to treatment with such radiopharmaceuticals. Unfortunately, it was quickly demonstrated that the ^{90}Y-DTPA-octreotide was unstable “in-vivo” with consequent release of high quantities of ^{90}Y that caused high bone marrow toxicity.

Recently, at Basel University a new octreotide derivative has been synthetized replacing Phe^3 with the more hydrophilic tyrosine and coupled with the macrocyclic chelating agent DOTA which is known to form stable complexes with ^{90}Y [13].

The new compound, named DOTATOC, has been efficiently labelled either with ^{111}In or ^{90}Y and tested in vitro for its affinity to SSTR and then in vivo in comparison with the commercial product Octreoscan [14].

Biodistribution experiments performed in animals with pancreatic tumour, have evidenced the tumoural capturing specificity of this compound and complete remissions of the tumour after treatment with ^{90}Y-DOTATOC [15].

These encouraging preliminary results have shown the possibility to treat SS-TR expressing tumours by receptor mediated radiotherapy.

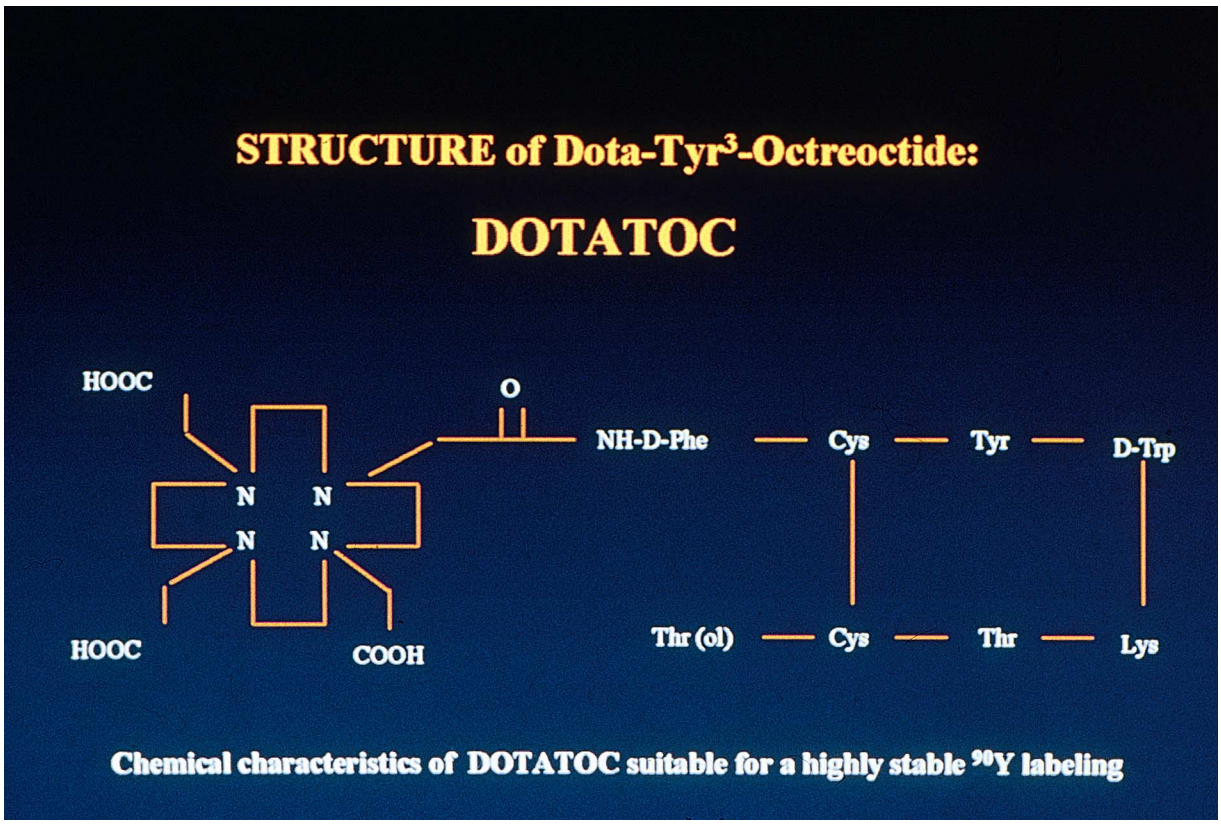

FIG. 2. Structure formula of DOTATOC.

We undertook a clinical trial to study the pharmacokinetics and biodistribution of ^{111}In-DOTATOC by performing scintigraphic studies in order to predict the in vivo behavior of ^{90}Y-DOTATOC in therapy trials.

Furthermore, the identification of the organs with the greater uptake would be useful in order to device preventive or adjuvant interventions minimizing serious or minor complications in therapy.

2. METHODS

To 30 μg of DOTATOC, 150 μl of 0.4 M Na Acetate/Gentisic acid (6.0 mg) pH = 5.0 are added. Then, 1.15 GBq (31 mCi) of Y-90 (usually in a volume of 50 μl of 0.04 M HCl) are added into the conical vial containing the peptide. The mixture is incubated for 25 min. at 90°C.

An aliquot is then removed, added with 20 μl of 1.0 mM DTPA solution, and the presence of free Y-90 determined by Sep-Pak C_{18} cartridge and HPLC analysis. Using this method, we routinely achieve a labelling yield >98% with a specific activity >60 GBq (1.6 Ci)/μmol.

Twenty patients with neuroendocrine tumours were injected with about 200 MBq of ^{111}In-DOTATOC. The patients were hospitalized for 3 days in order to collect all the necessary data and perform the scintigraphic examinations. A complete urine collection and a series of blood samples were withdrawn in order to study pharmacokinetics. The activity in the samples was determined by a ã-counter. Biodistribution was evaluated by scintigraphic images: planar images with anterior and posterior views were acquired 1, 3, 24, 48 h p.i and SPECT images were acquired 3 h p.i.

Whole body images were analyzed with the conjugate view method in order to evaluate the biodistribution of the tracer. ROIs were drawn around source organs (namely spleen, liver kidneys, lungs, tumours) and counts were corrected for background, attenuation and physical decay and converted in %ID vs. time, so that time-activity curves for source organs were obtained.

We set up a compartmental model to represent the organism, the activity distribution and exchange in and between organs. We considered the blood pool, the ECF, the spleen, liver, heart contents, kidneys and the excretion pathway. Each involved organ is represented by one or more compartments. Experimental data on pharmacokinetics and biodistribution were inserted in a special software (SAAM II) that gives the best fits describing the curves. The same software could calculate the area under these curves, namely the integral activity, and the residence time for each organ.

The dosimetric study was carried out by the MIRD (medical internal radiation dose committee) formalism by the MIRDOSE 3.1 software.

3. RESULTS

This is an example of the scintigraphic images obtained. This patient had lung metastases (left side) and liver metastases. We can see the anterior and posterior views at 3 and 24 h. The high uptake in tumours is evident from the first planar images and remains constant in the following acquisitions. These are the transaxial slices of a SPECT acquired 3 h p.i., showing a very high uptake in the lung tumours.

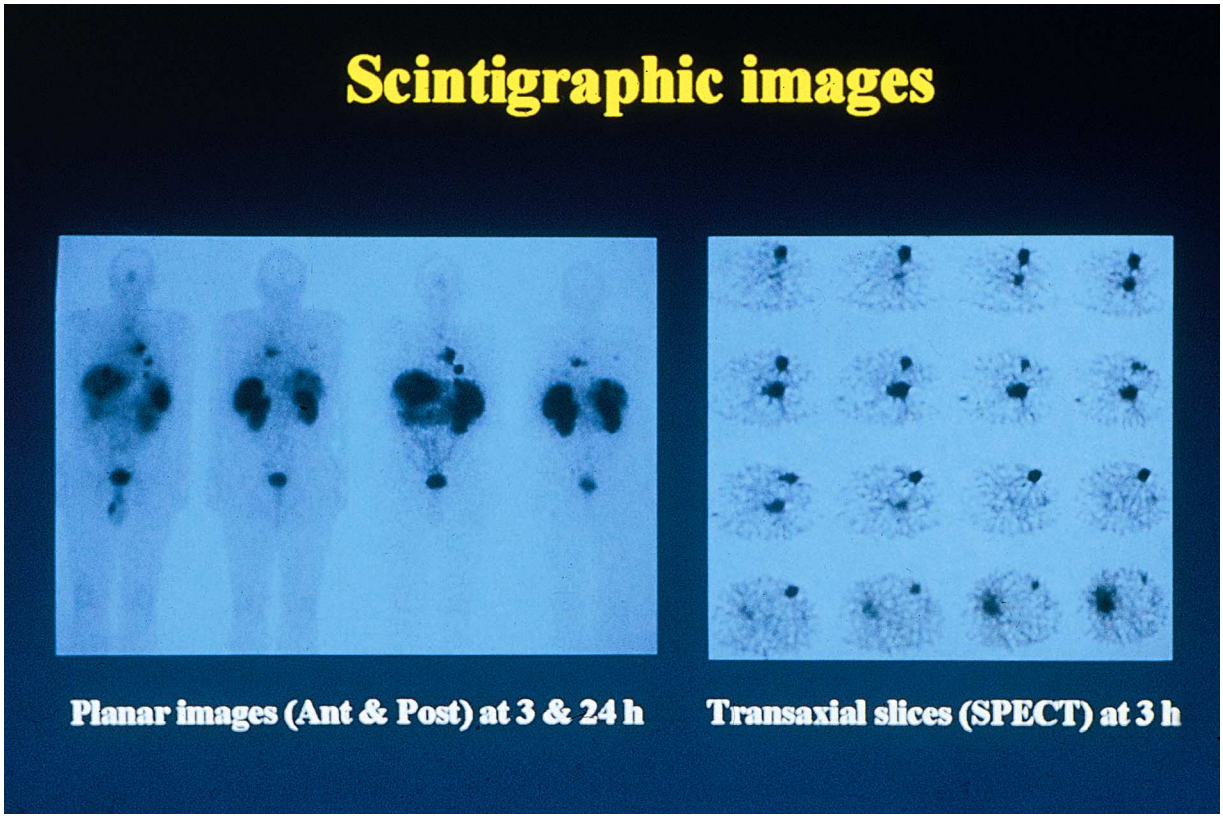

FIG. 3. Scintigraphic images acquired after the injection of 148 MBq of ^{111}In-DOTATOC in a patient affected by bronchial carcinoid with lung and liver metastases. The high uptake in tumours is evident from the first planar images (3h) and remains constant in the following acquisitions (24h). On the right side of the figure, the transaxial slices of a SPECT acquired 3h p.i., demonstrating an intense uptake in the lung tumours, are shown.

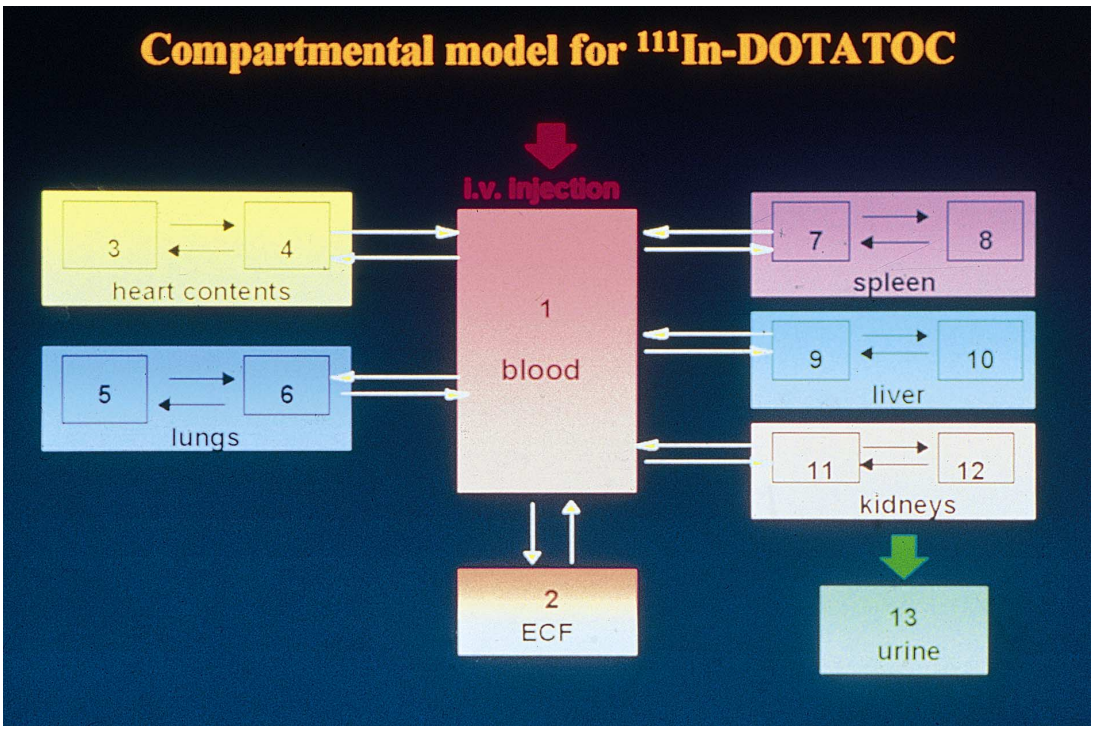

FIG. 4. Compartmental model developed to interpret the biokinetics from experimental data on biodistribution: each source/target organ is associated to compartments to represent the biodistribution and the exchange of ^{111}In-DOTATOC between organs. "In" and "out" arrows represent the activity injected in the blood and the activity excreted through the kidneys.

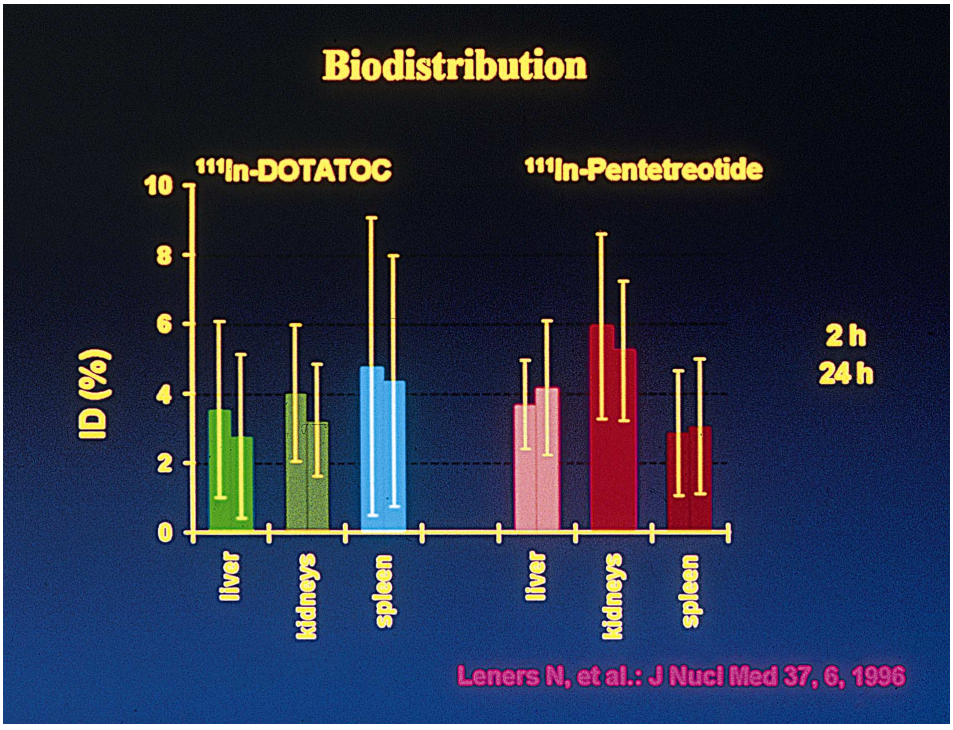

FIG. 5. Biodistribution of ^{111}In-DOTATOC in comparison with ^{111}In-Pentetreotide (Octreoscan). The histogram show the biodistribution (%ID) of ^{111}In-DOTATOC obtained from the analysis of scintigraphic images. The data for ^{111}In-Pentetreotide were obtained from literature.

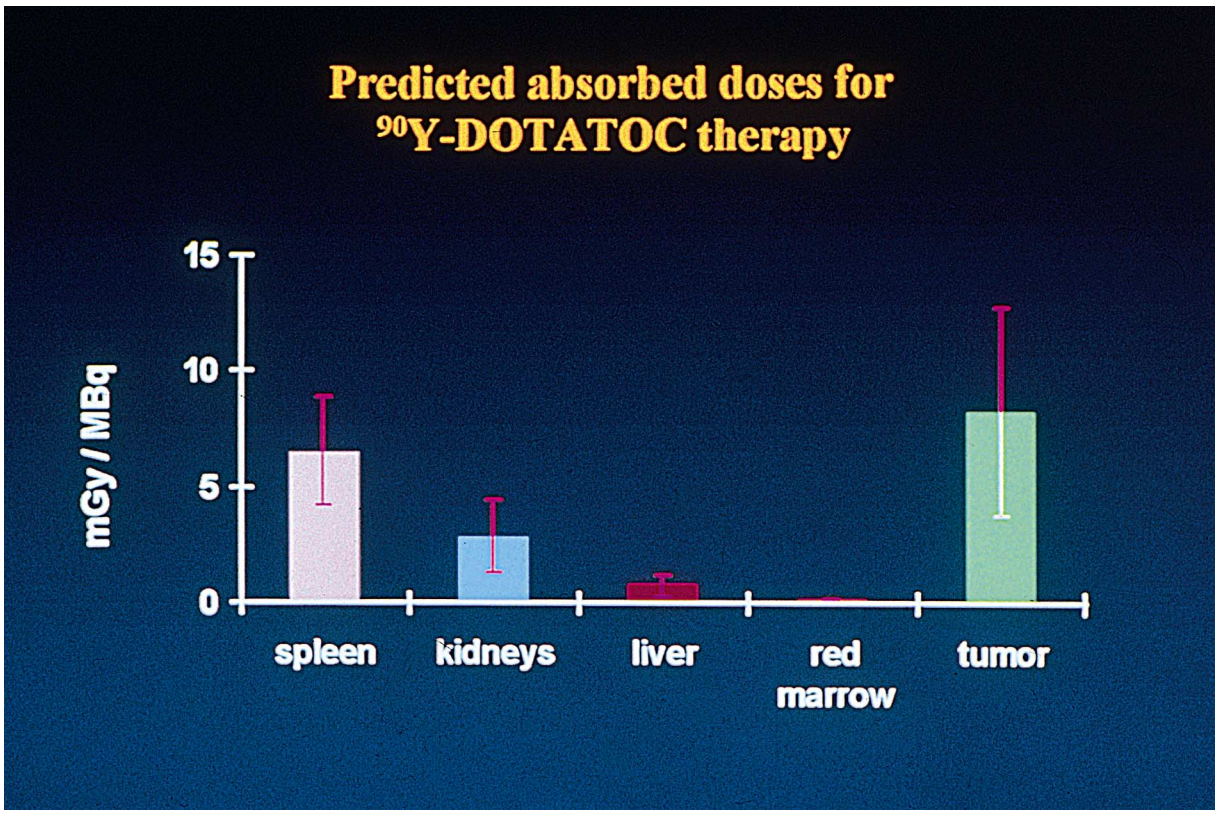

FIG. 6. Predicted absorbed doses for ^{90}Y-DOTATOC obtained by converting the residence times from ^{111}In into ^{90}Y.

A compartmental model was used to calculate the integral activity and the residence time for each organ.

The blood clearance of DOTATOC, plotted on a semilogarithmic scale showed three different slopes: between 0–2 h; 2–10 h; and then from 10 h on. Clearance was very fast: the activity in the blood pool after 10 h was already less than 1%.

The rate of excretion was obtained by a complete urine collection up to 3 days p.i. More than 70% of the injected activity was recovered in the urine within the first 24 h.

The biodistribution of ^{111}In-DOTATOC in comparison with the commercial Octreoscan is represented in figure 5.

The most affected organs are spleen, kidneys and liver. In particular, kidney uptake was lower for ^{111}In-DOTATOC while spleen showed higher accumulation of the radiotracer compared to Octreoscan.

The values of the residence time obtained for ^{111}In were converted into values for ^{90}Y and the absorbed doses to organs and tumour were predicted by the MIRDOSE3 software.

In case of therapy, the organs receiving the highest doses would be the spleen (with 6.5 ± 2.3 mGy/MBq) and the kidneys (with 2.9 ± 1.6 mGy/MBq). Usually the red marrow is identified as the critical organ for therapies with ^{90}Y. Instead, the absorbed dose to red marrow is very low (0.1 mGy/MBq) and this organ is no concern of future therapy.

As for the absorbed doses in tumour, the tumours considered here have different characteristics, for histology, dimensions and position, so it is preferable to look at the range (2–30 mGy/MBq) than at mean values, as from our experience, values are dependent from patient to patient.

In conclusions, the dose delivered to the tumour is high, being in the range from 2–30 mGy/MBq.

Absorbed doses to other organs including red marrow are acceptable, suggesting that high activities of ^{90}Y can be administered (>2 GBq) with low risk of myelotoxicity.

Finally, careful consideration has to be paid to spleen and kidneys in terms of possible complications following therapy.

The encouraging results of this dosimetric study towards future therapy with ^{90}Y-DOTATOC, have prompted us to explore the best labelling conditions of DOTATOC with ^{90}Y. The labelling procedure has now been optimized.

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MONOCLONAL ANTIBODIES TO THE PRETARGETING APPROACH: DEVELOPMENTS IN THE RADIOPHARMACEUTICALS FOR RADIOIMMUNOTHERAPY

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Abstract. In recent years, large experience has been accrued through the clinical application of radiolabelled monoclonal antibodies in the diagnosis and therapy of malignant disorders. While radioimmunoscintigraphy has established its role in the nuclear medicine practice, radioimmunotherapy has thus far gained limited acceptance mainly due to the low amount of radioactivity that can be targeted to the tumour and to the myelotoxicity which is typically the dose limiting factor. In an attempt to overcome the low uptake of label by the tumour and improve the tumour-to-blood ratio, various studies have examined the concept of tumour pretargeting based on the separate protocols, especially the 3-step approach, with respect to the use of directly labelled antibodies, lies in the lower toxicity observed which has allowed to administer high doses of therapeutic radionuclides, such as Y-90, without bone marrow toxicity. Pilot studies, applied to the treatment of advanced stage tumours, have shown that this approach interferes with the progression of tumours and produce tumours regression in patients no longer responsive to other conventional therapeutic modalities. The potency of pretargeting based on the avidin/biotin system may be exploited in the near future to convey a variety of cytotoxic substances, other than radioactivity, onto cancer cells.

1. INTRODUCTION

The utility of monoclonal antibodies (MoAbs) for targeting radioactive agents to tumour cells, for diagnostic and therapeutic applications, has been extensively studied [1–2].

Although several studies have been carried out in this area for almost two decades, many limitations when using radiolabelled MoAbs for treating solid tumours in humans have been encountered.

As far as the antibodies are concerned, the ideal tumour – specific antibody does not yet exist. However, advances in molecular biology and recombinant DNA technology is providing an increasing array of MoAbs capable of targeting several tumour antigens. In addition, directly labelled MoAbs circulate for days with maximum tumour concentration occurring at 1–2 days with continuing high blood concentration for several more days. Therefore, even when properly selected MoAbs are employed, only a small percentage (usually not more than 1%) actually localizes on the tumour [3]. The remaining 99% is distributed in the rest of the body and results in a high background signal.

Another problem is of a more complex nature and is related to the adverse intrinsic tumour characteristics.

Tumours often display intrinsic heterogeneity in antigen density. This factor, together with the non uniformity of tumour vascularization, capillary permeability, degree of tumour necrosis and difference in interstitial pressure, account for the heterogeneous distribution of antibodies in targeted tumours [4].

Tumour heterogeneity is partly overcome using radiolabelled MoAbs for therapeutic applications. High energy beta particles can penetrate up to several millimeters of tissues and the emissions can kill tumour cells which are antigen-negative and have no radiolabelled antibody localised on their surface (the cross fire effect).

In an attempt to overcome the low uptake of label by the tumour and improve the tumour-to-blood ratio, various studies have examined the concept of tumour pretargeting based on the separate administration of MoAbs and radiolabel [5–7]. Such systems require the use of a long-circulating targeting macromolecule (modified MoAb; first conjugates) having a high affinity for a small rapidly excreted effector molecule (second conjugates), which is administered after the MoAb has concentrated in the target tumour. Conceptually, the modified MoAb is administered first and allowed to distribute throughout the body, to bind to the tissues expressing antigen, and to clear substantially from other tissues. Then the radiolabelled second conjugate is administered and, ideally, it localises at sites where the modified MoAb has accumulated. Second conjugate injection time can be delayed to a time when most of the primary MoAb has been cleared from the blood and normal tissues, thereby decreasing non-tumour binding and achieving, with the use of this strategy, higher tumour to non-tumour ratios [8].

Several targeting macromolecule-conjugate/effector small molecule pairs have been proposed [9]. The second conjugate is not necessarily a radiolabelled molecule; a 3-step approach to target biotinylated tumour necrosis factor (TNF) onto tumour cells, potentially widening the therapeutic window of TNF, has been recently reported [10].

The methods, applied in the majority of clinical trials, are based on the avidin/biotin system, which has long and widely been used for in vitro applications [11].

Avidins are functionally defined by their ability to bind biotin with high affinity and specificity without recognising or binding any other physiological compound with any strength. For practical purpose, their binding can be regarded as irreversible [12] (Table I).

TABLE I. MOLECULAR PROPERTIES OF AVIDIN

	Avidin	***Streptavidin***
Molecular weight	64 000	60 000
Number of subunits	4	4
Subunit M.W.	16 000	15 000
Biotin binding sites/mole	4	4
Kd of biotin complex	10^{-15}	10^{-15}
Oligosaccharide/subunit	1	0
Mannose/subunit	4.5	-
Glucosamine/subunit	3	-
Isoelectric point	10.5	6

Avidins are small oligomeric proteins made up of four identical subunits, bearing a single binding site for biotin. Avidin is a 66 kDa glycosylated protein, commonly isolated from hen egg white, shows a strong positive net charge. Streptavidin, a non-glycosylated analogue, isolated from *Streptomyces avidinii*, in contrast to avidin is nearly neutral at physiological pH, with an isoelectric point of approximately 6.

Biotin is a 244 Da molecule constituted by a functional “head” region (bicyclic ring) which binds avidin, and a functionally irrelevant carboxyl “tail” end (valeric acid), which can be chemically altered with little or no effect on the molecule. A large series of biotin derivatives, obtained by modifying the carboxyl group, are commercially available and can be used to covalently link biotin to a variety of molecules containing primary amines, thiol groups aldehydes and so on.

Due to the flexibility of this system, several protocols have been devised and in particular two major methods are presently used in the clinical settings with the aim to improve the delivery of radionuclides to tumours.

2. METHODS

2 — step

This approach has been initially proposed based on the in vitro conjugation of streptavidin to the antibody which is administered first, followed, two to three days later, by the injection of radiolabelled biotin [13].

An alternative approach has also been used to target intraperitoneal tumours. In a typical protocol, biotinylated MoAbs are injected intraperitoneally (first step) followed 1–2 days later by the i.p. administration of radiolabelled streptavidin. This approach originated very high tumour to normal tissue (9:1) and tumour to blood ratios (14:1) suggesting that this two-step strategy might be superior to conventional radiolabelled MoAbs for intraperitoneal targeting of tumours [14].

3 — step

The locoregional approach is feasible when tumour is confined to the peritoneal cavity or in other locoregional approaches. In the presence of widespread disease, a systemic injection of the tracer is nonetheless required. A three-step approach has been designed for these cases, where conjugates need to be cleared not only from a well defined body cavity, but from the entire blood pool (15).

Briefly, a typical 3-step protocol involves the systemic injection of biotinylated MoAbs (step 1) followed by injection of avidin and streptavidin one day later (step 2). The second injection carries a two-fold purpose:

1) The removal of excess circulating biotinylated antibodies in the form of cold complexes via avidin (fast clearance) and 2) The targeting of tumour cells with streptavidin (slower clearance).

Thereafter, radiolabelled biotin which will selectively bind to streptavidin and thus to the tumour, is injected (step 3). The use of a second “chase” of biotinylated human serum albumin, with the purpose to decrease the radiation burden in circulation, administered few minutes prior to the radioactive biotin has been also proposed.

In this technique, the excess of circulating biotinylated MoAbs are removed as cold complexes, which are taken up and metabolized by the liver. This is the major factor in background reduction and is obtained prior to label injection.

3. RESULTS AND DISCUSSION

Moreover, the rapid blood clearance of the radiolabelled biotin allows imaging to be performed only 90–120 min after injection and with only very low background activity.

Figure 3 shows an example of anterior and posterior whole body images obtained 2h after an i.v. injection of ^{99m}Tc-biotin following a 3-step tumour targeting protocol. Note the rapid excretion of the radiotracer through the kidneys and the absence of activity in the liver and bone marrow (cold spine).

A therapy trial based on the three-step pretargeting has been conducted in 45 eligible patients with histologically confirmed grade III or IV glioma and documented residual disease or recurrence after conventional treatment.

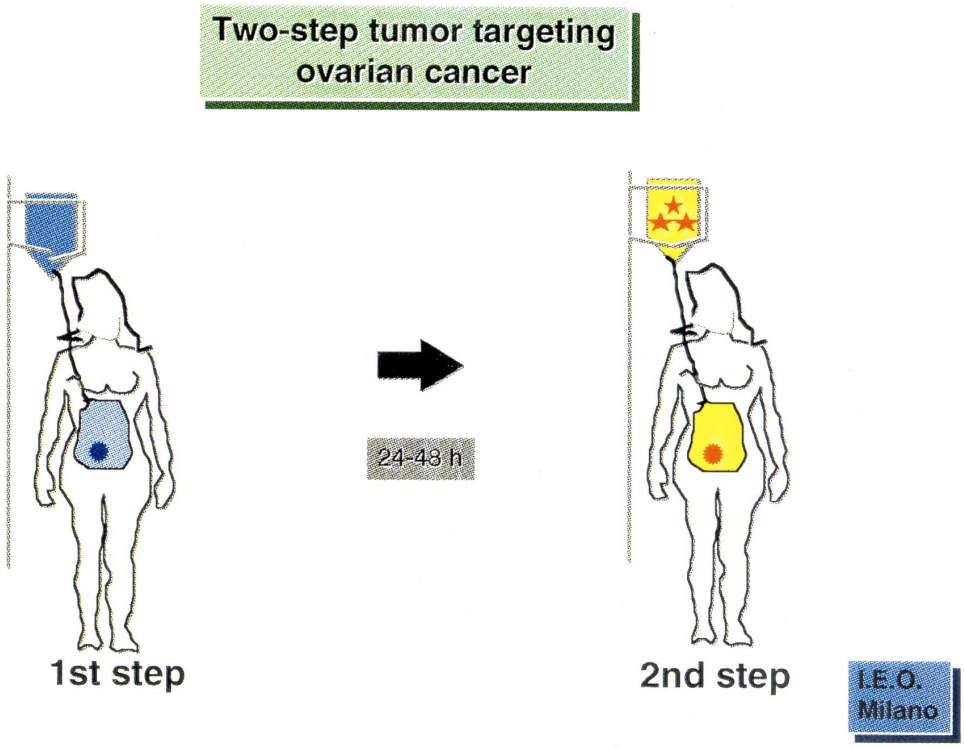

FIG. 1. Two-step strategy. Biotinylated MoAbs are injected (i.p.) and allowed to localize onto the target (1st step). Then 1–2 days later radioactive streptavidin is injected (i.p.) to target tumour.

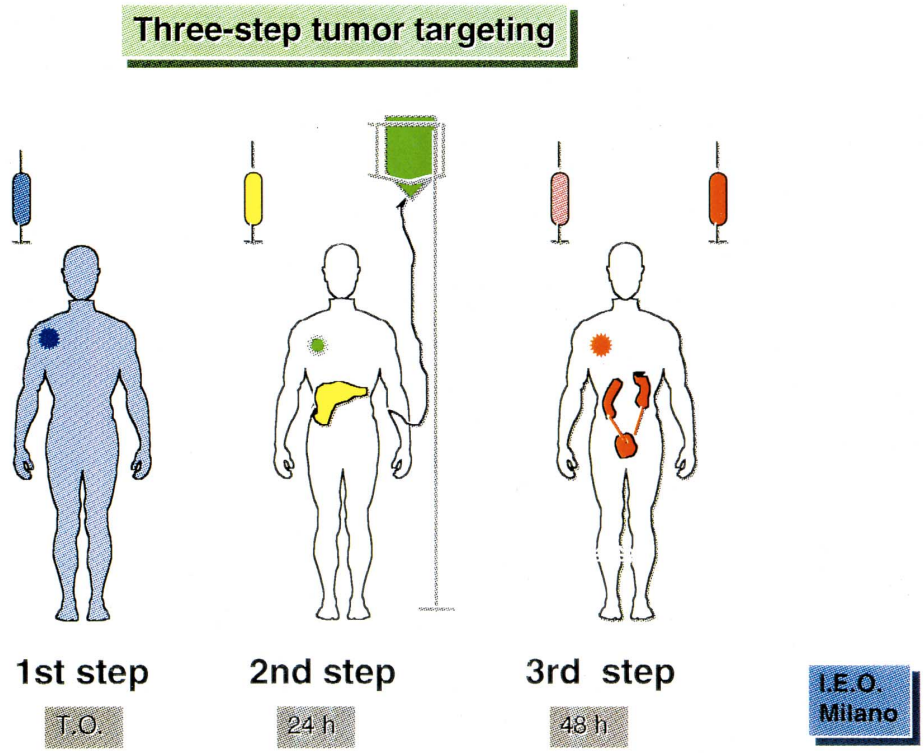

FIG. 2. Three-step strategy. Biotinylated MoAbs are injected (i.v.) and allowed to localize onto the target (1st step). One day later avidin and streptavidin are injected (i.v.) (2nd step). After 24 h, when unbound streptavidin and circulating avidin-MoAb complexes have been cleared from circulation, radiolabelled biotin is injected (i.v.) (3rd step).

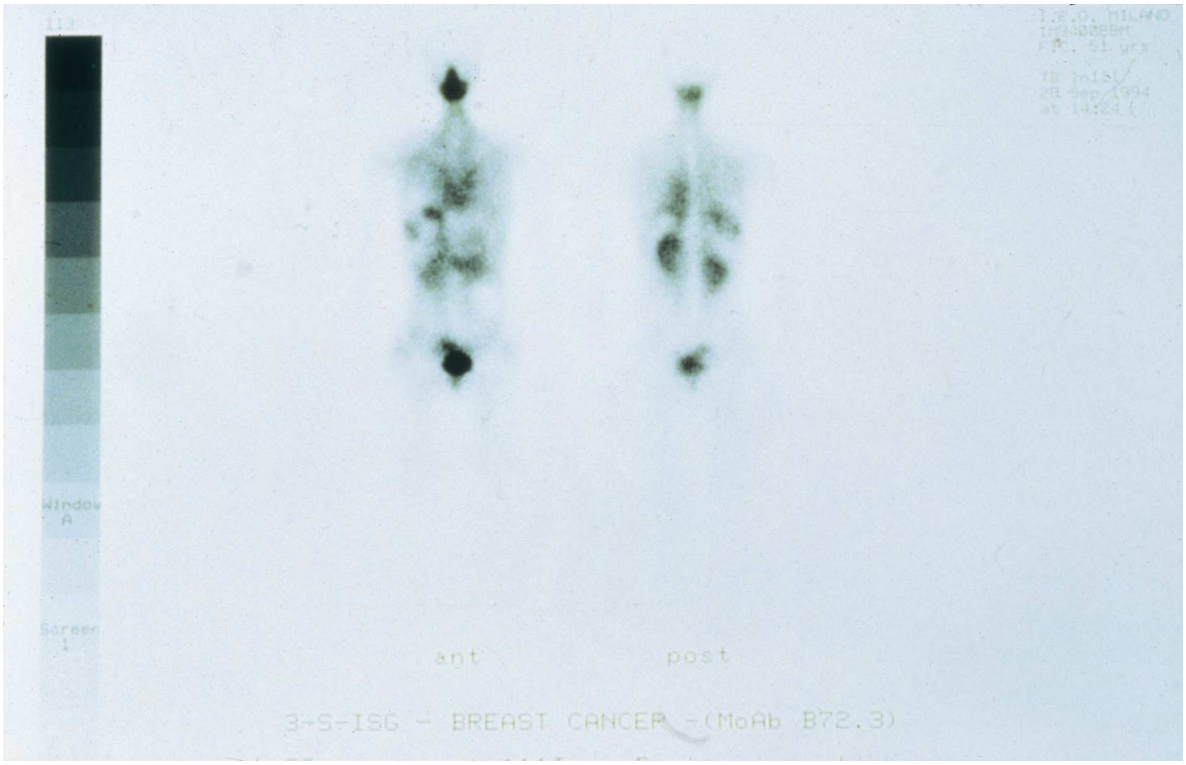

FIG. 3. Anterior and posterior whole-body planar images of a patient with breast cancer obtained 2h after the injection of ^{99m}Tc-biotin following a 3-step tumour targeting protocol with biotinylated B72.3 MoAb.

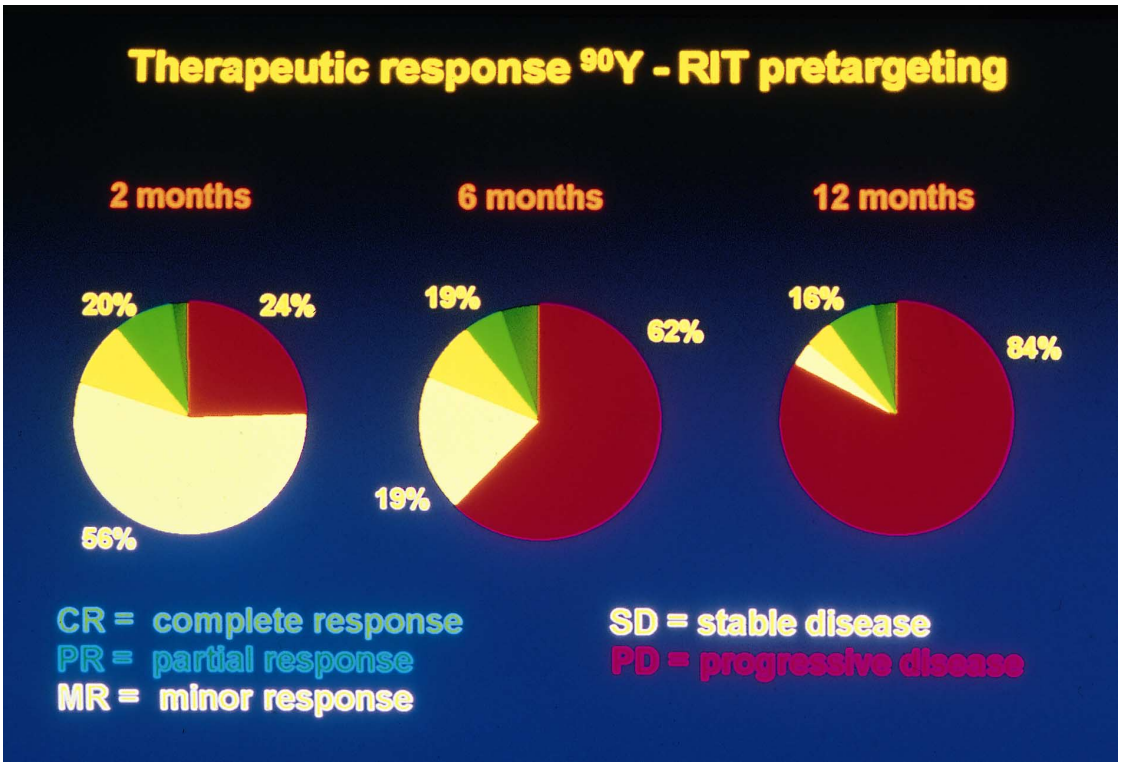

FIG. 4. Therapeutic responses obtained at 2, 6 and 12 months after ^{90}Y-biotin pretargeted radioimmunotherapy.

The first step of the protocol consisted of biotinylated anti-tenascin monoclonal antibodies in 100 mL of physiological saline, injected i.v. over a period of 20 min. at a dose of 35 mg/m^2. Avidin and streptavidin were then administered i.v. 24–36 h after the antibody as follows: 20–30 mg of avidin as a rapid bolus (first chase) and 50 mg of streptavidin in 100 mL of saline with 2% human albumin, 30 min. after the avidin.

Two mg of DOTA-biotin ligand, labelled with ^{90}Y-chloride, were administered 24 h after streptavidin infusion (third step) in a dose ranging between 2.22 to 2.97 GBq/m^2 (60 to 80 mCi/m^2) per cycle.

The clinical evaluation of all patients began 2 months after treatment. At this stage, tumour mass reduction occurred in 9/45 (20%) patients (MR+PR+CR); 56% of them had a stabilisation of the disease (SD) and 24% did not respond to therapy.

Of the 9 patients with reduced tumour mass at 2 months, 6 had grade IV and 3 had grade III glioma The proportion of patients with a decrease of tumour mass remained stable at 2, and 6 months of follow-up (respectively 20% and 19%) while the percentage of those in progression increased from 24% to 62%.

At 12 months follow-up, all 3 patients with anaplastic glioma were still in good conditions, while 1 glioblastoma patient had a minor response at 9 months and another is still in excellent condition more than two years after the first treatment (total of 11% MR + PR + CR).

Median survival from yttrium-90 treatment is 11 months for grade IV glioblastoma and 19 months for grade III anaplastic gliomas. Moreover, the maximum tolerated dose (MTD) was determined at 2.96 GBq/m^2 .

Three major conclusions emerged from this study. First, three-step radionuclide therapy with high dose ^{90}Y produces acceptable toxicity at the dose of 60 mCi/m^2 due to the extremely favourable biodistribution of ^{90}Y-DOTA-biotin, with the majority of the non-tumour bound activity excreted in the urine in the first 24 hours .

Second, an objective therapeutic effect was documented in an encouraging fraction of our patients, all of whom were no longer responsive to conventional treatments: in about 50% of cases, the disease did not progress any further (the majority of patients suspended cortisone, had reduction in epileptic seizure rate and improved quality of life), while significant tumour reduction occurred in 20% of patients.

Third, immune response to the murine MoAbs, known to interfere with the localisation of subsequent administrations, was less frequent than in patients treated with the directly labelled MoAbs used in other studies, possibly because of their shorter residence time in circulation with our procedure**;** however a rather potent immunogenicity of streptavidin was observed which may hamper repeated cycles of therapy [16].

Pilot trials, using pretargeting protocols, for the treatment of other solid tumours have been initiated.

A recent report describes the first case of complete clinical remission of an advanced oropharyngeal carcinoma induced by the combined treatment with external radiotherapy and 3-step pretargeted radioimmunotherapy with ^{90}Y-biotin [17].

The patient was in local relapse after surgery, chemotherapy and radiotherapy.

He received a cocktail of biotinylated MoAbs (anti CEA, B72.3), in order to target the largest number of tumour cells (first step) and 2.59 GBq of ^{90}Y-DOTA-biotin as third step. Dosimetric calculations showed estimated doses delivered to the tumour, liver, kidney and bone marrow (critical organs) of 13.4, 1.5, 3.8 and 0.7 cGy/37MBq respectively. Therefore the tumour received a dose of 10 Gy. MRI performed 7 months after RIT documented a complete response and after more than one year the patient was still alive and disease free by clinical examination and US.

4. CONCLUSIONS

The advent of the monoclonal antibody technology raised the hope that radiolabelled MoAbs would have the great advantage of sparing most normal tissues while targeting selectively malignant cells.

While radiolabelled MoAbs have established their imaging role in the nuclear medicine practice, their therapeutic applications have thus far gained limited acceptance mainly due to the low amount of radioactivity that can be targeted to the tumour and to the myelotoxicity which is typically the dose limiting factor. Remarkable high therapeutic response rates have been obtained for tumours that are refractory to other therapies through the use of locoregional administration that allows the delivery of higher radiation doses to produce cytotoxic effects. New strategies based on pretargeting techniques have shown that, on the contrary of directly labelled antibodies, higher doses of radioactivity can be administered systemically without associated bone marrow toxicity.

Pilot studies, applied to the treatment of advanced stage tumours, have shown that this approach interferes with the progression of tumours and produce tumour regression in patients no longer responsive to other conventional therapeutic modalities.

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MANAGEMENT OF HYPERTHYROIDISM

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Abstract. There are many clinical form of hyperthyroidism. It is necessary to determine the specific cause in order to direct the treatment strategy accordingly. The most common form is Graves' disease, an autoimmune disorder characterized by the presence of thyroid stimulating immunoglobulin that bind to and stimulate the thyrotropin receptor resulting in thyroid overactivity. Toxic nodular goitres, the next in prevalence, is more common in iodine deficient region and causes by autonomous hyperfunctioning thyroid nodules. The therapeutic approaches to treat hyperthyroidism are 1) antithyroid drugs to block hormones synthesis and release, 2) surgery and 3) radioiodine ablation of thyroid tissue. All therapeutic modalities are effective but the latter two methods are most probably the definitive means to achieve remission of the hyperthyroidism. Antithyroid drug therapy is the preferred treatment for all children with Graves' disease and patients with small goitres and short duration. However, a long term remission from antithyroid drug treatment is approximately 50%. Surgery is appropriate treatment for patient who has a very large goitre with symptoms of compression in the neck or patient with a cold nodule on thyroid scan. Currently radioiodine therapy is the most common therapy for Graves' disease. It is increasingly used as first-line therapy especially in elderly patients. It is the treatment of choice for patients with recurrent hyperthyroidism after antithyroid drug or surgical treatment. Radioiodine is also the preferred treatment for toxic nodular goitre. It is effective, safe and low cost. However it may aggravated Graves' ophthalmopathy. The only major disadvantage is high incidence of hypothyroidism. It is crucial that the patient be followed annually post treatment so that hypothyroidism can be detected early and proper treatment initiated.

1. INTRODUCTION

Hyperthyroidism has many causes that can be classified according to the sources of excess thyroid hormone. It is essential to distinguish thyrotoxic state that results from primary thyroid hyperfunction and those without hyperactivity in order to direct treatment effectively.

The most common form of hyperthyroidism is Graves' disease which is an autoimmune disease characterized by the presence of thyroid stimulating immunoglobulin (TSI) that binds to and stimulates the thyrotropin (TSH) receptor resulting in thyroid overactivity [1,2]. The next common form of hyperthyroidism is toxic nodular goiter which is characterized by the increased secretion of thyroid hormone by autonomous solitary or multiple thyroid nodules within the thyroid gland. Other forms of hyperthyroidism are relatively uncommon and will not be discussed in this presentation. Graves' disease is the focus of this discussion.

2. CHOICE OF TREATMENT

The therapeutic approaches to hyperthyroidism are antithyroid drugs (ATD), surgery and radioactive iodine (RAI). All are effective but no single method offer permanent euthyroidism. There is considerable disparity of opinion regarding the first choice for patient with Graves' disease [3–5]. Each modality has its own indication, contraindication, advantage and disadvantage. Guidelines are available to assist the appropriate choice for each patient. However, the use of RAI as first-line therapy for hyperthyroidism is growing especially at our institute and elsewhere [6]. It is the most common therapy in the United States [4,7]. Whatever approach selected, beta-adrenergic blocking agents are used to control adrenergic manifestations [2].

3. MEDICAL THERAPY

3.1. Thionamide drugs and mechanism of action

The most commonly used thionamide drugs are propyl thiouracil (PTU) and methimazole (MMZ). Their principle action is to inhibit the organification of iodide and coupling of iodothyronines which in turn suppress the synthesis of thyroid hormones. PTU has an additional peripheral effect that inhibit the conversion of thyroxine (T4) to tri-iodothyronine (T3) [8]. This effect probably inspires a preference for PTU rather than MMZ by some thyroidologists. All thionamide drugs also have a beneficial immuno-suppressive effect that reduces the TSH receptor antibody concentrations which suppress the immune-mediated hyperthyroidism of Graves' disease [2, 9, 10].

Propyl thiouracil is the preferred treatment for pregnant and lactating patients as it bound strongly to protein which make their passage cross the placenta or into breast milk limited [2, 11].

The half-life of MMZ in plasma is three to five hours, and that of PTU one to three hours [12]. In addition, MMZ has approximately 10 times the potency of PTU on weight basis, therefore PTU requires greater frequency of dosing and a greater number of tablets per dose, making compliance more difficult [2].

3.2. Indications and treatment regimens

Indications for ATD therapy includes hyperthyroidism in children and patient with mild symptom, small goiter and short disease duration. The initial dose of ATD is usually 200 to 600 mg of PTU in three or four divided doses or 20 to 60 mg of MMZ in single or divided doses. The maintenance dose is 100 to 200 mg of PTU or 5–20 mg of MMZ daily. The treatment it is usually continued for 12 to 18 months [2, 6, 11].

3.3. Outcome of treatment

Remission rate after ATD therapy is usually low. After a 12 month course of ATD therapy, remission rate is approximately 50% [2, 13]. Relapse if occurs is most likely evident within the first six months after withdrawal of ATD but may occur several years later [14].

3.4. Side effects

The most common side effects are mild allergic reactions such as skin rash, pruritus, fever, transient leukopenia. The most serious side effect is agranulocytosis which can occur in 0.1–0.5% of the patient and most commonly manifested during the first 3 months of the therapy [2, 11, 15]. Permanent hypothyroidism occurs about 0.6% per year after ATD treatment [16].

4. SURGICAL TREATMENT

4.1. Indications and contraindications

Currently thyroid surgery is rarely used to treat patients with Graves' disease. The major indications for surgery are large nodular goiter, very large goiter causing pressure symptoms in the neck, or patients with a nonfunctioning nodule on scintigraphic scan. The risk of malignancy in the nodule is much greater than that in euthyroid subjects and the carcinoma tends to be more aggressive [17] Surgery is also applied after antithyroid drug therapy failure and the patient refuses radioiodine therapy. Contraindications include severe concurrent disease, e.g. heart, lung and previous thyroid surgery.

The usual surgical approach is subtotal thyroidectomy in order to leave sufficient tissue to preserve the parathyroid and recurrent laryngeal nerves. To avoid the serious complication, it is important to refer the patient to a skilled surgeon. Prior to surgery, patients should be treated with ATD until euthyroid. The use of β-blockers alone is not an adequate preparation [6].

4.2. Results of treatment

The advantages of surgery include rapid reversal of hyperthyroidism and high cure rate. At 1 year after surgery, approximately 80% of the patients become euthyroid but permanent hypothyroidism occurs in 5 to 75% The prevalence increase with time [6, 18, 19]. Recurrent hyperthyroidism occurs in 1 to 3% of patients in the first year, most often occurs during the first five years after surgery [6, 18].

4.3. Complications

The prevalence of complications depends on the skill of the surgeon. Apart from hypothyroidism, which can be either transient or permanent, the specific post-operative complication of thyroid surgery include damage to recurrent laryngeal nerve and hypoparathyroidism [6, 18].

5. RADIOIODINE THERAPY

Hyperthyroidism has been treated with iodine-131 for over 50 years [20]. Currently radioiodine therapy is the most common therapy for Graves' disease. The cumulative experience on this therapy has confirmed its efficacy, safety and cost-effectiveness [2, 21]. It is used increasingly as a first-line therapy for adult and as treatment of choice for many clinical situations. At our institute, We have used iodine-131 for treatment of hyperthyroidism since 1959. Up to the end of 1998 , a total of 13 536 hyperthyroid patients were treated. The patients with hyperthyroidism who were referred for radioiodine therapy during the last 5 years was increased from 713 cases to 1240 cases per year.

The objective of radioiodine therapy is to destroy thyroid tissue sufficient for rendering euthyroid. The treatment goal is to administer enough radiation to achieve euthyroidism without causing hypothyroidism unless it is the intention of the physician to induce hypothyroid in some patients to avoid the risk of persistent hyperthyroidism. However, the most appropriate dose schedule remains controversial [6, 18, 21].

5.1. Indications and contraindications

There is general agreement that radioiodine therapy is the treatment of choice for hyperthyroidism in the elderly, cases of recurrence after thyroid surgery or medical therapy and severe concurrent disease, e.g. heart, lung, or chronic renal disease. Currently, the indication for radioiodine use includes young patients above a preselected age [2, 6]. At our institute, we consider to use radioiodine for patients over age 25. Several investigators reported their experiences in the use of radioiodine therapy in children and adolescents [22–24] Most clinics are reluctant to use I-131 therapy in children and adolescents although concerns about radiation-induced malignancy, mutagenic effect in offspring or impaired fertility have not been confirmed [2, 18].

Pregnancy and breast feeding mothers are absolute contraindicated for radioiodine therapy. Other contraindications are namely, suspected coexisting malignancy, patients below a preselected age limit and patients who fear of radiation. The use of radioiodine in patients with significant ophthalmopathy remains a contention [2, 6].

5.2. Patient preparation

Radioiodine therapy requires no medical preparation for most patients. However, in high-risk patients with severe hyperthyroidism, with complication especially cardiovascular diseases, and elderly patients. It is necessary to render the patients euthyroid with antithyroid drugs prior to radioiodine therapy to avoid the possibility of exacerbation of the disease due to hormone release after radiation [6, 21]. We usually gave antithyroid drug high dose for 4–6 weeks to bring the patient to euthyroid and stopped the drug for one week before administering I-131. However the medication can be discontinued as brief as 48–72 hours before radioiodine therapy or continue at a reduced dose during therapy [5] Continuing therapy with thionamides is prescribed 5–7 days after the administration of radioiodine. In patients with severe symptoms, a beta-blocker may be added to the pretreatment regimen. Digoxin should also be used in those patients with atrial fibrillation or heart failure [1]

5.3. Dose consideration

The most widely used method is to calculate the radioiodine dose in microcuries (μCi) per gram of thyroid tissue. The calculation required an estimated thyroid weight, the dose to be delivered per gram and the 24-hour thyroid uptake. Some clinics attempt to get a more precise estimated thyroid mass with ultrasonography. The following formula is generally used for I-131 dose calculation [2, 11, 18, 25]:

$$\text{Administered } \mu\text{Ci} = \frac{\mu\text{Ci/g desired} \times \text{estimated gland wt(g)} \times 100}{\text{24-hour radioiodine uptake (\%)}}$$

The desired dosage in microcuries to be delivered per gram of thyroid ranged from 50–200 μCi (1850–7400 kBq) [2, 11, 18, 25]. The radiation dose can be classified as low-dose (50–80 μCi or 1850–2960 kBq) medium-dose (100–120 μCi/g or 3,700–4,400 kBq) and high dose (150–200 μCi/g or 5550–7400 kBq).

We prefer to use low dose for hyperthyroid patient with short disease duration, small goiters and young patient especially the male because their thyroid gland is more sensitive to radioiodine.

The medium dose is most widely used for the majority of patient with moderately severe hyperthyroidism. Higher dose of radioiodine is suggested for patients with rapid radioiodine turnover, large goiter and those receiving antithyroid drugs before radioiodine therapy because larger gland and prior antithyroid therapy induce more resistance to radioiodine [2, 26–27].

High or ablative dose of radioiodine is generally prescribed for severely hyperthyroid patients or those with underlying cardiac or other serious disease or in elderly patients in whom the risks of persistent hyperthyroid are to be avoided. The associated higher incidence of hypothyroidism may be an acceptable consequence in such cases [18, 25, 26]. Some authors advocate ablative dose of radioiodine in patients with ophthalmopathy in order to eliminate any possibility of recurrence [26]. However, radioiodine therapy should be postponed in patients with progressive ophthalmopathy until their eye disease become stable by antithyroid drug therapy [29].

Toxic adenoma or toxic multinodular goiter are more radioresistant therefore the high — dose method is commonly employed [26–28].

5.4. Results of therapy

Usually hyperthyroid symptoms improve within 2 to 4 weeks after radioiodine therapy. However full clinical impact may not achieve until 3 months after therapy. The majority of patients require no supplement therapy during this period. Our experience have shown that about 75% of

hyperthyroid patients were cured with one dose of I-131 [30]. Once euthyroid has been achieved, hyperthyroidism rarely recurs [29]. Hypothyroidism occurring within the first six months after treatment may be transient or permanent. About 5% of our patients developed transient hypothyroidism and most of them spontaneously returned to normal within 3 to 6 months. If a patient is treated with thyroxine, reassessment for continuing therapy should be done after 6 months. For persistent hyperthyroidism, repeat I-131 treatment should be considered at least 3 months after the first dose.

5.5. Adjunctive medication

5.1.1. Beta-adrenergic blocking agents

Beta-adrenergic blocking agents (propanolol, atenolol, etc.) ameliorate some of the peripheral manifestations of hyperthyroidism through action on β-adrenergic receptors [29]. The major clinical effects of these drugs include reduction in heart rate and relief palpitations. Beta-blocking agents are particular useful for moderate or severe hyperthyroid patients since they make the patient more comfortable but do not interfere with most diagnostic tests or radioiodine therapy [25, 29]. The usual dose of propanolol is from 20–40 mg, 3 to 4 times a day [25], or atenolol 50–100 mg once a day [29]. Propanolol may be contraindicated in some patients with heart failure or asthma. Atenolol or metoprolol may be used with care in patient with asthma [25].

5.5.2. Thionamides

Thionamide may be used to control hyperthyroidism in elderly patient with moderate or severe symptoms or patient with cardiac disease. When euthyroid is achieved, the antithyroid drug should be tapered and eventually discontinued and the thyroid status should be evaluated.

5.5.3. Stable iodine (I-127)

Stable iodine may be used in patient in whom a partial response is achieved by I-131, but mild hyperthyroidism still persisted. Radioiodine treated gland seems particularly sensitive to iodides and this sensitivity may persist for months or years [31, 32]. About one to three drops of saturated potassium iodide solution per day are quite effective to control hyperthyroid symptoms.

5.5.4. Lithium

Lithium may prolong the retention of radioiodine thus enhance its effect. We often use as adjunct therapy for patient with rapid radioiodine turnover.

5.6. Side effects

5.6.1. Exacerbation of hyperthyroidism

Transient worsening of hyperthyroidism may occur within the first two weeks after radioiodine therapy but it rarely occurs in patients with adequate pretreatment with antithyroid drug. This exacerbation is caused by radiation thyroiditis and can be severe if a high ablative dose of I-131 has been given to a patient with a large overactive gland [26]. Thyroid crisis is a rare complication and should not occur if the most severely toxic patients are pretreated with antithyroid drugs and beta-blockers prior to radioiodine therapy

5.6.2. Ophthalmopathy

Graves' ophthalmopathy can occur before, during or after treatment. Whether Graves' ophthalmopathy will deteriorate after radioiodine therapy is controversial. However, a recent study reported a significant increased risk (33%) of new or worsening orbitopathy in patients treated with radioiodine compared to those treated with surgery (16%) or antithyroid drugs (10%) [34]. With current consensus that Graves' ophthalmopathy is the result of an immunologic attack on the orbit, because of some antigens shared with the thyroid, the removal of thyroid antigen becomes more sensible [35]. Radioiodine-associated exacerbation of eye disease can be prevented by concomitant administration of glucocorticoids [36].

5.6.3. Hypothyroidism

The only important complication of radioiodine therapy is the development of permanent hypothyroidism which may occur at any time after treatment. It is generally accepted that hypothyroidism is inevitable in patients with Graves' disease treated with I-131. Therefore, life-long follow-up of patient is crucial after radioiodine therapy. All patients should be followed annually post treatment so that hypothyroidism can be detected early and proper treatment initiated.

Hypothyroidism mostly occurred in the first 2 years following both surgery and radioiodine [25]. Various studies have shown that about 20 to 64% of patients became hypothyroid about 1 year after treatment. Subsequently, hypothyroidism appeared at a relatively constant rate of 3–5% per year after radioiodine treatment [1, 11, 25]. The late onset of hypothyroidism or cumulative hypothyroidism is not directly related to the amount of radioiodine received by the thyroid gland [37, 38]. Many think that in some patients, hypothyroidism is part of the natural history of Graves' disease [11]. Hypothyroidism is a frequent result of successful radioiodine or surgical ablation [2].

Toxic multinodular goiters and toxic adenoma treated with I-131 less often lead to hypothyroidism. This is due to the suppression of normal thyroid tissue by the hypersecretion of autonomic hyperfunctioning tissue [28, 33].

5.7. Follow-up

Thyroid functions should be evaluated at 2–3 months interval after radioiodine administration or more frequent if the patient is on antithyroid drug. When euthyroid has been achieved, thyroid status should be monitored annually thereafter life-long. Appropriate thyroid function tests include serum T3, free T4 and sensitive TSH. Although the serum TSH is most sensitive in the evaluation of thyroid function, it should be kept in mind that the serum TSH level may remain suppressed for several weeks or even months after the patient is euthyroid, so it is not a reliable monitor to follow in the first few months [18, 25]. Because transient hypothyroidism is not uncommon, it is generally recommended that patients should not be started on T4 therapy within 6 months unless they have clinical signs and symptoms of hypothyroidism [18]. It is important to demonstrate clearly that a patient has non-recoverable hypothyroidism before committing the patient to lifelong thyroxine treatment [31].

6. CONCLUSION

Selection of the appropriate mode of treatment of hyperthyroidism depends on various factors such as patient's age, disease severity, patient's preference, etc. Radioiodine therapy is most commonly used, most effective, simple and safe method in treatment of hyperthyroidism but carries a high risk of developing hypothyroidism. After successful treatment with any method, lifelong follow-up is necessary.

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CURRENT TRENDS IN RADIONUCLIDE THERAPY

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Abstract. Cancer treatment is the main theme. For this, there are four requirements: an understanding of cancer, of radiobiology, of the relevant physics, and of nuclear medicine. This small review high-lightens the major basic issues leading to receptor-targeted radiotherapy.

REVIEW

Cancer is now explained as a series of somatic mutations leading from the normal cell to the cancer cell. There are subtle differences between these two that can be exploited for therapy. The response to a mutation in DNA base pairs is repair. DNA that fails to be repaired triggers the P53 mechanism leading to apoptosis, death of the cell without release of contents. Mutations in the development of cancer overcome the DNA repair mechanisms, alter P53 so that it fails to trigger apoptosis and change the cell cycle from differentiation to proliferation. The control of these processes while inherent in the genetic material is triggered by events around the cell. These are sensed by receptors, responsive to hormones, cytokines, chemokines, and other biologically active substances. The information is transmitted from the cell surface to the nucleus by signal transduction for which a dozen pathways are described. Therapy of cancer therefore can be targeted at one or many of these processes. Cancer might be identified and treated by targeting the Oncogene with, for example, an antisense radiolabelled oligonucleotide such have already been labelled with Tc-99m but yet with therapy agents. Signal transduction can be targeted by gene therapy which needs monitoring and the use of FIAU labelled with I-131 appears promising. Targeting the cells' surface by receptor binding radionuclide therapy ligands or by radioimmunotherapy are in clinical trials.

Radiobiology developed for external beam therapy is inappropriate for nuclear medicine. External beam therapy delivers a high dose over a short time where single or double DNA strand breaks are the main consequence. Radionuclide therapy is delivered continuously in decreasing amounts over a long time depending on the half life of the radionuclide and its residence time, whereas calculations of effect tend to be related to external beam doses. Mechanisms for its success are much more subtle. They include triggering of apoptosis through the ceramide pathway by an effect of radiation on the cell surface. At low levels there is induction of free radical scavengers which prevent cytoplasmic damage to signal transduction, but higher levels can damage this process. There is a non uniform distribution of dose, particularly targeting and irradiating clonogenic cells which have the better blood supply and the higher compliment of receptors or antigens. Whereas external beam radiotherapy is fractionated to improved its effect, radionuclide therapy is a form of continuous fractionation. Radionuclide therapy may alter the cell surface so that it becomes immunogenic, a proposal made by Beierwaltes as part of the mechanism for treatment of thyroid cancer with I-131 many years ago. Cell necrosis as distinct from cell apoptosis generates an immunological response.

Physics. The choice of radionuclide for therapy is well illustrated by bone metastases. The question is whether to treat the invading edge of the metastases interacting with normal bone which generates pain for which a short path length soft radiation agent such as Sm-153 would be appropriate, sparing the marrow; or should one use an agent that also irradiates the marrow where the malignant cells are, to combine a palliative with a potential therapeutic response, but at the cost of damaging the normal marrow cells, such as with P-32 or Sr-89. Should one go for a short-lived radionuclide such as Re-188, a medium lived radionuclide such as Y-90 or Re-186 or a long lived radionuclide such as P-32. The same questions apply to radio-labelled peptides and antibodies. Is the radionuclide carrier bound to the cell surface , or internalised? For the former, a Beta emitter is required, for the latter an emitter of Auger electrons or even alpha particles can be considered. The range of the Beta particle is important. Should one go for cross talk because of heterogeneity of the distribution of cancer cells with a mix of receptor or antigen positive and negative as compared with

those that require homogeneity of receptor antigenic expression. The size of the tumour is another consideration, soft Betas for micrometastases, medium range Betas for small tumours and hard Betas for larger tumours. The site of the therapy is important. For therapy to cavities where there is a chance of escape of the radionuclide into the blood, then a high energy short lived radionuclide may be preferred, so that irradiation is completed before escape. For direct tumour injection when there is no escape as in a brain tumour, a longer lived radionuclide may be preferred. Where the tumour has good access in the blood such as leukaemia, then an intravenous injection of an alpha or beta emitter may be used, similarly for lymphoma, but for a solid tumour with poor access, a two or three stage approach, pretargeting the tumour is required to improve the therapeutic ratio. Differential residence time may also be required, making use of the rapid renal clearance of a small radiolabelled ligand from the blood as compared to its persistent residence on the tumour for which a long lived radionuclide such as P-32 would be preferred.

Nuclear medicine. The model for all radionuclide therapy is that of I-131 therapy for thyroid disease. The rules include:

Demonstration of uptake of a tracer dose of radio-iodine before initiating a therapy dose, rapid excretion of the agent that is not taken up by the tumour, a long lived beta emitter, lack of suppression of the immune response of the patient and repetition of the therapy at 6 monthly intervals. The use of I-131 tracer doses for reviewing the patient at 6monthly and yearly intervals may be replaced by a combination of thyroglobulin assays and I-123 imaging when thyroglobulin increases. The problem is that at 2mCi of I-131 tracer which avoids stunning is not sufficient to detect in some patients metastases subsequently seen by I-131 therapy, whereas a large dose of I-131, 10 mCi, causes stunning and a therapeutic effect reducing the efficacy of the subsequent I-131 therapy. We have shown that 5 mCi I-123 demonstrates metastases in over 95% of cases where 2mCi I-131 tracer doses have failed, yet subsequent I-131 therapy has shown uptake in metastases. The same rules generally apply to therapy with I-131 MIBG where I-123 MIBG is recognised as the appropriate tracer to prove uptake. In neuroendocrine tumours which fail to show concentration of MIBG, the use of Y-90 Lanreotide, the Mauritius project, or Y-90 DOTATOC are proving efficacious. Bifunctional genetically engineered designer molecules with a bivalent binding site for the tumour and a bivalent binding site for the radionuclide therapy ligand in a two or three stage procedure is the way forward for solid tumours. This is currently achieved using combinations of Avidin and Biotin such as the Paganelli procedure: Biotinylated antibody, Avidin chase and Biotinylated radionuclide ^{90}Y. Cancer specific targeted therapy is the goal.

EXPERIENCE WITH RADIOIMMUNOTHERAPY AND RADIO-PEPTIDE THERAPY

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Abstract. The expression of antigens and peptides on the cell surface of target cells has provided the use of monoclonal antibodies and peptides for experimental tumour therapy. Several studies using monoclonal antibodies (such as the anti-ovarian cancer monoclonal antibody ^{90}Y-HMCF1, the anti-CEA monoclonal antibody PR1A3 (ICRF), the anti-CD20 monoclonal antibody and the ^{90}Y-labelled lanreotide peptide are shortly discussed and highlighted regarding their clinical potential to treat cancer.

REVIEW

The additional requirements for radioimmunotherapy over radioimmunoscintigraphy are greater homogeneity of antigen expression, tumour penetration not just tumour surface uptake and an improved therapeutic ratio. Since only a few per cent of the injected radionuclide labelled antibody is taken up by tumour, the therapeutic ratio is inherently poor. The first approach therefore was to use intracavity injection of radionuclide radiotherapy with I-131 labelled monoclonal antibody HMFG 1 (ICRF) intraperitoneally for ovarian cancer. This was only successful in reducing the frequency of malignant ascites but had no benefit in terms of response. This was shown by paired intravenous and intraperitoneal injections of the same antibody with different radiolabels to be due to the lack of penetration to subserosal tumour. In addition a number of side effects were seen because peritoneal plaques of ovarian cancer are often on the serosal surface of the colon and bowel perforation occurred in two patients. The question was then asked as to whether the therapy was specific or non-specific and a multicentre trial in malignant ascites and pleural effusion compared HMFG 1 or H17E2 or AUA1 (ICRF) labelled with I-131 with a non-specific I-131 antibody. A trend for benefit was seen with the specific antibody. To overcome the lack of penetration, Yttrium-90 labelled antibody was substituted but in the patients with known peritoneal metastases no benefit was seen. However in a sub group of patients studied by Epenetos in whom no evidence of peritoneal malignancy was demonstrated by peritoneal washings, benefit was seen in Stage III and Stage IV ovarian cancer, with survivals of 80% in 5 years, twice that of historical controls. A multicentre trial of this agent is ongoing to test the specificity of this therapy.

Solid tumours require a different approach. Liver metastases can be treated by intra-hepatic arterial infusion of radiolabelled glass beads or microspheres. A study was undertaken with P-32 labelled anti-CEA monoclonal whole antibody called PR1A3 (ICRF). P32 was chosen because of its high Beta energy 1.71 Mev and its long half life, 14 days, so that after release from the antibody it would diffuse into the tumour and be incorporated into the RNA and DNA continuing its therapeutic benefit. To reduce the uptake by normal liver, angiotensin 2, a potent vasoconstrictor was infused just prior to the therapy. A P-32 monoclonal whole antibody SM3 (ICRF) was also tested in the treatment of polycythaemia on the basis that marrow side effects would be therapeutic by reducing the white count and platelets in such patients. This was found to be the case with platelets particularly sensitive to this form of P-32 antibody therapy. In no case in 4 patients did these values fall below normal.

For intravenous therapy a tumour type with good blood access is required for a single shot approach. Participation in the Coulter multicentre trial of using I-131 B1 anti CD20 monoclonal antibody in low grade B cell lymphoma has been undertaken in two stages, initially 8 patients in the Phase I study, and 5 patients to date in the Phase II study. These patients are selected in that they have failed several chemotherapy cycles of remission and relapse and whose outcome is invariably fatal., From the first series two patients showed a complete remission now for 2 years, and three patients showed a partial remission. These are similar to the findings of the much larger Kaminski series.

As well as cancer-related antigens, the increase in cancer-related receptors is well recognised. The use of radiolabelled octreotide analogues for imaging neuroendocrine tumours has now been augmented with Yttrium-90 labelled analogues such as Lanreotide, the Mauritius project. This we have used in one child (2 doses) infusing 15 mCi over 40 minutes, covered by prior and post amino acid infusion to reduce renal uptake. Total renal dose was calculated as 4.4 Gy but with no subsequent change in renal function or significant change in blood elements. Therapeutic trials with this agent have been successful in Vienna and our indication is it should be used for MIBG-negative octreotide-positive tumours. Progress will be to two or three stage radioimmunotherapy in the future using genetically engineered bivalent bifunctional agents with long-lived radionuclide ligands for therapy.

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THE ROLE OF MATHEMATICAL MODELS IN THE OPTIMIZATION OF RADIOPHARMACEUTICAL THERAPY

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Abstract. Mathematical models have been used in radiopharmaceutical therapy for over five decades. These have served to determine the amount of radioactivity required to treat disease, as in the therapy of hyperthyroidism with iodine-131, or, more frequently, to determine the largest amount of radioactivity that can be safely administered. Mathematical models are especially useful in the determination of fractionated radiopharmaceutical therapy. This review will briefly outline the historical development and current utility of mathematical models in radiopharmaceutical therapy, including thyroid disorders and radioimmunotherapy; and describe the potential of modeling in fractionated therapy. The extended application of such models to currently used radiopharmaceutical therapy based on indices of body mass or surface area, to alleviate toxicity and increase radiation dose to tumour, will be proposed. Finally, future applications of mathematical models in radiopharmaceutical therapy will be outlined.

REVIEW

In contrast to external beam radiotherapy, internally administered radiopharmaceuticals have inherent characteristics that affect effectiveness in cancer therapy, and determination of dose estimates to target and critical normal organs is therefore crucial to optimization of radiopharmaceutical therapy.

Existing mathematical models for calculation of maximum safe dose for differentiated thyroid carcinoma are being refined and extended to other therapies. There is a need for development of new models that address heterogeneity in radiation dose, permit fractionated therapy, and are directed toward optimizing radiation dose to tumour. This review will briefly address these issues.

The first application of mathematical models was in iodine-131 therapy for thyroid disorders (hyperthyroidism and differentiated thyroid carcinoma). Initial therapy with I-131, first used in 1942 [1], was with a fixed dose, and this continues to be an important method of treating patients [2]. However, the safety of radioiodine therapy in hyperthyroidism, and the ability to measure uptake and retention of ^{131}I in the thyroid gland, has led to increasing use of dosimetric models. In these models, an estimate of gland size is obtained, as is an estimate of ^{131}I retention in the gland. Using these estimates, it is possible to calculate the amount of ^{131}I required to treat the hyperthyroidism, using either a) the microcurie per gram desired [3, 4] or b) the desired radiation dose delivered to the gland [5]. The efficacy of such therapy is underscored by its ubiquity; most patients with thyrotoxicosis now receive ^{131}I as standard therapy, and surgery is no longer the standard of care. Similarly, calculated doses of ^{131}I have been used to ablate residual tissue in the thyroid bed after definitive surgery for differentiated thyroid carcinoma [6].

Almost all other models of radiopharmaceutical therapy have used dosimetry to estimate the largest safe amount of radioactivity that can be administered. Again, radioiodine therapy of thyroid carcinoma remains the paradigm [5, 6, 7]. As in ^{131}I therapy for hyperthyroidism, a small amount of ^{131}I is first administered, and serum and whole body clearance measurements used to calculate the largest safe (i.e. non-myelotoxic) amount of radioactivity that can be administered. Using this approach, developed at this Center, large doses of ^{131}I can be administered without significant myelotoxicity [6]. However, this approach does not take into account radiation dose to tumour; Maxon et al have shown the clear relationship between tumour response and radiation dose delivered to tumour [8].

In sharp contrast to ^{131}I therapy for thyroid disorders, radiopharmaceutical therapy for palliation of bone pain has not followed any dosimetric modeling. In patients receiving ^{89}Sr, it was not possible

to carry out whole body measurements (and, intuitively, serum measurements would not be representative of marrow dosimetry since strontium is a bone-seeker), because of the lack of a suitable radioactive emission. Consequently, the maximum tolerated dose of ^{89}Sr was arrived through escalation of the absolute amounts of radioactivity, rather than by radiation dose estimates [9]. It is rather unfortunate that this pattern was continued in the evaluation of other, gamma-emitting, radiopharmaceuticals used in the palliation of bone pain [10, 11]. The contiguity and lack of clear demarcation between the target and the critical normal organ are among the constraints that probably account for this feature.

Radioimmunotherapy trials also followed a methodology that involved escalation of radioactive amounts. Early trials [12, 13, 14] were carried out with murine antibody, precluding multiple infusions that would permit determination of whole body and serum dose estimates. Retrospective evaluation of a large body of data has made it clear that, while most radioimmunotherapy trials determined that the MTD was between 60 and 75 mCi/m^2 ^{131}I in most solid tumours, the most important predictor of hematopoietic toxicity is the radiation dose to the whole body and red marrow [15].

Patients with B-cell lymphoma constitute an exception to the rule that murine antibodies are immunogenic. It has thus become possible to carry out dosimetric estimates in patients receiving radioimmunotherapy with ^{131}I-labelled antibody; this has allowed the determination of a maximum tolerated dose (75 cGy) in patients receiving ^{131}I-anti-CD20 murine antibody with marrow rescue [16]. It has also allowed, in patients for whom marrow rescue is planned and thus for whom the critical organ would be other than the marrow, to determine the MTD to that critical organ and treat appropriately [17].

The development of relatively non-immunogenic antibody forms has made possible the use of dosimetric models in the therapy of cancer. This has rekindled interest in several related issues: a) how is tumour dose heterogeneity to be addressed; and b) is a single large dose of radiopharmaceutical preferable to fractionated therapy? These questions are perhaps less relevant to radioantibody therapy of leukemia and lymphoma; and extremely relevant to solid tumour radioimmunotherapy. It is unclear at this point what the optimum therapy schedule would be for bone metastases. The remainder of this review will focus on the role of mathematical models in radioimmunotherapy.

We have previously shown that antibody delivery to renal carcinoma is heterogeneous, despite homogeneous antigen distribution [18]. Various factors preclude successful targeting of radioantibody throughout a solid tumour, among them changes in vascular flow [19], interstitial pressure, and antigen heterogeneity. There is evidence that multiple administrations of radioantibody may result in more homogeneous distribution of radioantibody in tumour [20, 21].

Joseph O'Donoghue has constructed elegant mathematical models to study the effect of heterogeneity upon radiation dose. He has determined that dose heterogeneity results in inefficient tumouricidal effect, with the efficacy being inversely proportional to both the mean dose in, and the radiosensitivity of, the tumour.

The experimental data demonstrating the relative effectiveness of fractionated radioimmunotherapy to single large dose radioimmunotherapy led us to initiate a clinical trial in metastatic renal carcinoma. Mathematical models predicted that rapid fractionation would produce a longer duration of remission than a single large dose, though the latter would result in greater tumouricidal effect. This phenomenon was independent of tumour size, radiosensitivity, and other characteristics. We have thus far enrolled 9 patients, and demonstrated proof-of-principle: prediction of fractionation schema closely match actual patient clearance characteristics, both serum and whole body. An important finding has been the tremendous variability in whole body and serum clearance of

radioantibody between patients, which underscores the importance of mathematical models for use in therapy.

Decades of experience with thyroid cancer therapy have clearly demonstrated that the use of mathematical modeling allows for patient-specific therapy, with consequent minimization of side-effects. Currently, mathematical models are used primarily for determination of radiation dose to critical organs. Better tumour quantitation, to determine tumour volume and mass; to determine number of viable tumour cells, and to quantify amount and distribution of radiopharmaceutical in the tumour, will permit even more tailored therapy. The future of radiopharmaceutical therapy, therefore, is inextricably linked to the development of suitable mathematical models.

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RADIOIMMUNOTHERAPY: OPPORTUNITIES, OBSTACLES AND CHALLENGES, WITH SPECIAL REFERENCE TO DEVELOPING COUNTRIES

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Abstract. The targeting characteristics of, combined with the ease of radionuclide conjugation to, monoclonal antibodies makes them ideally suited for the selective delivery of potentially cytotoxic radioactivity to tumour. While early murine monoclonal antibodies were immunogenic, precluding repeat administration, genetic engineering has made possible the development of less immunogenic molecules, including fragments that can be grown in bacterial systems at relatively low cost. It is therefore currently feasible to produce relatively non-immunogenic tumour targeting antibody molecules at a reasonable cost, permitting their application in developing countries. As with chemotherapy, the utility of radioimmunotherapy has been most evident in lymphoma and leukemia. Progress in solid tumours has been exciting but slow. As with thyroid cancer therapy, the most utilized radionuclide in radioimmunotherapy has been iodine-131. The use of radioimmunotherapy as first- or second- line therapy in lymphoma and leukemia is being studied, and it appears likely that radioimmunotherapy could be a suitable, lower-cost alternative to chemotherapy in the treatment of these disorders, especially in developing countries. The cost-benefit of radioimmunotherapy compared to chemotherapy is especially stark when the cost of treating complications of chemotherapy is taken into account. Radioimmunotherapy as cost-effective therapy in developing countries is therefore feasible and has tremendous potential. This review will highlight milestones and pitfalls; suggest guidelines for future development; and outline potential clinical utility for radioimmunotherapy in developing countries.

REVIEW

Monoclonal antibodies are ideal for use in targeted therapy. They offer considerable tumour specificity, with high (nanomolar) affinity. They can be conjugated with cytotoxic agents. Radionuclides of therapeutic potential, when labelled to antibodies, offer the promise of selective deposition of cytotoxic radioactivity in/around cancer cells, with minimization of side-effects.

Early studies with polyclonal antibodies [1] demonstrated proof-of-principle. The development of monoclonal antibody technology [2] permitted production of antibody with reproducible characteristics; clinical trials with radiolabelled antibodies against antigens, notably CEA [3, 4], followed shortly thereafter. The immunogenicity of murine monoclonal antibodies led to the characterization and production of potentially less immunogenic antibody forms, including chimeric [5] and humanized [6] immunoglobulins (usually IgG), and "truncated" forms, including fragments (Fab' [7] and sFv [8]).

As with chemotherapy, therapeutic progress with radioimmunoconjugates has been most evident in the non-solid tumours. B-cell lymphoma was an ideal first opportunity; the diseased B-cell would be less likely to mount an immune response to murine antibody. Seminal work with anti-CD20 antibodies (murine and chimeric) both with [9] and without [10] bone marrow rescue showed significant complete and overall major responses in patients with refractory transformed low-grade or intermediate grade lymphoma. Current clinical trials with iodine-131 labelled anti-CD20 antibody have shown promising results; an important feature of this multi-national, multi-center trial has been the ability to ship ^{131}I-antibody from a central facility to remote locations in different countries, without loss of immunobiologic function.

Initial work with a murine anti-CD33 antibody in myelogenous leukemias demonstrated [11] the characteristics of rapid targeting and therapeutic efficacy, especially in acute promyelocytic leukemia [12]. The antibody was invariably immunogenic, precluding repeat therapy. Subsequent studies with humanized M195 have shown that targeting is comparable [6] , while *in vitro* and *ex vivo* analysis has shown that there is significant internalization of antibody subsequent to interaction with

the CD33 receptor. Early work, using radiometals with varying half-lives and emission characteristics, has shown promise [13].

Larson et al [14] pioneered radioimmunotherapy in solid tumours (using both intact immunoglobulins and fragments); progress in solid tumours has since been disappointing (again as in chemotherapy). As with radioimmunotherapy in hematologic neoplasms, primary dose-limiting toxicity has been hematologic [15]. Murine antibodies have been immunogenic [15, 16]. Several radioimmunoconjugates, some recently approved by the FDA for radioimmunodetection of occult, solid tumours, consist of antibody Fab' fragments conjugated to technetium-99m [7, Verluma^{TM}], and these smaller forms may be less immunogenic. Apart from confirming selective targeting of radioactivity to tumour, these trials demonstrated that myelotoxicity was primarily a function of radiation dose to marrow [17]. Clinical trials with chimeric and humanized radioimmunoconjugates are under way; while responses are few and far-between, these radioimmunoconjugates are certainly less immunogenic [6, 18], and future trials must therefore increasingly focus upon using initial "scout" doses of radioimmunoconjugate to calculate absorbed dose to critical organ and administer the corresponding radioactive amount of radioimmunoconjugate; this methodology is already widely used in calculating the "safe doses" of ^{131}I therapy in differentiated thyroid carcinoma [19].

Iodine-131 has been the most widely used radionuclide. Its beta-minus emissions offer therapeutic potential, and its gamma emissions permit imaging. Advances in radiochemistry have made possible the conjugation of an array of radionuclides with therapeutic potential — from those with beta-minus emissions, such as yttrium-90, and other similar nuclides (^{186}Re, ^{188}Re, ^{177}Lu) to those with alpha emissions, such as ^{211}At and ^{213}Bi — and trials with these radioimmunoconjugates are under way.

The primary challenge to radioimmunotherapy is increasing selective radiation dose to tumour. Decreasing the size of the molecule increases tumour:non-tumour ratios of antibody uptake, more than offsetting any decrease in absolute tumour uptake. We have studied antibodies not only against tumour-associated cell surface antigens, but also against fibroblast activation protein (FAP), found selectively on tumour stroma [20]. Bi-specific antibodies have been developed; one arm of the immunoglobulin recognizes the tumour-associated antigen, while the other arm interacts with another ligand [21, 22, 23]. Several clinical trials have been initiated using this methodology; while it is possible to give higher amounts of radioactivity, it is as yet unknown whether the overall tumour:non-tumour ratio is higher.

Radioimmunotherapy, using non-immunogenic proteins, has particular attraction for developing countries. Most importantly, the therapy offers specificity. Toxicity is hematopoietic, and has been reversible. The development of dosimetric models to predict myelotoxicity will minimize toxicity. While the cost of radioimmunoconjugate is not trivial, it should be comparable to chemotherapy, and its selectivity should permit cost savings by minimizing side-effects such as seen after chemotherapy. The safety and potential of antibody therapy has been underscored by FDA approval of unlabelled antibody, for the treatment of B-cell lymphoma and, more recently, for breast cancer. Central facilities would dispense radioimmunoconjugate much as is currently done, in India and in other countries, with ^{131}I for thyroid carcinoma. As with thyroid cancer, early and aggressive use of radioimmunotherapy should result in successful disease control, with minimal side-effects. And just as the therapy of differentiated thyroid carcinoma (an allegedly radioresistant tumour) with iodine-131 is being used extensively and increasingly throughout the developing world, so will radioimmunotherapy be used in preference to other more toxic, less specific therapies, for an increasing range of diseases.

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RHENIUM RADIOISOTOPES FOR THERAPEUTIC RADIOPHARMACEUTICAL DEVELOPMENT*

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Abstract. Rhenium-186 and rhenium-188 represent two important radioisotopes which are of interest for a variety of therapeutic applications in oncology, nuclear medicine and interventional cardiology. Rhenium-186 is directly produced in a nuclear reactor and the 90 hour half-life allows distribution to distant sites. The relatively low specific activity of rhenium-186 produced in most reactors, however, permits use of phosphonates, but limits use for labelled peptides and antibodies. Rhenium-188 has a much shorter 16.9 hour half-life which makes distribution from direct reactor production difficult. However, rhenium-188 can be obtained carrier-free from a tungsten-188/rhenium-188 generator, which has a long useful shelf-life of several months which is cost-effective, especially for developing regions. In this paper we discuss the issues associated with the production of rhenium-186- and rhenium-188 and the development and use of various radiopharmaceuticals and devices labelled with these radioisotopes for bone pain palliation, endoradiotherapy of tumours by selective catheterization and tumour therapy using radiolabelled peptides and antibodies, radionuclide synovectomy and the new field of vascular radiation therapy.

1. INTRODUCTION

The availability of therapeutic radioisotopes at reasonable costs is important for applications in nuclear medicine, oncology and interventional cardiology. Rhenium-186 (Re-186) and rhenium-188 (Re-188) are two reactor-produced radioisotopes which are attractive for a variety of therapeutic applications. Rhenium-186 has a half-life of 90 hours and decays with emission of a β-particle with a maximum energy of 1.09 MeV and a 136 keV (9%) gamma emission which permits imaging. In contrast, Re-188 has a much shorter half-life of 16.9 hours and emits a β-particle with a much higher energy of 2.12 MeV (E_{max}) and a 155 keV gamma photon (15%) for imaging.

While Re-186 is unavailable from a generator system and must be directly produced in a nuclear reactor (Table 1), Re-188 can also be directly produced in a reactor with high specific activity, but is more conveniently and cost-effectively available as carrier-free sodium perrhenate by saline elution of the alumina-based tungsten-188 (W-188)/Re-188 generator system [1–2].

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TABLE I. PRODUCTION AND PROPERTIES OF RHENIUM-186 AND RHENIUM-188

	Rhenium-186	**Rhenium-188**
Half-Life	90 hours	16.9 hours
Beta Particle, MeV	1.09	2.12
Gamma Photon, keV (%)	136 (9%)	155 (15%)
Direct Production Mode	$^{185}Re(n,\gamma)\ ^{186}Re$	$^{187}Re(n,\gamma)\ ^{188}Re$
Cross Section (σ) for Direct Production	112 b	76.4 b
Calculated Specific Activity — mCi/mg Target for 2-day Irradiation		
10^{14} neutrons/cm^2/sec	500	600
10^{15} neutrons/cm^2/sec	5000	6000
Generator Production	None	$^{186}W(2n,\gamma)\ ^{188}W(\beta^-) \rightarrow\ ^{188}Re$
Cross Section (σ) for Generator Parent Production	.....	$^{186}W(n,\gamma)\ ^{187}W$ 37.9±0.6 b $^{187}W(n,\gamma)\ ^{188}W$ 62±10 b

2. PRODUCTION OF RHENIUM-186 AND RHENIUM-188

2.1. Rhenium-186

One important advantage of using Re-186 is that it can be produced in many nuclear reactors throughout the world by direct neutron activation of enriched Re-185 (Figure 1), and the 90 hour half-life can often permit distribution to sites distant from the production facility. Which reactors can be used for routine production of Re-186 (Figure 2), and the shelf-life of Re-186 inventories, however, depend upon the specific activity requirements. While very high specific activity Re-186, for instance, is required for antibody and peptide radiolabelling [3], preparation of phosphonates for bone pain palliation [4] and use for intravascular radiotherapy for inhibition of coronary restenosis after angioplasty (*vide infra*) is possible with lower specific activity Re-186. The thermal neutron flux required for production of Re-186 will thus depend upon the particular application.

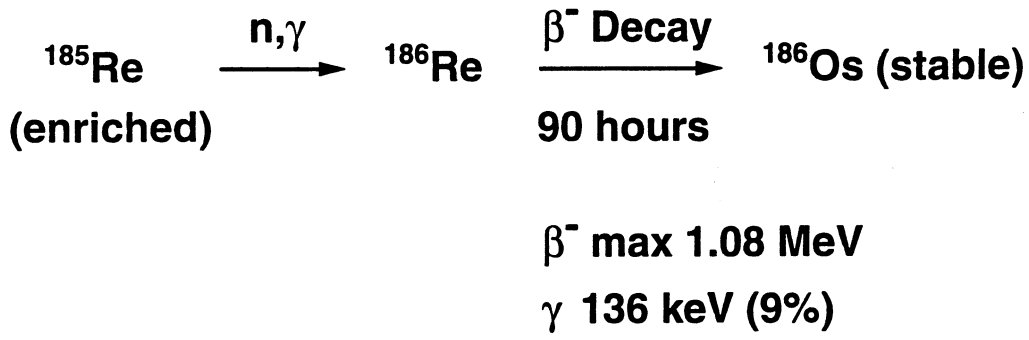

FIG. 1. Reactor production of rhenium-186.

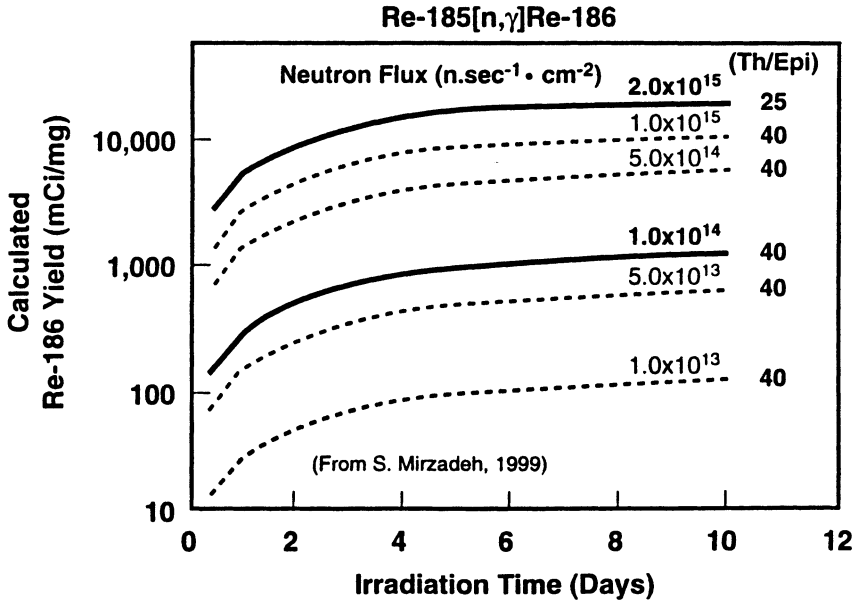

FIG. 2. Calculated specific activity of reactor-produced rhenium-186 at various thermal neutron flux values.

2.2. Rhenium-188

Rhenium-188 can also be produced with relatively high specific activity by direct production in a nuclear reactor (Figure 3) by irradiation of enriched rhenium-187 (Figure 4). A major advantage for use of Re-188, however, is its carrier-free availability as Re-188-perrhenate from the W-188/Re-188 generator in the clinic at any time, since elution every 24 hours provides about 50% yields of Re-188. The availability of Re-188 on demand from this high performance generator provides great versatility for development of a range of Re-188-labelled therapeutic agents and the generators have a long useful shelf-life of > 6 months. Recent research and introduction of new agents labelled with rhenium radioisotopes has by far primarily focused on the use of Re-188 (Table 2).

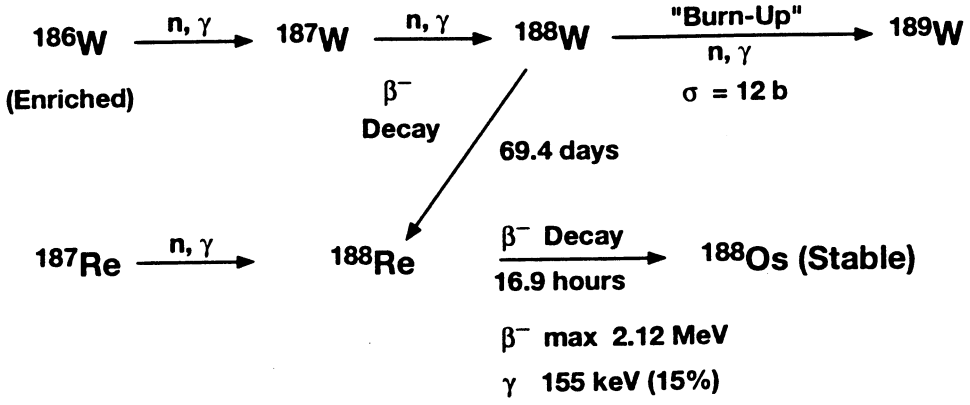

FIG. 3. Reactor production and decay scheme for tungsten-188.

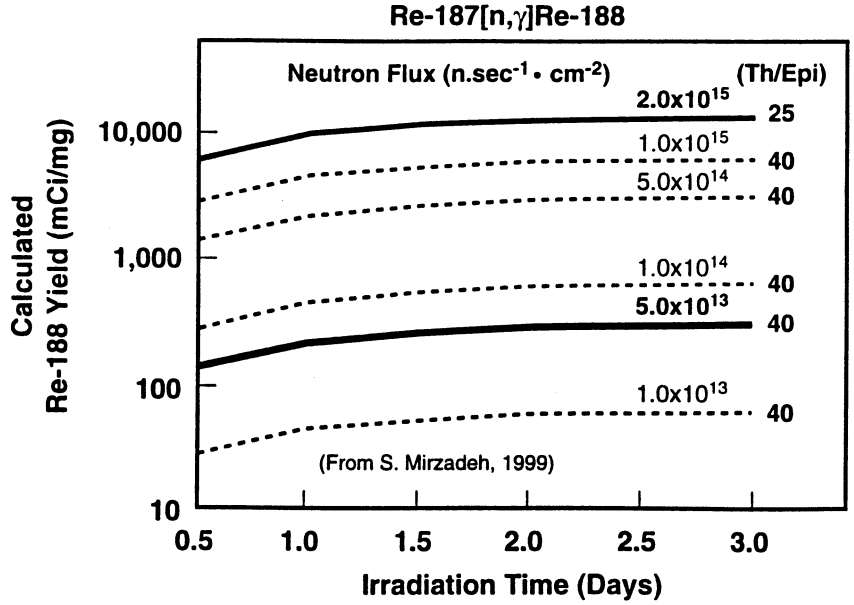

FIG. 4. Calculated specific activity of "direct" reactor-production of rhenium-188 at various thermal neutron flux values.

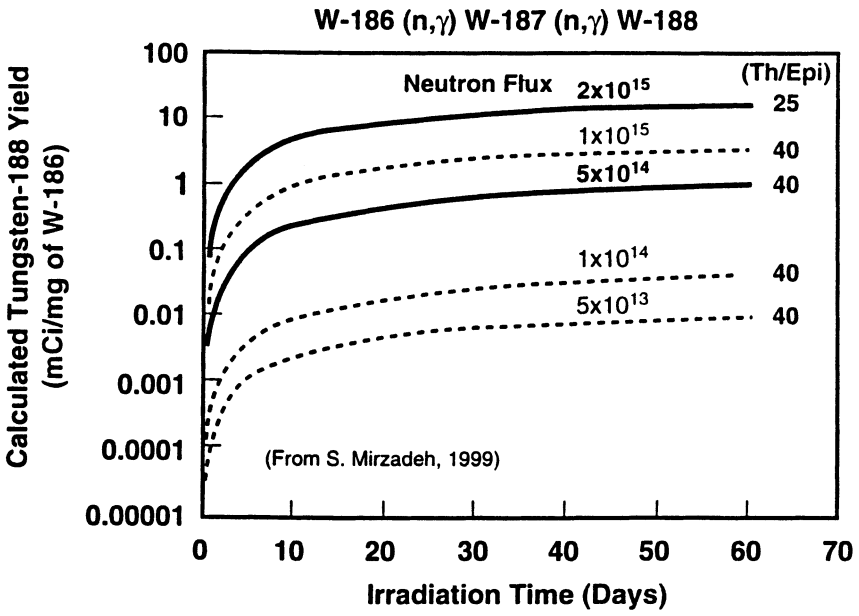

FIG. 5. Calculated specific activity of reactor-produced tungsten-188 at various thermal neutron flux values

Although there are only a few high flux reactors available for production (Figure 5) of the W-188 parent [5], the logistics for production and processing of W-188 and the distribution of the W-188/Re-188 can be easily coordinated. Use of inexpensive disposable tandem concentration units [6] is simple and provides very high specific volume solutions of Re-188 (i.e. > 700 mCi/mL from 1 Ci generator). The W-188/Re-188 generator is especially important for providing a reliable source of this versatile therapeutic radioisotope to remote sites, especially in developing regions, which involve long distances and expensive distribution costs.

3. THERAPEUTIC AGENTS FOR TREATMENT OF MALIGNANT DISEASE LABELLED WITH RHENIUM-186 AND RHENIUM-188

3.1. Agents for bone pain palliation

Rhenium-186-*HEDP* is widely used in Europe for the palliative treatment of bone pain from skeletal metastases [4,7]. As alternatives, both Re-188-*HEDP* [8–10] and Re-188(V)-*DMSA* [11] have been developed for bone pain palliation. Patient studies with Re-188-*HEDP* are in progress in Bonn [8] and Dresden [9], Germany, in Montevideo, Uruguay [10], and several other sites, and the Re-188(V)-*DMSA* is being evaluated in patients at the Canterbury and Kent Hospital in Great Britain [11]. Imaging of the 155 keV gamma photon is an advantage which provides an opportunity for estimation of radiation dose to metastatic sites.

TABLE II. EXAMPLES OF CURRENT PRECLINICAL AND CLINICAL TRIALS WITH RHENIUM-188-LABELLED AGENTS

Re-188 Agent	Application	Institution
Re-188-HEDP	Bone pain palliation	Bonn and Dresden Germany; Montevideo, Uruguay; Szeged, Hungary; Athens, Greece
Re-188-(V)-DMSA	Bone pain palliation	Kent and Canterbury Hospital, Great Britain
Re-188-Perrhenate	Endovascular radiation therapy	Cedars Sinai Medical Center, Los Angeles; Perth, Australia
Re-188-MAG3	Endovascular radiation therapy	Columbia University, New York
Re-188-Peptides	Tumour therapy	Preclinical — Bonn, Germany
Re-188-Particles	Endoradiotherapy of Tumours — catheter administration	Preclinical — Dresden, Germany; Seoul, Republic of Korea; Kaichung, Taiwan (China)
Re-188-Labelled Antigranulocyte Antibodies	Marrow ablation prior to stem cell rescue	Ulm, Germany

3.2. Labelled antibodies and peptides for tumour therapy

Various tumour-specific antibodies have also been labelled with Re-186 and Re-188 [3,12]. More recently, somatostatin analogues radiolabelled with therapeutic radioisotopes are of interest for tumour treatment and the RC-160 somatostatin analogue has been directly labelled with Re-188 and evaluated in nude mice having human mammary gland, prostate and small lung cell carcinoma tumours resulting in significant reduction or elimination of the tumours [13]. The extremely short vascular stability of this agent, however, requires the direct tumour or cavity administration. More recently, the P829 and P773 peptides have been directly labelled with rhenium-188 and are being evaluated for therapy of non small cell lung tumours in a CD1 nu/nu nude mice tumour model [14].

3.3. Labelled particles for tumour therapy

Rhenium-188-labelled particles (Table 3) are also being evaluated for direct tumour injection or for endoradiotherapy by administration to the tumour arterial supply *via* a catheter. In one study, Re-188-labelled Aminex A27 microspheres (15–20 μm) [15] were directly injected into tumours from N1-S1 hepatoma cells in the lobes of the livers of Sprague-Dawley rats. About 80 per cent of the treated rats survived over 60 days after intratumoural injection, while only about 26 per cent of the non-treated rats survived during the same time period. The stability of several other Re-188-labelled

microspheres has also been evaluated by incubation with human plasma and by biodistribution studies in rats [16]. The most favorable biodistribution properties were found for the Re-188-*B-20 HSA* microspheres (Mallinckrodt; 15–20 μm). The Re-188-labelled sulfur colloid is also simple to prepare [17], with a tight particle size range (86% = 5 μm) , with most activity retained in the liver *via* both intravenous and hepatic artery injection.

TABLE III. EXAMPLES OF RHENIUM-188-LABELLED PARTICLES BEING EVALUATED FOR TUMOUR THERAPY

Particle	Size	Application	Refs.	Status	Comment
B20 HSA Particles	15–20 microns	Endoradiotherapy of tumours	15–16	Preclinical	Dresden, Germany — Planning of Clinical Trials in Progress
Aminex A27	15 microns	Endoradiotherapy, Synovectomy	19	Preclinical	Taichung, Taiwan (China)
Sulfur Colloid	1–5 microns	Endoradiotherapy, Synovectomy	20	Preclinical	Seoul, Republic of Korea; Taichung, Taiwan (China)

4. THERAPEUTIC AGENTS FOR TREATMENT OF NON-MALIGNANT DISEASE LABELLED WITH RHENIUM-186 AND RHENIUM-188

4.1. Radiation synovectomy

An important treatment of inflammatory disease is the use of Re-186-labelled sulfur colloid particles for therapy of rheumatoid arthritis of the synovial joints [18–19]. Rhenium-186-labelled particles are commercially available in Europe, for example, for this clinical application, but are not yet available in the USA. Because of expected cost effective on-site preparation in the nuclear pharmacy when required, several groups are also exploring the use of the Re-188-labelled particles for this application [20-22].

4.2. Intravascular radiation therapy

We have also proposed and evaluated Re-188-labelled agents for the use of Re-188 liquid-filled angioplasty balloons inflated at low pressure following coronary angioplasty for the inhibition of coronary restenosis by high dose delivery [23–25]. Angioplasty balloons are filled at low pressure (2–3 atmospheres of inflation pressure) with a solution of Re-188-perrhenate or Re-188-*MAG3* following high pressure angioplasty to deliver a dose of 2500–3000 rad at 0.5 mm of depth. This application is expected to be important for the inhibition of the hyperplastic component of coronary restenosis. Swine studies have also demonstrated the inhibition of restenosis with the Re-188 liquid filled balloon approach after coronary overstretch injury [25] and patient studies are in progress at several Institutions in the USA, Europe and Australia (Table 2). The use of Re-186-liquid-filled balloons for restenosis therapy is also being evaluated [26].

5. SUMMARY AND CONCLUSIONS

Because of their attractive radionuclidic and chemical properties and relatively ready availability, rhenium-186 and rhenium-188 continue to be of interest for the radiolabelling of a variety of therapeutic agents for applications in nuclear medicine, oncology and interventional cardiology. The use of rhenium-188 is of particular interest, since the availability of the tungsten-188/rhenium-188 alumina-based generator system represents a convenient system to provide the rhenium-188 for a variety of therapeutic applications.

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2. RADIOPHARMACEUTICALS

^{90}Y-PREPARATION AND SOME PRELIMINARY RESULTS ON LABELLING OF RADIOPHARMACEUTICALS

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Abstract. The production of ^{90}Y by ^{90}Sr-^{90}Y generator was studied. ^{90}Sr was adsorbed at a column with Aminex A-5 resin. The daughter radionuclide ^{90}Y was eluted with 0.7 M α-hydroxyisobutyrate (α-HIB, pH5.4). Radionuclidic, radiochemical and chemical purities were >98% and yield >85%. After converting into chloride form $^{90}YCl_3$-solution (pH:1) was used for preparing injectable yttrium citrate and labelling some other radiopharmaceuticals such as antibody or methylene diphosphonate (MDP). Furthermore, a fast ITLC-method for determination the content of ^{90}Sr in ^{90}Y-eluate was developed.

1. INTRODUCTION

The interest for application of radiopharmaceuticals for the direct management of serious illness and exceptionally the cancerous and rheumatism's illness has increased during the last decade. At the moment radioisotopes of ^{89}Sr, ^{186}Re, ^{153}Sm, ^{90}Y and ^{211}At are used in the routine practice of the medical clinics. The tendency of this camp is to concentrate studies for designing radiopharmaceuticals that fulfil the following requirements:

- to realize a high absorbing doses in malignant cells in the shorter time interval
- don't damage the healthy cells.

These requests fulfil radioisotopes that emit αand β particles. The number of radioisotopes that accomplishes this task it not high and between β–radioisotopes of great interest is the ^{90}Y. ^{90}Y has a LET useful for therapy, $E_{\beta max}$ = 2.3 MeV, $T_{1/2}$ = 64.1 h with no gamma emissions.

The aim of the work was:

1. Preparation of new type of ^{90}Sr-^{90}Y generator with relatively high activity (3.7 GBq);
2. Separation of ^{90}Y with high radioactivity concentration in chloride state and with high radiochemical and chemical purity;
3. Labelling of different biomolecules (somatostatin, antibodies, porphyrins) and ligands (MDP) with ^{90}Y to study their properties as new potential radiopharmaceuticals for treatment soft tissue tumours;
4. Production of ^{90}Y-citrate from the $^{90}YCl_3$-solution for palliative treatment of skeletal metastases;
5. Developing a new and fast method for evaluating the radiochemical and nuclear purity of $^{90}YCl_3$ or -citrate;

2. METHODS

2.1. Generator of ^{90}Sr-^{90}Y

^{90}Sr-^{90}Y-generator (FIG. 1.) was loaded with 3.7 GBq ^{90}Sr as $^{90}Sr(NO_3)_2$ in 0.97 M HNO_3. The ion exchange column (Aminex-A5, dimension: 7 × 50, particle size: 13 ± 2 μm Bio-Rad company) had a capacity for strontium of 43.5 mg/mL. The breakthrough of ^{90}Sr was checked by measuring ^{85}Sr (added as $^{85}SrCl_2$ in 0.5M HCl on the head of columns, specific activity: 402.19 MBq/mg, radioactivity concentration: 462.5 MBq/mL, total activity: 185 MBq). α-hydroxyisobutyrate (α-HIB, SIGMA, pH: 5.4) was used for elution of ^{90}Y from the first two columns. The elution process was controlled by measuring the ^{90}Y-bremstrahlung with a NaI(Tl) detector.

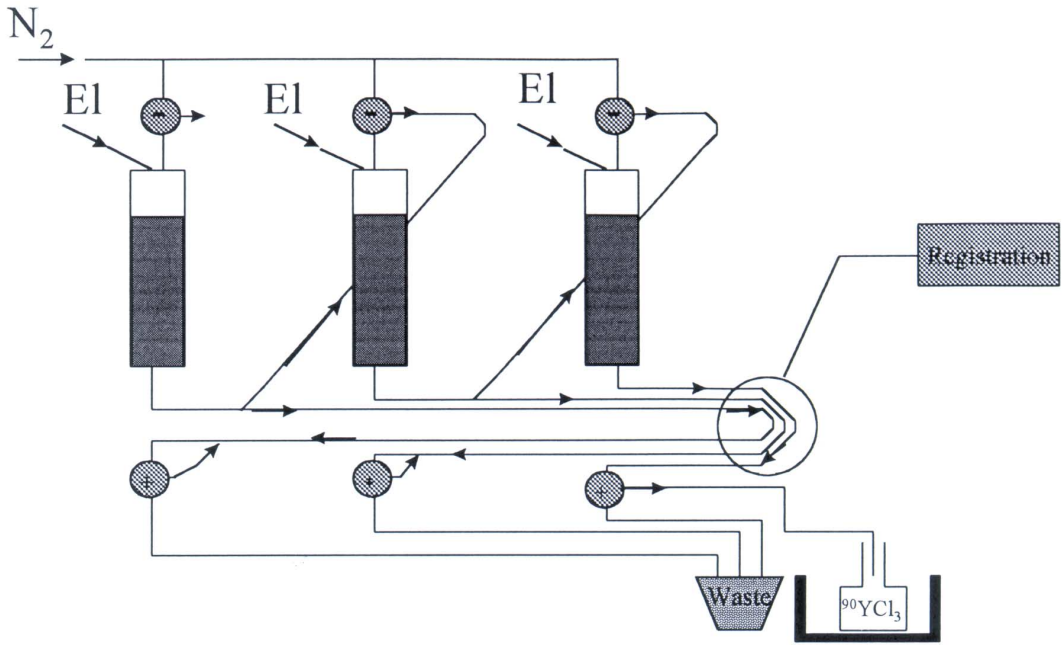

FIG. 1. General scheme of generator.

2.2. Procedure

a) Preparation of the ion-exchange column

After 3–4 fold treating the Aminex-A5 with 0.05M HCl, centrifugation, removing the liquid phase and rinsing the resin 5 times with double distilled water and 3–4 times with 0.05M NH_4OH it remained in contact with 0.05 M NH_4OH during the night. After that resin was rinsed 3–4 times with double distilled water. Finally the columns were loaded with resin prepared in the described manner. The column was rinsed with double distilled water until getting a neutral pH.

b) Preparation of the α-HIB solution

The stock solution of 1 M α-HIB was prepared by dissolving 10.41 g of α-hydroxiisobutyric acid in 90mL double distilled water and increasing pHup to 5.4 by adding NH_4OH. α-HIBA with concentrations of 0.14 and 0.07M were prepared by diluting this solution with double-distilled water.

c) Loading of ^{90}Sr and ^{85}Sr at column.

3.7 GBq ^{90}Sr in 5 mL were mixed with 37 MBq ^{85}Sr in 0.1 mL and 1.3 mL 0.2 M NaOH to increase the pHup to 4–5. Than, the solution was transferred at the first column using a “pneumatic”

system. The mixture of two radioisotopes was adsorbed on the column with a flow rate of 10 drops/min.

d) Elution of ^{90}Y

From the first and second columns:

After passing of the solution through the column the first elution was carried out with 10 mL 0.07 M α-HIB, and after that with a 0.14 M α-HIB to remove iron and other metallic impurities from the column. Eluate with yellow colour indicating the presence of iron but without radioactivity was collected as waste, whereas the eluate with radioactivity passed a second column. After that the elution process was carried out in the same manner as on the first column.

From the third column:

The ^{90}Y-α-HIB complex can not be used for medical purposes because of its toxicity and low in vivo stability. Therefore a conversion into chloride form was carried out by re-adsorption of the ^{90}Y on the third column after adjusting pH1 of ^{90}Y-α-HIB-solution with HCl. After that the ^{90}Y was eluted with 6 M HCl — probably as hexachloro-complex — collected and evaporated to dryness by mild heating. Finally, the radioactivity was collected in 1.5 mL 0.03N HCl. This solution was used as starting material for labelling different compounds (see above).

2.1.2. Results

a) Efficiency of ^{90}Sr-retention

No ^{90}Sr-break-through could be detected in the eluate during adsorption of the $^{90}SrNO_3$ on the first column. That means: the ^{90}Sr adsorption was completely.

b) Elution process

The profile of the elution process is shown in FIG 2. The eluate was collected in 1–1.5 mL fractions. The efficiency of ^{90}Y elution was 75–90%. These values are in accordance with published data[1–6] for generators which were loaded only with relatively low ^{90}Sr radioactivity's (kBq — up to few MBq). The ^{90}Y-radioactive concentrations was dependent on the time elapsed from the last elution. Assuming that the elution is carried out every day, the achievable radioactivity concentration of the final solution is 333 MBq/mL.

c) Radionuclidic and radiochemical purity

The main problem is a possible contamination of ^{90}Y by ^{90}Sr. The ^{90}Sr-breakthrough was measured by different methods including gamma-spectroscopy for the presence of ^{85}Sr, measurement of ^{90}Sr by radiochemical separation from eluate, determination of radioactive decay of ^{90}Y in comparison with a standard, and by a new developed method for fast chromatographic separation of ^{90}Sr from ^{90}Y.

The content of ^{90}Sr radioactivity was less than 200 Bq per 3.14 GBq ^{90}Y ($<5.4*10^{-3}$ μCi/85 mCi ^{90}Y). The determined values of the half-life were 64.2 h for our product and 64.1 h for the standard supplied by Amersham. These data correspond to published results [7].

Radiochemical purity was checked by a new developed ITLC method. ITLC.SG strips (Gelman Science, 16 × 140 mm) were used for this purpose. 0.1 M Tris-buffer (Tris-hydroxymethyl-aminomethan, Merck Art.1.06448), pH7 served as eluent. A typical radiochromatogram is presented in FIG. 3. A sample of contaminated solution was investigated until one month after elution.

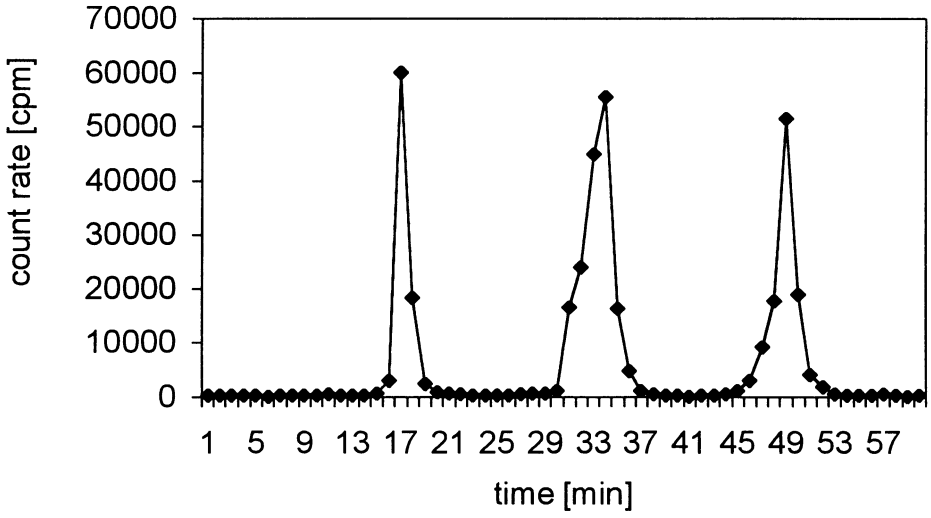

FIG. 2. Profile of elution process.

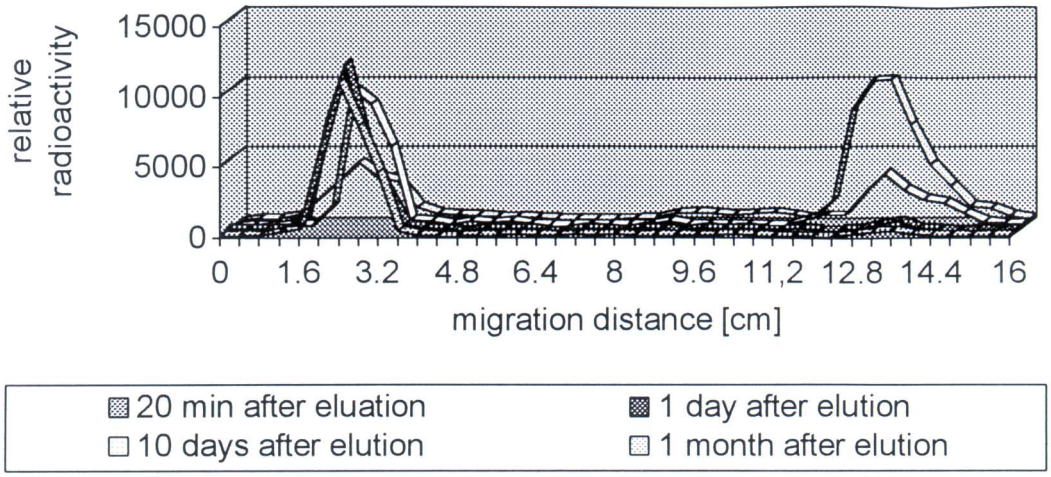

FIG. 3. Radiochromatogram of ITLC.Sg strip.

d) Trace elements as chemical impurities

The content of trace metals can influence the labelling procedure very significantly and is, consequently, an important parameter of the quality of ^{90}Y. If ^{90}Y is used for labelling of antibodies coupled with DTPA, somatostatin-DTPA, etc. the presence of trace metals in the labelling medium leads to a competition of non-radioactive metal ions with ^{90}Y for binding sites resulting in a low labelling efficiency. According to Hnatovich et al. [8,9] the effect of trace metals can be measured by testing the labelling yield on low concentrations of free DTPA in presence of ^{90}Y. In our experiments were used solutions of DTPA which were prepared with normal distilled water and double distilled water. In this case the differences are obviously.

For these investigations we used DTPA-concentrations of 25.4; 2.54; 0.254 and 0 μmol/l. ^{90}Y was added as acetate at pH=5 with radioactivity's >74 MBq/mL.

After a reaction time of 1–2 h between DTPA and ^{90}Y an ascending chromatography using Whatman No.1 paper was carried out. 0.1M tris buffer at pH= 7 was used as eluent. The ^{90}Y-DTPA moved with the front and the free ^{90}Y stayed at the start. The results of these investigations are demonstrated at Table I.

TABLE I. RESULTS OF CHROMATOGRAPHY FOR THE CONTROL OF TRACE ELEMENTS

DTPA-conc. [μmol/l]	Distilled water		Double distilled water	
	% of radioactivity Rf = 0	% of radioactivity Rf = 1	% of radioactivity Rf = 0	% of radioactivity Rf = 1
*25.4	-	100	-	100
2.54	2.5	97.5	2.9	97.1
0.254	11	89	1.9	98.6
0	99.7	0.3	99.8	-

*1.0μg/100μl.

2.2. Preparation of ^{90}Y citrate

The method to prepare radiopharmaceutical ^{90}Y-Citrate is relatively simple and fast.

Radioactive ^{90}Y-solution (activity depends on request) was evaporated to dryness. 3 mL of isotonic citrate solution (11.4 mM $Na_3Cit*2H_2O$ and 77 mM NaCl) were added to the residue for 10 min to allow a complete resolution. The radioactive solution was filtered aseptically by using sterile Sartorius membrane-filter (0.22 μm exclusion size). This solution is ready for using and is recommended to store it in refrigerator on 4^0C. The radiopharmaceutical fulfils all requirements for radiopharmaceuticals in general. Radiochemical purity was available to check by above-mentioned method (ITLC.SG strip10*100mm and mobile phase 0.1 M Tris at pH~ 7). The results were the same as in the case that has been used electrophoresis's method.

The radiopharmaceutical was used for treatment of pain resulting from bone metastases of 50 patients in Oncology clinic of Tirana University Hospital Center. The primary tumours were determined to be mamma carcinoma (28 cases) or prostate carcinoma (22 cases) . All the patients have been with diffuse bone metastases could be demonstrated by the scintigraphy in all the patients. The radiopharmaceutical was administered i/v in a dose 5–10 mCi. Just one case has had 39^0C temperature, after the injection was performed. The temperature declines some hour's letter. We have not notice any other side effects.

All to be said is that when the administered dose had been 7.5 mCi, the results had been more positive. For the lower doses the results have been at an average degree. means that effect has been for the shorter time.

2.3. Some problems in labelling of antibody

Mab B72.3 murine monoclonal antibody of the IgG_1 has been used for the labelling is directed against a high molecular weight tumour associated glucoprotein (TAG-72). For labelling properties' antibody is conjugated with linker chelator glycyl-tyrosyl-N-ε-diethylen triamine penta acetic acid-lysine (GYK-DTPA). This is obtained in form of kit ONCOSCINT CR 103 that is used for labelling with ^{111}In for radio-immunodiagnostic purpose. Antibody [10] weight is 1 mg Mab B72.3-GYK-DTPA in 2 mL of phosphate buffered saline at pH=6. ^{90}Y was carrier free solution in form of YCl_3 in 0,03N HCl, home made [11]. Concentration of radioactive solution was more than 30 mCi/mL. The solution of ^{90}Y before using was checked for labelling availability, as a significant parameter of quality of radioactive solutions. The presence of trace metals in labelling medium led to a competition of non-radioactive metal ions with ^{90}Y for binding sides resulting in a low labelling efficiency.

Solution of ^{90}Y was used in acetate form. Solution of ^{90}Y was added to 1mL solution of sodium acetate at pH6. Desired activity from above mixture is added to vial with antibody and is let to stay at room temperature for incubation. The results obtained shows that the process of labelling is relatively fast. Table II. shows the dynamic of the yield of labelling.

TABLE II. DEPENDS OF THE RATE OF LABELLING

Time, min	0	10	30	60	100	150	200
Activity, × 10^3 cpm	0	30	45	43	46	46	47

From the data given in Table II it appears clearly that 30 min were enough for labelling and after this increasing the time of incubation was without practical interest.

Influence of pHon the value of labelling is measurement in two manners.

- From the mixture of antibody-^{90}Y it was taken the sample and was analyzed for the yield of labelling directly by ITLC
- Mixture of antibody-^{90}Y was filtered through 0,22mμ membrane filter and filtrate was analyzed by ITLC.

Data obtained are represented in graphical form on FIG. 4.

The influence of pHwas obvious, so for pHvalue 1–2 it was obtained the yield of labelling 30% and for pHvalue 7, the yield of labelling was 66%, ceaselessly after 12-min contact between DTPA and the labelled mixture antibody-^{90}Y.

Premises of evaluation the yield of labelling

It is interesting the fact, that conditions on which chromatographic analyses was performed play important role and has apparent influence on the results. We have checked influence of pHof DTPA-solution and the contacting time between sample and the solution of DTPA. In low pHof DTPA-solution it was always obtained high value of the yield of labelling. That case we can explain with aggregation of antibody at low pHvalue. The difference in results between pHvalue 1–2 and 7 was around 2.5 times.

Also contacting time between sample of labelled mixture antibody-^{90}Y and DTPA was important. FIG.5. represents this influence and that fact can be seen: from 1min to 12 min in contact the results vary 1,5 times

This situation we think can be explained by ion exchange between antibody-DTPA-Y and DTPA-Y. Maximum amount content of antibody-DTPA is 1mg and if we assume that each molecule of antibody is coupling with one molecule DTPA, content of DTPA connected with antibody is in range μg, but meanwhile content of DTPA that we have added in solution is around 18 mg, and in this case in the following reaction:

$$A_b{}^{90}Y + DTPA \rightleftharpoons A_b + DTPA\text{-}{}^{90}Y$$

it is shifted at right side. Reaction between free ^{90}Y and DTPA is instantaneous, so 1 min contact is full satisfactory

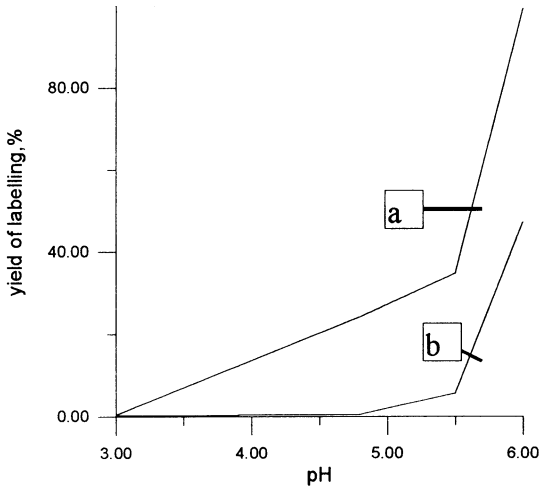

FIG. 4. Influence of pHon yield of labelling.

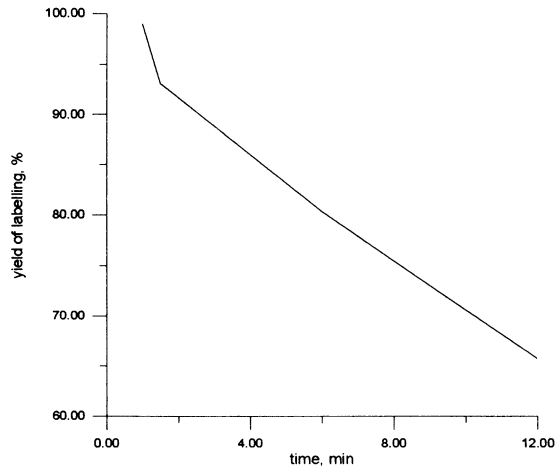

FIG. 5. Influence of contacting time.

2.4. Labelling methylene diphosponate (MDP)

MDP labelled with ^{99m}Tc has played a significant role in the diagnostic practice of nuclear medicine during last years. MDP and his substitute HO-MDP have a high accumulation in the bone metastases and fast blood clearance. In this background we undertook the study for labelling MDP with ^{90}Y as a potential radiopharmaceutical for therapeutic treatment of cancer metastases.

By the HPLC technique was studied the possibility of forming the complex between MDP and ^{90}Y. FIG. 6. shows elution curve of Y as carrier(2), pure MDP(1) and mixture(3) MDP + Y(carrier). The elution curve was investigated by measuring the absorption of light on λ=254 nm. It is apparent the difference 2 min between them in the R/T.

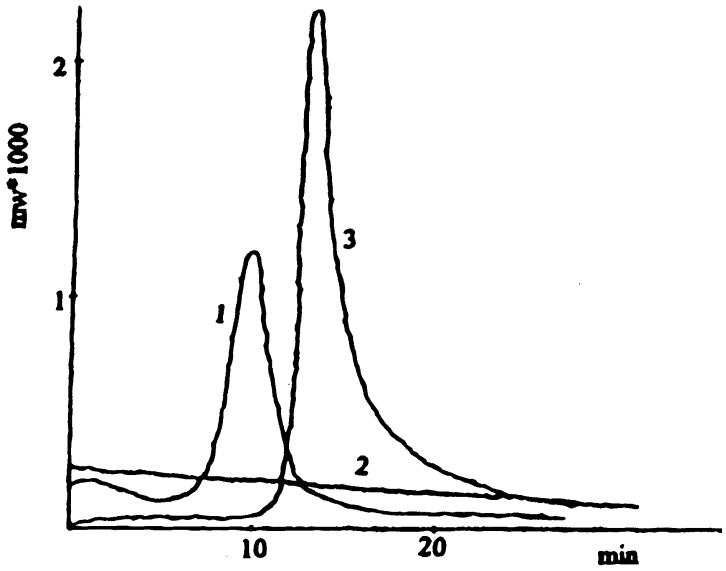

FIG. 6. Elution curve of MDP(1), Y(2) and complex Y-MDP(3).

We have study the correlation between the amount and concentration of MDP in the yield of labelling. Table III. shown part of these studies.

TABLE III.. DEPEND OF YIELD OF LABELLING FROM THE CONCENTRATION OF SOLUTION.

Variant	Amount of MDP mg	Vol.(μL) of MDP solution	Vol.(μL) of ^{90}Y solution	Total volume μl	MDP concentration mg/mL	Yield of labelling %
1.	0.8	83	50	133	6.0	95
2.	1.6	166	50	216	7.4	96
3.	3.2	330	50	380	8.4	95
4.	4.8	500	50	550	8.7	96
5.	7.2	750	50	800	9.0	-
6.	9.6	1000	50	1050	9.1	97

It's clear that amounts of MDP in such conditions do not influence on the yield of labelling. The yield of labelling is practically constant, meanwhile concentration of the ^{90}Y from the first variant to the sixth change ~8 times.

Concerning the influence of pHon the yield of labelling it seems that lowest value (87%) obtained in the band of pH4–5.

Evaluation of the stability of complex in the sera medium was done by HPLC equipment Pharmacia and as column was used Superdex HR 10/30 and elute phosphate puffer at pH= 7.

From the data obtained by HPLC we could give following commentary:

In FIG. 7. is shown that our chromatographic system is sufficient to separate MDP-^{90}Y and the R/T was 39.63 min. From the FIG. 8 seem that free ^{90}Y during incubation with the sera was complexes with macromolecules of serum blood, probably with serum albumins. The R/T in this case was 16.32 min. It is an evidence that if the complex ^{90}Y-MDP is unsteady and released ^{90}Y, this ^{90}Y will be binding this fraction of serum albumin's and will be reflected as a separate peak in the HPLC curve.

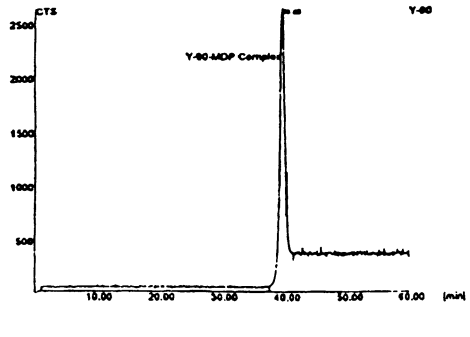

FIG. 7. Elution curve of water solution of ^{90}Y-MDP.

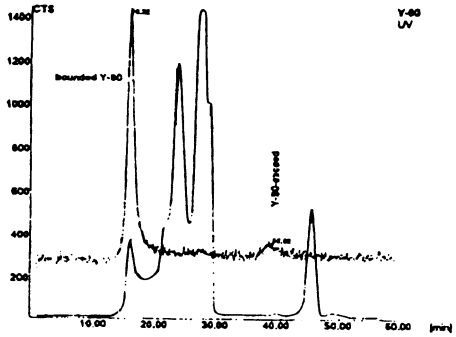

FIG. 8. Elution curve of ^{90}Y and sera.

FIG. 9. shows that ^{90}Y-MDP complex does not bind any component of the human sera. It seems that does not exist any accordance between peaks that were obtained by measurement in UV spectrum with peaks were obtained by measurement with radioactive probe. The complex ^{90}Y-MDP has appeared with same value of R/T as not having sera; meanwhile content of free ^{90}Y was insubstantial. For the curves in the FIG. 10, 11 and 12 comments are the same as in case of FIG. 9. Again the content of free ^{90}Y was insubstantial.

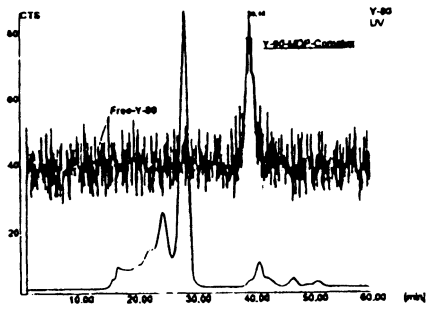

FIG. 9. Elution curve of ^{90}Y-MDP and sera after 1h incubation. Sera product ratio 9:1.

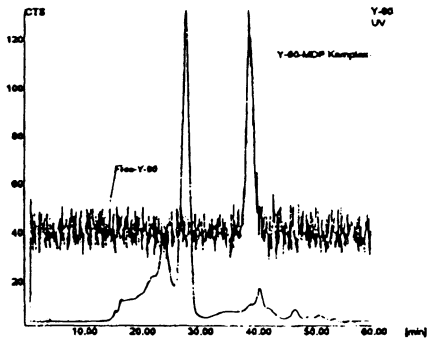

FIG. 10. Elution curve of ^{90}Y-MDP and sera after 2h incubation. Sera product ratio 4:1.

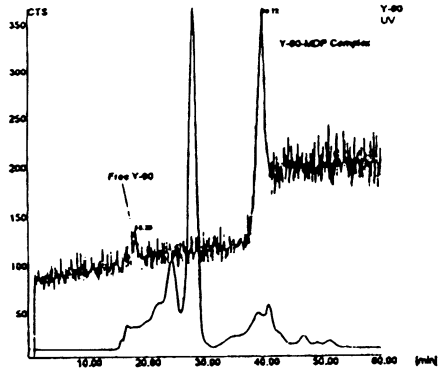

FIG. 11. Elution curve of ^{90}Y-MDP and sera after 4h incubation. Sera product ratio4:1.

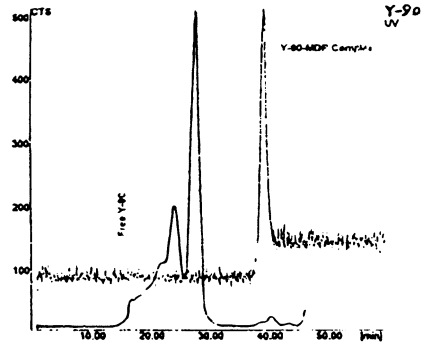

FIG. 12. Elution curve of ^{90}Y-MDP and sera after 1h incubation. Sera product ratio 3:1.

From this presentation we can conclude that the complex is stable in sera's medium for a long time. We think that 1-h incubation is enough for estimate the stability of complex. We assume that the clearance from the blood is fast, in similar to properties of ^{99m}Tc-MDP complex.

ACKNOWLEDGEMENTS

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^{90}Y OF HIGH PURITY FOR MEDICAL APPLICATIONS

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Abstract. Several ^{90}Sr/^{90}Y-generator systems have been developed and used to produce ^{90}Y. The most important parameter of the ^{90}Y to be assayed is ^{90}Sr content. In addition, when labelling monoclonal antibodies for therapy trace metal quantities accompanying ^{90}Y (Fe^{3+}, Zn^{2+}, Cu^{2+}, ZrO^{2+}, etc.) are to be kept as low as possible in order to obtain high labelling efficiencies. Generally generators' lifetime is limited due to the ^{90}Sr breakthrough which increases in eluates as a result of the radiolytic degradation of the resin used as support. In the study a described procedure for ^{90}Y purification from metal contamination is modified in order to lower the amount of ^{90}Sr present in eluates from generators. As a result a very low ^{90}Sr content is always assured (^{90}Sr/^{90}Y < 10^{-6}).

1. INTRODUCTION

Nowadays ^{90}Y (a pure beta emitter) is a radioisotope widely used for therapy. It is obtained from ^{90}Sr, a high yielded fission product.

Although several methods have been published, the most frequently used for its production are ion exchange (ionic chromatography) and solvent extraction. The first one is employed at MBq scale production and the second one is used preferentially at larger scale, say, GBq quantities due to the effect of radiolysis on the resins employed in the chromatographic methods.

Generator systems are mostly based on ionic chromatography for its simplicity and rapidity when compared to solvent extraction. Among these systems can be mentioned those employing Dowex 50 WX8 resin and lactate [1], citrate [2], ethylenetriaminetetraacetic acid disodium salt [3, 4] (EDTA), oxalate [5] and methanol-acetate [6] as eluants. The best results are achieved with the EDTA chelating agent that gives the highest elution efficiency in the smallest volume and a very low ^{90}Sr breakthrough [4]. In addition, the pHof this solution does not need to be adjusted when prepared by dissolution of the salt [3].

The aim of our study was to find a procedure to purify ^{90}Y eluates from generators lowering their ^{90}Sr content and other metal contamination. ^{90}Sr contamination level is the most important parameter to be assayed if ^{90}Y is intended for clinical application. In addition, when labelling monoclonal antibodies the amount of trace metals has to be kept as low as possible in order to attain high labelling efficiencies.

Samples collected from two generators are included in this work to show the results of the elaborated procedure.

2. METHODS

Reagents

Nitric, sulphuric, and hydrochloric acids used in this research were both Analar and ARISTAR quality from BDH. DTPA, EDTA, and salts of iron, zinc, copper, zirconium and yttrium were reagent grade and from BDH. Dowex 50Wx8 (50–100 mesh) resin for the preparation of generators was from

BDH too. AG50 Wx8 (100–200 mesh) and Chelex-100 resins (both from BioRad), Fluoran scintran LS cocktail (BDH) and $^{90}Sr/^{90}Y$ standard solution (Amersham) were supplied by IAEA authorities through CUB/2/011 project. The water used throughout the study was grade 2 (according ISO 3696:1987 [E]) and further purified with Chelex-100 resin and filtered through a 0.22 μm pore size membrane filter. Purification glass for ^{90}Y columns were 0.7 cm wide by 18 cm high.

Equipment

Wallac 1209 LS counter was used for ^{90}Y and ^{90}Sr measurements. Collection of effluents from columns was made by means of an Eldex fraction collecting equipment.

Preparation of generators

$^{90}Sr/^{90}Y$ generators involved in this study were prepared following the method described first by Skraba [3] and more recently by Hnatowich [4]. Generators are of different activities and ages. The oldest one with 185 MBq activity and one and a half year old has been eluted irregularly varying the time between two consecutive elutions from 1 week to 3 months. The volume employed in its elution has also been irregular. The other one of 740 MBq is only six months old and has been milked regularly every week.

Purification procedure. Determination of elution volumes

A purification method known as Strelow´s procedure [4] to eliminate the excessive metal contamination of ^{90}Y eluates was first studied. This method consists of making the eluate from generator 0,5 mol/L H_2SO_4 and passing it through a column containing AG-50 WX8 (100–200 mesh) resin in H^+ form. After washing with 0,5 mol/L H_2SO_4 and 2 mol/L HCl solutions ^{90}Y is recovered with 4 mol/L hydrochloric acid.

In order to find the elution volume of these acids for our system (a 0,7 cm wide and 18 cm long column with 4,4 g or 14 cm^3 of AG-50WX8 resin) 50 mL of each were consecutively used in the same order mentioned above. Samples of different cations (Fe^{3+}, Zn^{2+}, Cu^{2+}, ZrO^{2+} and $Y\text{-}^{90}Y$ each separately) in quantities equivalent to the 3% of total resin capacity and samples of about 37 kBq of ^{90}Y without carrier were passed through the resin at a flow rate of 0.8 mL/min. Effluents from the column were collected in 10-mL fractions except for the samples of ^{90}Y where 2-mL fractions were taken. Each one was properly treated and its metal content determined by volumetric analysis with 0.01 mol/L EDTA solution to find recovery. Samples containing $Y\text{-}^{90}Y$ were measured by liquid scintillation counting.

Synthetic samples with about 37 kBq of $^{90}Sr\text{-}^{90}Y$-equilibrium solution were submitted to Strelow's procedure. After varying the volume of 2 mol/L HCl and adding a wash-step with 2 mol/L HNO_3 samples of similar composition ($^{90}Sr\text{-}^{90}Y$ solution) were passed through the system by the application of this modified procedure.

Determination of ^{90}Sr and ^{90}Y

^{90}Y and ^{90}Sr were measured by liquid scintillation counting. The double energetic window method described by Moreno [7] and his co-workers was used in the case of ^{90}Sr. Most og generators samples was measured after almost total decay of ^{90}Y. When it was needed, radiochemical separation of ^{90}Sr from ^{90}Y was performed using an AG 1X4 (100–200 mesh) resin in OH^- form and DTPA chelating agent obtaining decontamination factors for ^{90}Y higher than 10^7.

3. RESULTS AND DISCUSSION

Elution volumes in purification procedure

Recovery of each metal is shown in table I. The elution of Zr in 0.5 mol/L H_2SO_4, Fe, Zn, Cu in 2 mol/L HCl and Y in 4 mol/L HCl and the experimental volumes are as they were expected from the values of the Distribution coefficients (Kd) reported by Strelow [8, 9].

TABLE I. RECOVERY OF METALS ELUTED FROM PURIFICATION COLUMN (AG 50WX8 100–200 MESH)

Metal	Eluant	Kd†	EVTR (mL)*	Recovery (%)
Zr(IV)	0.5 mol/L H_2SO_4	4.6	20	97 ± 2 **
Fe^{3+}	2.0 mol/L HCl	5.2	20–30	99 ± 2**
Cu^{2+}	2.0 mol/L HCl	4.3	20	98 ± 2**
Zn^{2+}	2.0 mol/L HCl	3.7	20	98 ± 2**
^{90}Y-Y	4.0 mol/L HCl	8.6	25	96

** Experimental volume for reported recovery in the table.*
*** Recoveries found by volumetric titration with EDTA.*
Recovery calculated from direct measurement of Y-90 activity.
† Distribution coefficients reported by Strelow [8, 9]
Errors reported correspond to standard deviations for n=5.

However, in figure 1 it can be seen that there is a small difference in the position of elution peaks of ^{90}Y with and without carrier. As the case of no carrier addition is closer to real operation conditions and considering that similar behaviour may be expected from the other metals an increase of 10 mL was made to the final elution volumes in order to obtain a more complete removing of metal traces.

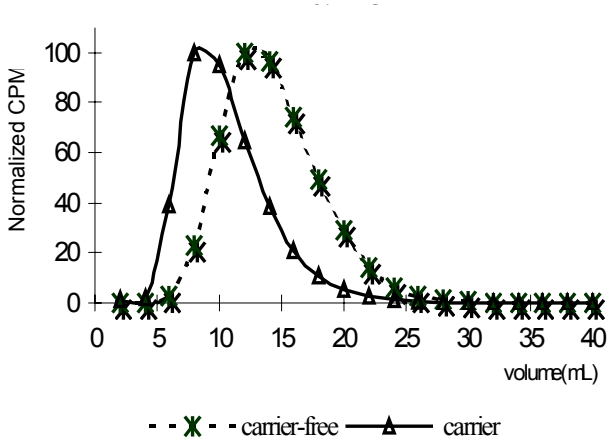

FIG. 1. Elution of Y-90 from purification column with 4 mol/L HCl.

^{90}Sr content

Data regarding ^{90}Sr contamination levels in eluates from generators is shown in Table II. Samples are arranged in descending order according to their elution date and are not consecutive ones.

TABLE II. ^{90}Sr CONTENTS REFERRED AS $^{90}Sr/^{90}Y$ RATIO IN SAMPLES ARISING FROM GENERATOR # 1 (1½ YEAR OLD) AND GENERATOR # 2 (SIX MONTHS OLD). IN THE CASE OF GENERATOR # 1 THE SAMPLES WERE COLLECTED FROM 97/04 TO 98/08 AND FOR GENERATOR # 2 FROM 98/05 TO 98/10

	$^{90}Sr/^{90}Y$	
Samples	Generator # 1 185 MBq	Generator # 2 740 MBq
1	2.4×10^{-5}	2.9×10^{-6}
2	4.7×10^{-6}	2.1×10^{-6}
3	6.7×10^{-6}	2.0×10^{-6}
4	5.0×10^{-6}	2.1×10^{-6}
5	4.0×10^{-6}	1.6×10^{-6}
6	5.4×10^{-6}	3.1×10^{-6}
7	6.7×10^{-6}	5.1×10^{-6}
8	1.6×10^{-5}	8.2×10^{-6}
9	1.8×10^{-5}	2.9×10^{-5}

**All measurements of activity were carried out with a relative error less than 1%.*

In the case of generator # 1 the contents of ^{90}Sr from sample # 2 to sample # 7 correspond to the required values ($^{90}Sr/^{90}Y$ ratio has to be kept bellow 10^{-5} for in vivo use of the Y [10]). In sample #1 (which is the first elution) the ^{90}Sr contamination level is higher than in the next eluates probably due to the fact that only a small volume of eluant was used to wash the column immediately after loading the activity. Samples # 8 and # 9 hold a higher ^{90}Sr breakthrough. They correspond to elutions after a 3 months' period of time during which the generator was not operated. These higher ^{90}Sr values could be associated to a more serious damage caused to the resin by radiolysis.

The last two samples (# 8 and # 9) of the second generator show an important increase in the ^{90}Sr content. The age of generator # 2 is about a third of generator # 1. In addition, the fact that samples under analysis cover the entire range of their lifetime it can be presumed that generator # 2 has been affected faster by radiolytic degradation despite that it was more regularly milked because of the higher activity. (A more detailed study, which includes dose calculation, should be carried out in order to arrive to more consistent conclusions)

The result of the application of the purification procedure of Strelow to the ^{90}Sr-^{90}Y-mixtures is shown in figure 2 where no separation of these two elements is achieved. The introduction of a washing step with 2 mol/L nitric acid as main modification of the previous procedure brings a totally different result. In figure 3 a very good separation can be observed (^{90}Sr recoveries from the mixtures are between 96 and 98%). By decreasing the used volume of 2 mol/L HCl the volume of HNO_3 can be raised. This is important in order to assure a good separation and avoid the breakthrough of ^{90}Y from the column with HNO_3. On the other hand the washing with 2 mol/L HCl should not be eliminated otherwise the volume of nitric acid needed to elute the contaminating metals (chiefly Fe^{3+}) would be too large. (Details of this study are not given in order to gain in brevity and they will be further published). The 4 mol/L hydrochloric acid is removed by evaporation. The addition of a 1:1 conc. H_2SO_4:HNO_3-mixture and its evaporation to eliminate any organic residues is followed by dilution of ^{90}Y into a small volume of 0.01–0.05 mol/L HCl as final product. (A pre-concentration step is under study to avoid the evaporation of such large volumes of concentrated acids)

Table III shows ^{90}Sr contamination levels of some samples after being treated by this modified procedure. In every case the $^{90}Sr/^{90}Y$ ratio is kept well bellow 10^{-6}.

No trace metals' determination was carried out by any specific technique such as ICP spectrometry. However the high (always >90%) DTPA labelling-efficiencies achieved with this purified Yttrium (60% was the maximal value attained with ^{90}Y directly obtained from generators) indicates that just as the established procedure this modified one is also suitable for reducing the levels of metal contaminants accompanying ^{90}Y.

TABLE III. ^{90}Sr CONTENT AS $^{90}Sr/^{90}Y$ RATIO OF SAMPLES PURIFIED WITH THE MODIFIED PURIFICATION PROCEDURE

Sample	$^{90}Sr/^{90}Y$
1	1.5×10^{-7}
2	1.1×10^{-7}
3	9.9×10^{-8}
4	7.6×10^{-8}
5	1.8×10^{-7}

**All measurements of activity were carried out with a relative error less than 1%.*

4. CONCLUSIONS

We have improved the described procedure of Strelow for purification of ^{90}Y from metal contamination by making it work as well for ^{90}Sr, decreasing in this way its content in the final product. Although generators involved in this study have low activity, one must expect at higher ones the ^{90}Sr breakthrough levels to grow even faster. So this modified procedure which can be used at laboratory scale brings the possibility to solve two problems at the same time: ^{90}Y purification from metal contaminants and from ^{90}Sr. The latter is the most important parameter if it is intended for clinical use. Because this procedure guarantees very low ^{90}Sr content in the final ^{90}Y product, it enlarges the usage of generators that do not necessarily have to be dismantled when the degree of ^{90}Sr breakthrough goes over the established limits.

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^{90}Y AND ^{105}Rh LABELLED PREPARATIONS: POTENTIAL THERAPEUTIC AGENTS

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Abstract. ^{90}Y and ^{105}Rh formulations were studied with an aim to prepare therapeutic radiopharmaceuticals. ^{90}Y obtained from a ^{90}Sr-^{90}Y generator as chloride was complexed with known ligands such as DTPA, EDTMP and DOTA as well as a few other phosphonate ligands. Particulates such as ^{90}Y labelled ferric hydroxide macroaggregates (FHMA) and ^{105}Rh-sulphur colloid were prepared and studied for their stability in buffers and human serum. The studies on the complexation of ^{90}Y and the preparation of radiolabelled particulates are described. ^{90}Y complexed nearly quantitatively with DTPA, DOTA and EDTMP under optimised conditions of reaction pH, temperature and ligand concentrations. Both ^{90}Y-FHMA and ^{105}Rh-S colloid could be prepared in high yields under optimised conditions. The labelled particulates were measuring 20–100 μm and 1–20 μm, respectively and were found to be very stable in buffers as well as human serum at 37 °C. The particulates have the potential for use as radiosynovectomy agents and for therapy of cancers such as hepatomas.

1. INTRODUCTION

The potential of ^{90}Y ($T_{1/2}$ 64.4 h, β^- decay, E_{max} 2.25 MeV) and ^{105}Rh ($T_{1/2}$ 35 h, β^- decay, E_{max} 0.56 MeV,70% & 0.25 MeV,30%, γs 319 keV,19% and 306 keV,5%) as therapeutic radionuclides has been realised since long [1–3]. Apart from their amenable physical characters for therapy, the feasibility of producing carrier free grade ^{90}Y (from a ^{90}Sr-^{90}Y generator) and ^{105}Rh {^{104}Ru (n, γ)^{105}Ru → ^{105}Rh} adds to the potential for their use in therapy. ^{90}Y labelled antibodies have been of interest since long [4] and have been studied in many laboratories [5–7]. Complexation properties of the ^{90}Y obtained from a locally developed ^{90}Sr-^{90}Y generator was studied with the ligands such as DTPA, DOTA, EDTMP to adjudge the product for further use in radiolabelling proteins and polypeptides, and are described here.

^{90}Y labelled particulates were also prepared with an aim to use them for radiosynovectomy. Treatment of arthritis with radionuclides in the form of colloids and particulates such as ^{198}Au-colloids have been reported since long. Leaching of activity from the synovial joints has been the major problem causing poor efficacy of such agents. Currently, agents such as ^{166}Ho-chitosan, ^{166}Ho-FHMA, ^{90}Y-FHMA, ^{165}Dy-FHMA and ^{166}Ho-HA are under clinical trials in different countries [8]. Availability of a wide range of beta emitting radionuclides and the feasibility of making radionuclide incorporated bio-degradable particulates have facilitated a great deal of studies in this area [8]. Attempts to prepare labelled Ferrichydroxide macroaggregates (FHMA) and Sulphur colloid with ^{90}Y and ^{105}Rh were made and the preparations were studied for their stability. The studies on these particulates are also described here.

2. MATERIALS AND METHODS

DTPA (diethylene triamine pentaacetic acid) was purchased from Sigma Chemical Co. EDTMP (ethylene diamine tetramethyl phosphonate), DOTA (1,4,7,10-tetraaza cyclododecane N,N′,N″,N‴tetraacetic acid) and a cyclic phosphonate, tetraaza cyclo tetradecane N,N′,N″,N‴ tetramethylene phosphonate (CTMP) were synthesised in the laboratory by following reported procedures. All other reagents were either from Sarabhai M.Chemicals or S.D. Fine Chemicals, India. The solvents used in these studies were from Merck (India).

^{90}Y was supplied as chloride, by the Fuel Reprocessing Division, BARC from a ^{90}Sr-^{90}Y generator developed by them [9]. The absence of ^{90}Sr was tested by beta spectrometry in the initial

stages. The purity of ^{90}Y and absence of ^{90}Sr were also ascertained by following the decay of ^{90}Y activity. ^{105}Rh-chloride, processed from irradiated Ru target [10] was supplied by our colleagues.

Both ^{90}Y and ^{105}Rh were counted in NaI(Tl) scintillation detector; Bremstrahllung radiations from ^{90}Y were measured while the window was adjusted for the γs from ^{105}Rh.

2.1. Preparation of ^{90}Y complexes

^{90}Y complexes of the ligands DTPA, DOTA, EDTMP and CTMP were optimised for pH, time, reagent concentrations etc. Initially excess reagents were reacted for a period of 4 h or more, to study the reaction at different pH, ranging from highly acidic (<2) to pH≥ 9.5. 1 M acetate buffer was used to maintain a pHupto 5.5–6, 1 M phosphate buffer for pH7–7.5 and 1 M Bicarboante buffer for pH9.5. 2.5 μM–25 mM solutions of the ligands were prepared in double distilled water. Reagent concentrations and time of reaction were then optimized under the optimum pH. Typically, 25 μL of ^{90}Y-chloride (500–900 kBq) was mixed with 50 μL of the appropriate buffer, to which was added 50 μL of the ligand solution. In the case of DOTA, the effect of heating on reaction yield and time required was also studied. After incubation of the reaction mixture, the complexation yields were determined by paper chromatography/TLC using pyridine:ethanol:water (1:2:4) elution, in all the cases.

2.2. Preparation of ^{90}Y -FHMA

^{90}Y-FHMA was prepared by precipitating ferrichydroxide as fine particulates in the presence of ^{90}Y under alkaline conditions [11]. ^{90}Y-chloride, (both at tracer levels as well as with addition of ~0.3 nanomoles inactive YCl_3) was added to 0.5 mL of 0.02 M ferrous sulphate solution and mixed well. 3 mL of 0.2 N NaOH was added dropwise to this mixture with stirring. The solution was mixed well and then centrifuged to remove the supernatant, washed with N saline thrice and resuspended in N saline. The labelling yield was calculated by determining the percentage of ^{90}Y activity associated with the particulates. The effect of addition of polyvinyl pyrollidone (net 0.6%) during precipitation on the yield of labelling and on the particle size distribution was studied.

2.3. Preparation of ^{105}Rh-Sulphur Colloid

^{105}Rh labelled sulphur colloid was prepared as reported for rhenium labelled sulphur colloid [12]. 18–37 MBq of ^{105}Rh chloride was added to 2 mL of 0.1 M sodium thiosulphate containing 1% Haemmacel. This solution was acidified with 1 mL of 1 N HCl. The colloidal sulphur formed was then mixed and placed in a water bath at 80°C for 4–5 minutes followed by rapid cooling in an ice bath for 5 minutes. The colloid was centrifuged to remove the acidic supernatant and reconstituted in saline. This procedure was repeated twice to remove traces of acid. The yield of ^{105}Rh-sulphur colloid was determined as in the case of ^{90}Y-FHMA. The effect of the presence of 0.1% Haemmacel in the reaction mixture on the reaction yield and particle size distribution was also studied.

2.4. Stability Studies

The stability of the particulate preparations, namely, ^{90}Y-FHMA and ^{105}Rh sulphur colloid was studied in saline and phosphate buffered saline (0.04M, pH7.5) at ambient temperature (25°C) and in human serum at 37°C. At each time point, the particulates were centrifuged, separated from the liquid phase and counted to estimate the extent of leaching of activity from the particles.

2.5. Estimation of the particle Sizes

In order to estimate the particle size distribution, both Y-FHMA and Rh-Sulphur Colloid were made under optimal conditions, using inactive yttrium chloride and rhodium chloride, keeping the amounts of Y and Rh identical to those in active preparations. The particles were dispersed in N

saline and analysed by laser diffraction for size distribution at the Powder Metallurgy Division, BARC.

3. RESULTS AND DISCUSSION

3.1. ^{90}Y complexes

^{90}Y was free from any detectable ^{90}Sr activity as estimated by following the decay of separated ^{90}Y. DTPA, EDTMP and DOTA showed good complexation with ^{90}Y. Paper chromatography using pyridine:ethanol:water (1:2:4) solvent was found to be the most suitable with R_f of the complex ~1 and the uncomplexed ^{90}Y retained at R_f value of 0. The cyclic phosphonate, CTMP, did not complex ^{90}Y under various reaction conditions, perhaps indicating the importance of the cavity size available for complexation, which is observed with most metal ions including Y and several lanthanides.

The effect of pHon the complexation yields is depicted in Figure-1.

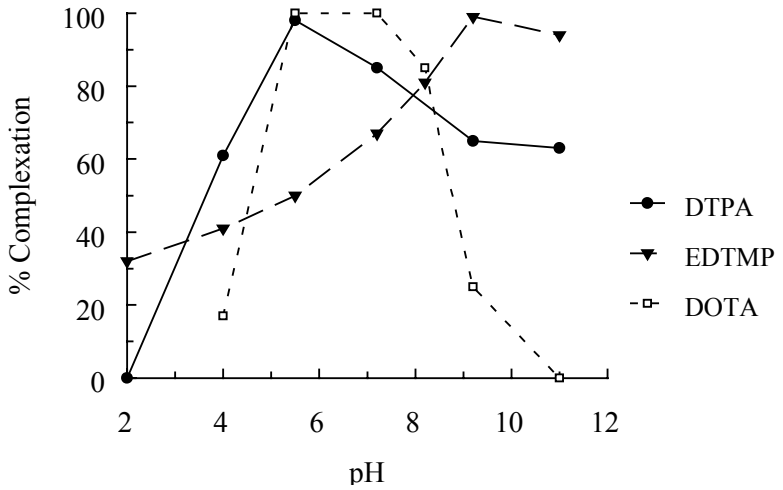

FIG. 1. Effect of pHon the complexation yields

It was observed that pHwas an important factor and DTPA complexed ^{90}Y at a pHof ~ 5–5.5 in acetate buffer to the extent of >99%. In the case of EDTMP, it was imperative to maintain the reaction pHat ~ 6–7 to obtain reasonable complexation yields and high yields of complexation was possible only under stronger alkaline conditions, pH≥ 9. Acetate buffer was found to be suitable for the range 5–6 pHand often ^{90}Y complexes are reported via formation of an acetate prior to the addition of the ligand. However, in the case of EDTMP where the complexation required pH>9, acetate addition did not alter the yield and was not necessary. As reported by several workers earlier, DOTA formed stable complexes with Y, at a pH~ 5.5–6. Figure-2 gives the effect of ligand concentration on complexation yield for the three ligands, DTPA, EDTMP and DOTA.

It was observed that a minimum of 1 μg (~2.5 nanomoles) of DTPA was required to completely complex trace amounts of ^{90}Y (~0.5 picomoles). However, when the complexation was carried out with ~270 picomoles of carrier Y (which corresponds to 0.185 GBq of ^{90}Y activity) the amount of ligand required was much higher (80 μg) corresponding to a ligand: metal ratio of ~750:1. In the case of EDTMP, a much larger amount, 50 μg was required for complete complexation of even trace amounts of Y, but the addition of carrier Y did not alter the complexation yield. At least 5 μg of DOTA was necessary to quantitatively complex trace levels of "no carrier added" (n.c.a.) ^{90}Y. Addition of carrier Y (0.3 nanomoles) however resulted in better complexation at lower amounts of DOTA and quantitative complexation could be obtained at ~2.5 μg of DOTA. Figure 3 shows the complexation yields of the three ligands with respect to time.

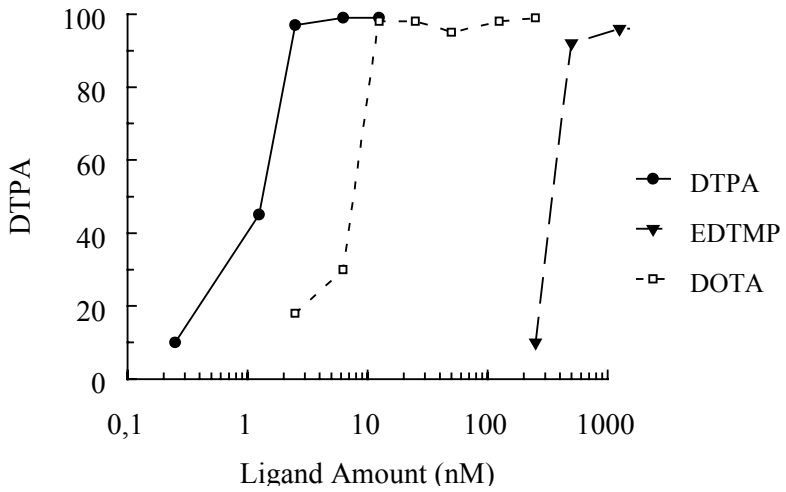

FIG. 2. Effect of the ligand amount on the complexation yields.

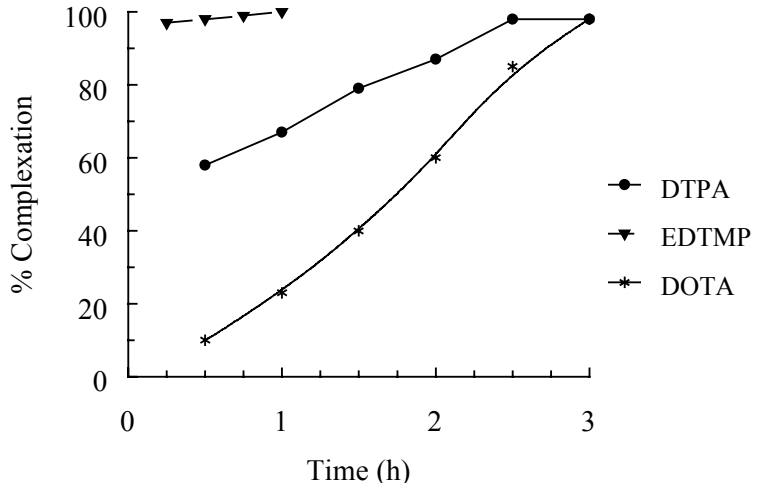

FIG. 3. Effect of Reaction Time on the Complexation Yield.

Mild heating (~37°C) of the reaction mixture helped in reducing the reaction time from a minimum of 2.5 h to 1 h for DTPA, and from 3 h to 2 h for DOTA. But there was no effect on the minimum ligand amount required for near quantitative complexation. In the case of EDTMP, there was no complexation when the reaction mixture was heated. On placing the ^{90}Y-EDTMP complex in a water bath at 37°C, disintegration of the complex was observed. In brief, the ligands DTPA, EDTMP and DOTA complexed ^{90}Y quantitatively under optimal conditions of pHand reagent concentrations and required a minimum of 2.5 h, 15 min and 3 h, respectively for completion of reaction at ambient temperature. Of these, the EDTMP complexes were not stable, pre-empting their use for in-vivo applications.

3.2. ^{90}Y -FHMA and ^{105}Rh-sulphur colloid particulates

^{90}Y-FHMA could be prepared in very high yields of 98 ± 3% (n = 5) with ease, both at tracer level and when carrier Y was added. Although the precipitation of ferric hydroxide could be observed with lower amounts of alkali, the variations in the acidity of the ^{90}Y-chloride solution warranted different amounts of alkali to be used for complete precipitation. Hence a moderate excess of alkali was used in the optimised procedure. The particles were ranging between 20–100 μm size and addition of polyvinyl pyrrolidone did not affect the yield or the particle size distribution.

^{105}Rh-Sulphur Colloid formed in considerable yields and 85 ± 4% activity was associated with the colloid. Addition of 0.1% Haemaccel was essential to keep the colloidal particles from clustering and sticking to the walls of the container. However, Haemaccel did not have any effect on the labelling yield. The Rh-suphur colloid particles were smaller in size and ranged between 1–20 μm.

Both the preparations were stable and no significant activity (<0.5%) was lost on repeated washings with water, saline or buffer. Table I shows the percentage of ^{90}Y or ^{105}Rh activity retained with the particulates along with the storage duration. Both ^{90}Y-FHMA and ^{105}Rh-S-Colloid particles are seen to be very stable even in human serum at 37°C for several days.

TABLE I. STABILITY OF ^{90}Y-FHMA AND ^{105}RH-S-COLLOID PARTICLES

Preparation	% Activity retained		Storage duration
	In PBS, 25°C	In human serum 37°C	
^{90}Y-FHMA	>99.5	~98	10 days
^{105}Rh-S colloid	>99	~97	5 days

4. CONCLUSION

^{90}Y complexes could be made with ease with the tested ligands such as DOTA, DTPA and EDTMP, which proves the usability of the locally made ^{90}Sr-^{90}Y generator. The particulate preparations, ^{90}Y-FHMA and ^{105}Rh-S-Colloid, could both be obtained in good yields and were found to be very stable. These particles hence may have potential for use in radiosynovectomy and the ^{105}Rh-S-Colloid in treatment of cancers such as hepatomas.

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RADIOCHEMICAL PROCESSING OF RADIONUCLIDES (^{105}Rh, ^{166}Ho, ^{153}Sm, ^{186}Re and ^{188}Re) FOR TARGETED RADIOTHERAPY

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Abstract. Radiopharmaceuticals are now increasingly used for therapy of cancer, palliation of pain caused due to bone metastasis and for the treatment of rheumatoid arthritis. Isotopes decaying by the emission of β^- particles are preferred in most of these applications. The half life, decay characteristics and energy of the emitted particles are the factors which govern the selection of a radionuclide for therapy. An yet another major consideration is the ready availability of the isotopes in adequate quantities. Hence, reactor produced isotopes are preferred for therapeutic applications. Radioisotopes meant for *in vivo* applications should meet the stringent quality control requirements with respect to radionuclidic purity, radiochemical purity and specific activity. This paper deals with the production and radiochemical processing of a few of the reactor produced therapeutic radionuclides. A major part of the radioisotopes used in India are produced in the 100 MW Dhruva reactor. The neutron flux for radioisotope production in this reactor varies from 0.6–1.4 × 10^{14}n/cm^2/s when the reactor is operating at its full rated power. Radioisotopes for which production and radiochemical processing are standardised and reported in this paper include ^{105}Rh, ^{166}Ho, ^{153}Sm, ^{186}Re, ^{188}Re.

1. INTRODUCTION

The development of therapeutic radiopharmaceuticals involve research in a number of diverse areas. These include production and radiochemical processing of the isotopes, synthesis of ligands, complexation studies with radiometals, conjugation of the ligands/complexes with carrier molecules and bio-evaluation of the products in suitable animal models. Of all the above, the production and radiochemical separation of isotopes for therapy is the primary requirement. However, this work is confined to a few laboratories which have access to nuclear reactors. Most of the laboratories depend on commercial sources for the supply of radioisotopes.

A large number of radioisotopes are proposed to be used for therapeutic applications [1–5]. The three major qualities which are looked in a radioisotopes to be used in *in vivo* medical applications are radionuclidic purity, specific activity and radiochemical purity. The requirement with respect to specific activity and radiochemical purity depend on the type of carrier molecules used. However, the radionuclidic purity of the isotope should be very high irrespective of the type of application and the type of carrier molecule selected. Relatively low specific activity radioisotopic preparations will suffice for the formulation of particulate radiopharmaceuticals, whereas, medium to high specific activity will be needed when the isotopes are used either as inorganic ions or as complexes of chelating molecules. Extremely high specific activity radioisotopes are essential when they are to be used for the preparation of labelled peptides and antibodies. Like wise, the requirement with respect to radiochemical purity of the radionuclide could also differ depending on the radiopharmaceutical application. The radiochemical purity requirement is very high for the preparation of complexes as well as labelled antibodies and peptides. The isotope produced should also be free from other metal contamination especially when used for the labelling of peptides and antibodies. Other metal ions if present could drastically reduce the complexation/labelling yield as the amount of chelating molecules available in a modified peptide or antibody is relatively low.

This paper describes the production and radiochemical separation of some of the radioisotopes which are used for targeted therapy. ^{105}Rh is one of the radioisotopes which can be used for targeted therapy. ^{105}Rh decays by the emission of β^- particles of 560 keV (70%) and 250 keV (30%); and γ rays of 319 keV (19%) and 305 keV (5%). ^{105}Rh has a half life of 35 h and can be prepared in reasonable quantities in a medium flux reactor [6]. Rh forms inert and stable complexes with a number of multidentate ligands which could be used as carrier molecules either directly or after linking with an antibody or peptide in order to direct ^{105}Rh to the target tumour cells [7–10].

^{153}Sm ($T_{1/2}$ 47 h) is a radionuclide which is widely used for therapeutic applications [11]. ^{152}Sm has a high thermal neutron capture cross section (204 b) and hence ^{153}Sm of adequate specific activity which can can be prepared in medium flux reactors. This could be used for palliative therapy, radiosynovectomy and for labelling antibodies and peptides

^{166}Ho ($T_{1/2}$ 26.9 h) decays to stable ^{166}Er by emitting 1.8 MeV β^- radiations and γ rays of 81 KeV (6%). ^{166}Ho could be used for both bone pain palliation and for the preparation of radiosynovectomy agents [12].

^{186}Re and ^{188}Re are the two isotopes of rhenium which are excellent choices for therapeutic applications [13–14]. Both ^{186}Re ($T_{1/2}$ 90h, E_β 1.07, 0.93 MeV, E_γ 137 keV) and ^{188}Re ($T_{1/2}$ 17h, E_β 2.1 MeV, E_γ 155 keV) can be prepared in nuclear reactors with adequate specific activities by using enriched targets. In the present paper, we describe the production and radiochemical processing of $^{186/188}Re$. In addition, the development of a ^{188}W-^{188}Re generator based on zirconium tungstate gel is also discussed [15–16].

2. MATERIALS AND METHODS

All the target materials used for neutron irradiation were of high purity grade (>99.99%). All natural targets, Sm as Sm_2O_3 and Ho as Ho_2O_3 were procured from American Potash Chemical Corporation, USA. W as WO_3 was from E. Merck, Germany. Re and Ru as metal powders were procured from Johnson Mathey. Enriched target of Sm, Sm_2O_3 (99.7% as ^{152}Sm) was from Bionucleonics Inc., USA. All the chemicals used were of A.R. grade procured from reputed suppliers.

Whatman 3 chromatography paper (30 × 2.5 cm) was used for paper chromatography as well as for paper electrophoresis studies.

2.1. Production and radiochemical processing

2.1.1. $^{105}RhCl_3$

Natural Ru powder (100 mg) was sealed in an Al can of 18 mm × 42 mm dimensions and irradiated at a neutron flux of 3×10^{13} n/cm^2/s for 7 days. The irradiated sample was cooled for a minimum of 24 h prior to radiochemical processing.

The irradiated Ru powder was transferred into a 250 mL beaker and 2 g each of KIO_4 and KOH pellets were added followed by the addition of 50 mL of double distilled water. Complete dissolution of the sample was accomplished by gentle warming. The alkali ruthenate formed was converted into RuO_4 by acidifying the solution with ~10–15 mL of dil. H_2SO_4. The end point of the reaction was monitored by a colour change from deep red orange to golden yellow. The solution was extracted with 4 × 50 mL of CCl_4, RuO_4 was extracted into CCl_4 and the aqueous phase carrying Rh fraction was evaporated to dryness. The contents were dissolved in 20 mL of 6 M HCl acid and warmed with 2–3 mL of 30% of H_2O_2 solution. This solution was extracted with 3 × 20 mL of n-tributyl phosphate pre-equilibrated with 6 M HCl to remove any Ir activation product. At the end of each stage the aqueous layer was warmed with 2–3 mL of 30% H_2O_2 solution. Aqueous layer from the TBP extraction was evaporated, suspended in ~5 mL of Conc. HCl, cooled and centrifuged to remove bulk of the KCl. The supernatant was taken in 15–20 mL of 1 N HCl and passed through a cationic exchange column loaded with 10 g of Dowex 50 (50–100 mesh) resin. The column was further washed with 10 mL of 1 N HCl. The effluent and washing were collected. The solution was evaporated and dissolved back in 5 mL of double distilled water. The last step was repeated till the pHof the solution was about 4–5.

2.1.2. $^{166}HoCl_3$

5 mg of Ho_2O_3 powder was sealed in an aluminium can and irradiated in a flux of 1.8×10^{13} neutrons/cm^2/s for a week. The irradiated sample was processed by dissolving in 5 mL of 0.1 N HCl acid followed by gentle warming. The solution was evaporated to near dryness and reconstituted in 10 mL of 0.1 N HCl solution.

2.1.3. $^{153}SmCl_3$

Approximately 10 mg of natural or 1.5 mg of enriched (^{152}Sm 99.7%) Sm_2O_3 was sealed in a quartz ampoule and irradiated in a neutron flux of $1.8x10^{13}$ n/cm^2.s. for 7 days. The irradiated sample was dissolved in 0.1 M HCl.

2.1.4. $Na^{186/188}ReO_4$

5 mg of natural Re metal was irradiated in a flux of $1.8x10^{13}$ neutrons/cm^2/s for 7 days and cooled for 1–4 days. The irradiated target was dissolved in 5 mL of 2.0 M HNO_3 solution by gentle warming. 5 mL (3 mg, 16.1 μM) of rhenium solution prepared above was aliquoted in a vial and the contents were evaporated to dryness by heating. 1 mL of 25% ammonia solution was added to the dry residue. Excess ammonia was removed by heating and the ammonium perrhenate residue was dissolved in 5 mL of 5 M NaOH solution.

Rhenium activity was extracted into 5 mL of methyl ethyl ketone (MEK) and the extraction efficiency was estimated by determining the radioactivity in equal aliquots of MEK and aqueous phase. Extraction was repeated once more with an equal volume of MEK and both the extracts were pooled together. MEK was removed by gentle heating and the residue was dissolved in 5 mL of normal saline.

2.1.5. $^{188}W/^{188}Re$ Generator

2 g of natural WO_3 was sealed in an aluminium can and was irradiated for 4 months in a neutron flux of 3×10^{13}n/cm^2/s. Irradiated target was dissolved in 2 M NaOH solution by gentle heating and Na_2WO_4 was precipitated as zirconium tungstate by mixing with a solution containing equimolar amount of $ZrOCl_2.8\ H_2O$ in water. The precipitated gel was boiled for 10 minutes and filtered. The precipitate was washed with 200 mL of hot double distilled water followed by 50 mL of ethanol. The gel was dried in air and powdered and loaded on a small glass column of ~5 mL size. The column was eluted with 0.9% NaCl solution. The eluent was further passed through an alumina column loaded with 5 g of acidic alumina.

2.2. Quality control tests

2.2.1. Assay of activity

Radioactive assay was carried out using a pre-calibrated ion chamber. Wherever a calibration factor for a radionuclide was not available, appropriately diluted activity was assayed using a HPGe-4 K multichannel analyzer connected to a computer with Nucleus Inc MCA-ADC card and corresponding PCA software. HPGe detector was pre-calibrated with a standard ^{152}Eu source obtained from Amersham International and the energy vs. efficiency chart was prepared. The sample to be assayed was counted by carefully preparing an aliquot of appropriate strength and the counting was done in the same geometry for which the detector was calibrated. The net activity corresponding to the counts at the respective gamma photopeaks were calculated by making use of the efficiency factor of the detector for that gamma energy.

2.2.2. Radionuclidic purity

Radionuclidic purity of the processed isotopes was estimated by using the HPGe MCA set up as described above. Wherever needed, the samples were stored for appropriate periods of time before determining the isotopic contents by this method.

2.2.3. Radiochemical purity

Radiochemical purity was estimated by paper chromatography and paper electrophoresis studies. Paper chromatography was performed using Whatman 3 MM paper (1 × 15 cm strip). 5μL portion of the test solutions was applied at 1.5 cm from the lower end of the strip. The strips were developed in different solvents such as acetone or normal saline. The strips were dried, cut into equal segments and the radioactivity was estimated in an appropriately adjusted single channel analyzer.

Paper electrophoresis studies were carried out by using Whatman 3 MM chromatography paper (30 × 2.5 cm). 5 μL samples were spotted, 10–12 cm from the cathode and paper electrophoresis was carried out for 1 h at 300 V in 0.02 M phosphate buffer at pH7.5. After run, the strips were cut into 1– cm segments and the radioactivity was measured.

3. RESULTS AND DISCUSSION

The work carried out for the preparation of radionuclides for the development of some of the therapeutic radiopharmaceuticals is elaborated in this paper. All the radioisotopes were produced in the 100 MWt Dhruva reactor. The reactor has a large irradiation volume dedicated for the production of radioisotopes. The flux varies from 0.6–1.8 × 10^{14} neutrons/cm^2/s. at full power operation. However, many of the irradiation reported in this paper were done at low flux irradiation positions.

3.1. $^{105}RhCl_3$

The nuclear reaction leading to the production of ^{105}Rh is given below.

$$^{104}\text{Ru (n, }\gamma\text{) }^{105}\text{Ru} \xrightarrow[4.54\text{ h}]{\beta^-} {}^{105}\text{Rh} \xrightarrow[35.5\text{ h}]{\beta^-} {}^{105}\text{Pd (n,}\gamma\text{) }^{106}\text{Pd}$$

Irradiation of an enriched target of ^{104}Ru and cooling for a period of 24–48 h should produce ^{105}Rh with radionuclidic purity >99%, the only radionuclidic contamination in that case will be ^{105}Ru, which will eventually decay to ^{105}Rh. Some amount of palladium isotopes will also be formed which are non-radioactive. However, the metal ion contamination in the isotopic preparation will be very high as all the unreacted Ru will be present in the sample. Hence, such a preparation will be of very little use for radiopharmaceuticals application and hence a radiochemical separation will be essential to separate unreacted Ru target from the ^{105}Rh. Therefore, the use of an enriched target is not of much help in ^{105}Rh production. When natural Ru target is used, activation products are possible from ^{96}Ru (5.51%), ^{102}Ru (31.61%) and ^{104}Ru (18.1%) leading to the production of ^{97}Ru ($T_{1/2}$ 2.9 d), ^{103}Ru ($T_{1/2}$ 40 d) and ^{105}Ru ($T_{1/2}$ 4.4 h). After a 24 h cooling >95% of ^{105}Ru is converted to ^{105}Rh. The presence of Ru isotopes could be beneficially used as a tracer to monitor the removal of the unreacted target from the product. When natural Ru is used as the target, traces of ^{192}Ir and ^{194}Ir are also formed by the activation of Ir impurity present in the target. However, a highly purified target material will significantly reduce these RN impurities. Table 1 shows the amount of different activation products formed during irradiation. The decontamination of ^{97}Ru, ^{103}Ru and ^{192}Ir from the ^{105}Rh finished product was used as one of the parameters to monitor the efficiency of the radiochemical processing.

The dissolution of irradiated Ru target using KIO_4 and KOH was found to yield better results than the reported method of using Cl_2 gas and KOH [6]. The dissolution was found to be faster and there was no need to handle corrosive gases. Salting out of KCl as well as $KClO_3$ from their saturated

solution was observed in the reported method using Cl_2 and KOH. Results of the solvent extraction studies with carbon tetrachloride are given in Table 2. The results suggest that a four step CCl_4 extraction could remove >98% of the Ru isotopes. The TBP extraction which follwed CCl_4 extraction was found to effectively remove >98% of ^{192}Ir.

After TBP extraction, ^{105}Rh was present in a mixture of cationic as well anionic species as seen by the movement of activity in the paper electrophoresis strip to both towards cathode and anode. The evaporation of the aqueous phase after TBP extraction and dissolution of it back in Conc. HCl salts out KCl present in the solution. Bulk of the KCl is removed by centrifugation. The column filled with cationic exchanger is used to remove traces of K^+ ions present. The paper electrophoresis of the final product showed that the Rh is present in anionic form possibly $RhCl_n(H_2O)_{6-n}$. Paper chromatography of the final product in saline showed that the Rh activity remains as a single species at the point of spotting. The suitability of using the product for labelling a number of different amine-phenol ligands were studied and labelling efficiency >95% could be obtained.

TABLE I. THE ACTIVATION PRODUCTS FORMED DURING THE IRRADIATION OF 100 MG OF NATURAL RU AT A FLUX OF 3×10^{13} NEUTRONS/CM^2/SEC FOR 7 DAYS AND AFTER 24 HOUR COOLING

Batch No.	Flux n/cm^2.s	Irradiation time (d)	Cooling time (h)	^{105}Rh (MBq)	^{97}Ru (MBq)	^{103}Ru (MBq)	^{192}Ir (MBq)
1	3×10^{13}	7	24	1665	207	814	3.3
2	3×10^{13}	7	24	1591	174	962	2.7
3	3×10^{13}	7	24	1576	155	762	3.1
4	3×10^{13}	7	24	1721	211	903	3.7

TABLE II. RESULTS OF CARBON TETRACHLORIDE EXTRACTION. THE ACTIVITY VALUES GIVEN ARE PRE AND POST SOLVENT EXTRACTION WITH CARBON TETRACHLORIDE (4 × 50 ML)

Batch No	^{105}Rh (MBq)			^{97}Ru (MBq)			^{103}Ru (MBq)		
	Prior	Post	% Recovery	Prior	Post	% Removed	Prior	Post	% Removed
1	1665	1631	98.0	207	1.6	99.3	814	6.7	99.2
2	1591	1550	97.4	174	0.9	99.5	962	4.6	99.6
3	1576	1558	98.8	155	0.9	99.4	762	5.3	99.3
4	1721	1669	97.0	211	1.5	99.3	903	6.7	99.2

3.2. $^{166}HoCl_3$

The target nuclide viz. ^{165}Ho is present in 100% isotopic abundance. A good activation cross section value (66 barns) results in the production of high specific activity (~3.6 TBq/g, 96 Ci/g). Radionuclidic purity evaluation by gamma spectrometry did not reveal the presence of any other gamma emitting impurities. The processed $^{166}HoCl_3$ was used for labelling HA particulates as well as for complexation with phosphonate ligands. The labelling efficiency of the particles as well as ligands were very high [17].

3.3. $^{153}SmCl_3$

The production and radiochemical processing of ^{153}Sm in $SmCl_3$ form was found to be fairly straight forward. The specific activity of ^{153}Sm was around ~82 Ci/g (3.1 Tbq/g) and ~260 Ci/g (9.6 TBq/g) while using natural and enriched ^{152}Sm target, respectively. Though the specific activity achievable with natural Sm target is adequate for therapeutic applications, the contamination of the sample with ^{155}Eu ($T_{1/2}$ 4.9 Y) formed by the activation of ^{154}Sm is one of the problems [18].

$$^{154}\text{Sm (n,}\gamma\text{) } ^{155}\text{Sm} \xrightarrow{\beta^-} ^{155}\text{Eu} \xrightarrow{\beta^-} ^{155}\text{Gd}$$
22.6%, 5.5 b 22 min 4.9 Y

A sample containing ~70 mCi (2.59 GBq) of ^{153}Sm prepared by irradiating natural Sm, when counted in the HPGe MCA showed ~30 μCi (1.1 MBq) of ^{155}Eu. No other gamma emitting impurities could be identified in the gamma spectrum. Hence, a therapeutic dose of 70 mCi (2.59 GBq) delivered to a patient will invariably contain ~30 μCi (1.1 MBq) of ^{155}Eu which strictly speaking is a R.N impurity. The gamma spectrum of ^{153}Sm obtained from enriched sample also showed presence of ^{154}Eu photopeaks. However, the amount formed was practically negligible. Formation of ^{154}Eu was due to the long irradiation time (7 d) followed by us. The ^{153}Sm prepared could be used for labelling phosphonate ligands [19].

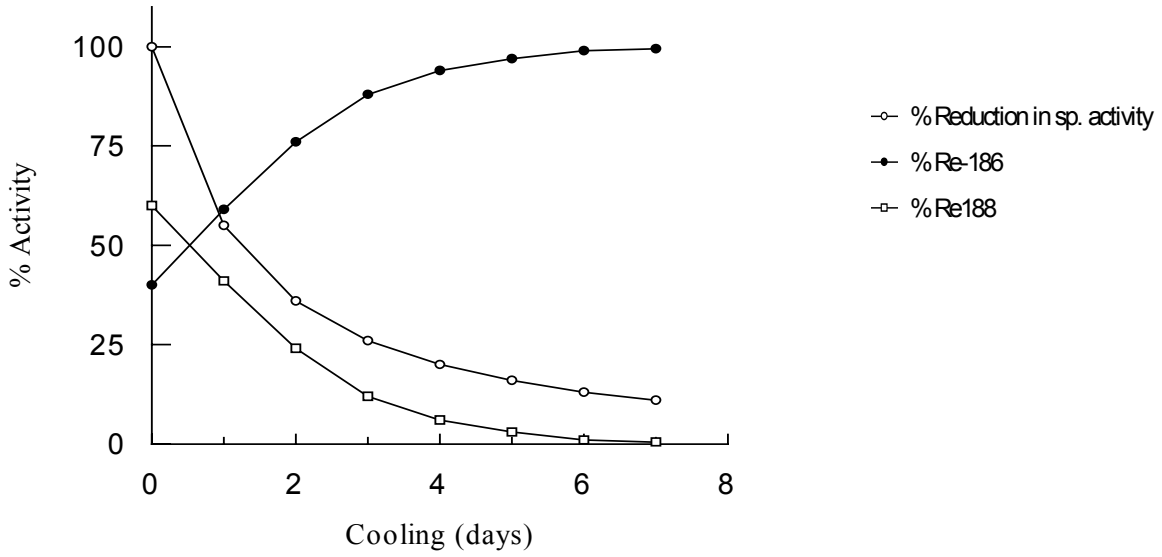

FIG. 1. Specific activity and l yields of $^{186/188}$Re with cooling period.

3.4. $Na^{186/188}ReO_4$

The naturally occurring Re consists of 37.3% ^{185}Re (110 b) and 62.3% ^{187}Re (74 b) mixture and irradiation results in the production of a mixture of ^{186}Re and ^{188}Re in 40:60 proportion at EOB. A cooling of 4 days will reduce the ^{188}Re contamination to <6%. However, the specific activity of the end product also undergoes a drastic reduction during this period (Figure 1). As both ^{186}Re and ^{188}Re are radionuclides suitable for therapy, one could use the activity at 24 h post irradiation at which time the ^{186}Re and ^{188}Re will be present in 60:40 ratio. A mixed radionuclide therapy is proposed using this preparation [20,21].

The radiochemical processing of irradiated Re to convert it to NH_4ReO_4 is essential for its further use for labelling studies. The MEK extraction which we have followed has given consistently good quality perrhenate solution which gave very high labelling yields with a number of ligands [21–23]

3.5. ^{188}W/^{188}Re generator

Radionuclidically pure ^{188}Re can be prepared by direct irradiation of ^{187}Re enriched target. However, the high cost of the target and the short half life ($T_{1/2}$ 17 h) do not allow this mode of

preparation of ^{188}Re for therapeutic applications. A viable alternative is the ^{188}W-^{188}Re generator, which could give carrier free ^{188}Re. However, the parent nuclide ^{188}W is produced by a double neutron capture of ^{186}W as shown below. The natural isotopic abundance of ^{186}W is only 26%.

$$\underset{38\ b}{^{186}W\ (n,\gamma)}\ \underset{64\ b}{^{187}W\ (n,\gamma)}\ ^{188}W \xrightarrow[69.4\ d]{\beta^-} {}^{188}Re \xrightarrow[16.9\ h]{\beta^-} {}^{188}Os$$

The general equation for the build up of ^{188}W by the above reaction could be written as [24]

$$^{188}W \text{ activity (Bq)} = \phi^2\ \sigma_1\ \sigma_2\ N_1 0\ (1 - e^{-\lambda_3 t)} \div \lambda_2$$

Where ϕ = neutron flux, σ_1 and σ_2 are reaction cross sections for the respective reactions.

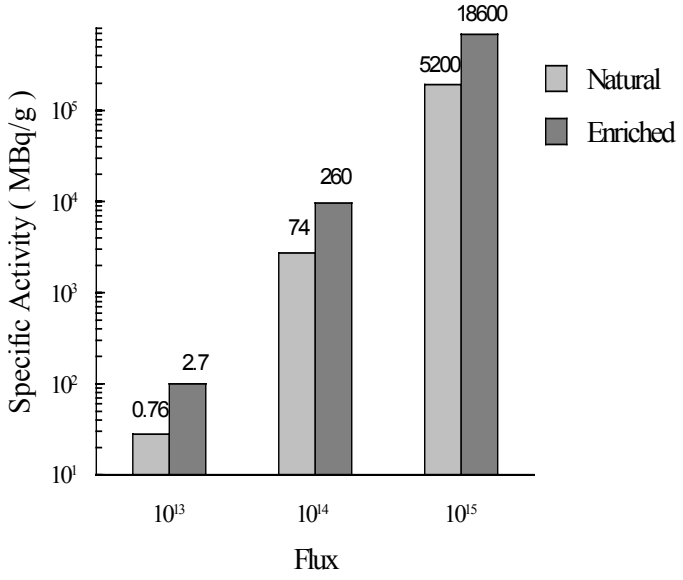

FIG. 2. Theoretical specific activity of ^{188}W at different neutron fluxes.

The amount of activity formed in a double neutron capture reaction is generally low as it is a function of $\sigma_1 \times \sigma_2$. The neutron flux (ϕ) is one of the most critical factors determining the activity build up. As ϕ^2 term comes in the reaction, the yield could increase nearly a 100 fold with an one order increase in the neutron flux. The yield of ^{188}W formed at different flux values are given in Figure 2. However, the actual build up could be much less as part of the ^{188}W will be lost due to burn up (σ 12.5 barns) [25]. The use of enriched target could give a 3.5 fold increase in activity. However, the use of enriched target in a medium flux reactor is not economical. Hence, for the preparation of any meaningful quantities of ^{188}W with adequate specific activity, a flux of 5×10^{14} and above will be essential. Though zirconium tungstate gel was initially used for the development of ^{188}W-^{188}Re generator, ^{188}W prepared in high flux reactors is now used for preparing alumina based column generators [25].

The amount of activity formed by irradiating 2 g of natural WO_3 at a flux of 4×10^{13} n/cm^2.s for 180 days was around 2–3 mCi only. The gel generator developed by us could be used for the preparation of small amounts of ~1mCi (37 MBq) of $^{188}ReO_4^-$form. Purification of the eluent from the gel column by passing it through a small alumina column was found to be essential to get perrhenate solution suitable for labelling studies.

4. CONCLUSION

The availability of suitable radionuclides for therapy with appropriate specific activity, radionuclidic purity, radiochemical purity and chemical purity is the primary requirement for the production of therapeutic radiopharmaceuticals. The neutron flux available in the reactor limits the specific activity, though it can be increased to an extent by using enriched targets. Among the isotopes for which radiochemical processing is discussed, ^{153}Sm and ^{166}Ho can be prepared for therapy in adequate quantities with appropriate specific activities using medium flux (10^{13}–10^{14}) reactors. Though ^{105}Rh of very high specific activity can be prepared in medium flux reactors, the amount formed is relatively small due to the low reaction cross section and hence this isotope may not find much use in therapy. The radiochemical processing is also more complicated. Production of $^{186/188}$Re by medium flux reactor is feasible. The mixed radionuclide therapy using a preparation containing both ^{186}Re and ^{188}Re obtained from natural target is advantageous and worth pursuing. Due to the very small amount of ^{188}W formed in a medium flux reactor, it is practically impossible to develop ^{188}W-^{188}Re generator for any therapeutic applications.

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STUDY ON THE PREPARATION AND STABILITY OF ^{188}Re BIOMOLECULES VIA EHDP

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Abstract. A direct labelling technique via ethane-1-hydroxy-1,1-diphosphonic acid (EHDP) as a weak competing ligand was developed for the preparation of several biomolecules: ^{188}Re-monoclonal antibody ior cea1 against carcinoembryonic antigen (^{188}Re-MoAb), biotinylated ^{188}Re-MoAb (^{188}Re-MoAb-biotin), ^{188}Re-polyclonal IgG (^{188}Re-IgG), ^{188}Re-peptide (somatostatine analogue peptide b-(2-naphtyl)-D-Ala-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-amide), ^{188}Re-MoAb fragments (^{188}Re-F(ab')$_2$) and biotinylated ^{188}Re-F(ab')$_2$ (^{188}Re-F(ab')$_2$-biotin). The reaction conditions such as pH, temperature, weak ligand concentration and stannous chloride concentration were optimized during the radiolabelling of each biomolecule. Before the labelling procedure, disulphide bridge groups of the biomolecules were reduced with 2-mercaptoethanol (2-ME). To obtain ^{188}Re labelled antibodies and peptides in high radiochemical yields (>90%) via EHDP, it was necessary to use acidic conditions and a high concentration of stannous chloride to allow the redox reaction $Re^{+7} \rightarrow Re^{+5}$:$Re^{+4}$. The labelling of MoAb and F(ab')$_2$ with ^{188}Re via EHDP was also evaluated employing a pretargeted technique by avidin-biotin strategy in normal mice, demonstrating that the ^{188}Re-labelled biotinylated antibodies are stable complexes *in vivo*. The ^{188}Re-peptide complex prepared by this method, was stable for 24 h and no radiolytic degradation was observed.

1. INTRODUCTION

Increased effort has been made to label monoclonal antibodies (MoAb) and peptides with rhenium-188 because of their potential role in the radioimmunotherapy of cancer and the availability of Re-188 from a W-188/Re-188 generator [1–3].

The β-(2-naphthyl)-D-Ala-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-amide peptide is a somatostatin analog with cytostatic effect on small lung cancer cells [4].

Whereas the avidin-biotin system, has shown that target-to-nontarget radioactivity ratios and radioimaging scanning can be significantly improved by introducing a two-step or three-step system. One two-step approach is based on the administration of streptavidin conjugated to the antibody followed by a radioactive biotin derivative or biotinylated antibody is injected followed by radioactive streptavidin [5]. In the three-step system, biotinylated antibody is injected followed by an excess of cold avidin or streptavidin and, as a third step, radioactive biotin is administered. An avidin "chase" of biotinylated antibody has been also reported improving clearance of radiolabelled MoAb without decreased accumulation in the target tumour [6].

In this report, the MoAb murine anti-CEA IgG1 designated ior cea1 (Havana, Cuba), its F(ab´)$_2$ fragments and the β-(2-naphthyl)-D-Ala-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-amide peptide, were labelled with Re-188 with high radiochemical purity based on the direct labelling method ^{188}Re-EHDP-MoAb [7]. The labelling of biotinylated MoAb ior cea1 and its F(ab´)$_2$ fragments with Re-188 from instant freeze dried kit formulations was also performed. The biodistribution and dosimetry of these radioimmunoconjugates were determined in Balb/c mice after injection of avidin as a "chase" under the hypothesis that if the ^{188}Re-labelled biotinylated MoAb ior cea1 and its ^{188}Re-labelled biotinylated F(ab´)$_2$ fragments prepared by this method, are stable complexes *in vivo*, an increased blood clearance of the radiolabelled agent with reduction of radiation dose would be obtained.

2. MATERIALS AND METHODS

2.1 Preparation and Purification of the F(ab´)$_2$ Fragments

F(ab´)$_2$ fragments of ior cea1 were prepared by digestion with pepsin following by a purification step using a Protein A-Agarose column and a ProteinPak 300SW (1 mL loop) HPLC size-exclusion column. Finally, F(ab´)$_2$ fragments were concentrated by ultrafiltration (Ultrafree-PFL 30,000 NMWL, Millipore Co.) to obtain a concentration of approximately 10 mg/mL.

2.2 Biotinylation of Antibodies and Antibody Fragments

To prepare biotin labelled whole MoAb or F(ab´)$_2$ fragment, 5.0 mg of succinimidyl-6-(biotinamido)hexanoate (ImmunoPure NHS-LC-Biotin, PIERCE Co.) were dissolved in 30 µL DMSO and 0.1M phosphate buffer was added to a final volume of 0.5 mL. Immediately, 40 µL of the NHS LC-Biotin, were added to 1.0 mL of the MoAb solution (≈10 mg/mL) and the mixture was incubated with gentle stirring for 30 minutes at 18°C. The biotinylated antibody was separated from the unreacted NHS-LC-Biotin employing a ProteinPak 300SW (1 mL loop, Waters) HPLC size-exclusion column run in 0.1 M phosphate buffer (pH7.4). Under these conditions, ratios of 4–5 moles of biotin per mole of whole antibody and 2–3 moles of biotin per mole of F(ab´)$_2$ fragment were obtained.

2.3 Biomolecules reduction

To 0.5 mL of peptide or MoAb solution were added 25 µL of 2-mercaptoethanol (2-ME) previously diluted with distilled water 1 :10. After allowing the mixture to react at room temperature for 30 min with continuous rotation, the resulting solution was purified on a ProteinPak 125 (Waters) HPLC size-exclusion column, using 0.1 M phosphate buffer (pH7.4) as mobile phase at a flow rate 1.5 mL/min This system produced retention times of 20–21 min, 8–8.5 min and 4–4.5 for the peptide, 2-ME and MoAb respectively. The U.V. spectrum of each compound was obtained with a HPLC Photodiode Array Detector In the case of peptides, the radiolabelling was carried out using reduced and unreduced molecule.

2.4 Preparation of ^{188}Re-biomolecules

The general procedure for the preparation of ^{188}Re-biomolecules was as follows: EHDP and 5 mg of gentisic acid were dissolved in 0.5 mL of stannous chloride solution ($SnCl_2$ in 0.06 M HCl), and 1.0 mL of reduced or unreduced biomolecule was added followed by addition of 1.5 mL of ^{188}Re-perrhenate solution (Oak Ridge National Laboratory).

2.5 Radiochemical Purity

The radiochemical purity for MoAb, F(ab')$_2$ and IgG was determined by a combination of instant thin layer chromatography (ITLC) and HPLC as reported previously [7]. The evaluation of the radiochemical purity for peptides was determined by ITLC-SG analysis (Table I) and C-18 Sepak cartridges (Waters). The immunoreactivity of the labelled antibodies and its fragments was evaluated using affinity thin layer chromatography (ATLC) as Zamora et al reported [8].

2.6 Biodistribution and dosimetry

Female Balb-c mice (27–30 g) were used in Biodistribution studies. 50 µg of avidin (Pierce Co.) was injected 15 min after injection of the biotinylated radioimmunoconjugate.

TABLE I. SYSTEMS EMPLOYED DURING THE DETERMINATION OF ^{188}Re-PEPTIDE RADIOCHEMICAL PURITY BY ITLC-SG ANALYSIS (1 × 10 cm STRIPS)

Solvent:	0.9% NaCl	Acetone	Acidified ethanol (10% HCl 0.01N)
Rf $^{188}ReO_4^-$	1.0	1.0	1.0
Rf $^{188}ReO_2$	0.0	0.0	0.0
Rf ^{188}Re-peptide	0.0	0.7–1.0	1.0
Rf ^{188}Re-EHDP	1.0	0.0	1.0

Cumulative activities and residence times in all organs studied were calculated from the biological data obtained in the animals. Human absorbed dose calculations were performed according to the methods outlined by the MIRD committee using the computer program MIRDOSE3 developed at Oak Ridge Associated Universities.

3. RESULTS AND DISCUSSION

The reduction of intrinsic disulphide bridges within the antibody molecule by the use of the reductant 2-ME, was an essential step during the preparation of ^{188}Re-MoAb complexes. However, contrary to the labelling studies with MoAb's, the reduction of peptides is not necessary to obtain ^{188}Re-peptides complexes in high radiochemical yields. This result is expected as the Sn(II) ion works strongly in the acidic region reducing the rhenium to a reactive species, and reducing the peptide for subsequent chelation to the metal.

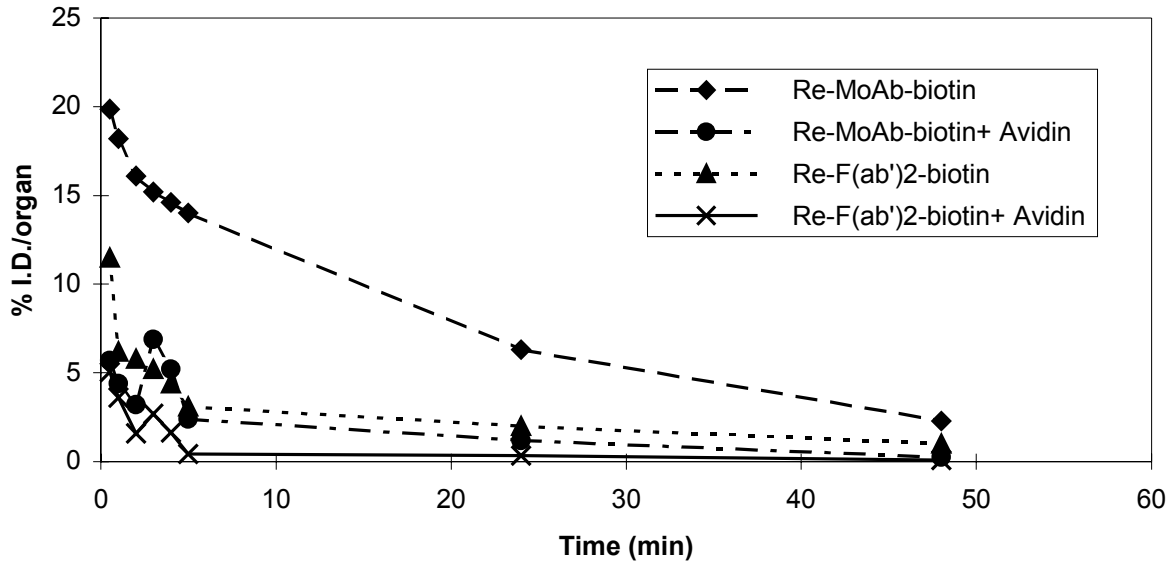

FIG. 1. Blood clearance in mice of ^{188}Re-MoAb-biotin and ^{188}Re-F(ab´)2-biotin with or without avidin as a chase.

^{188}Re-MoAb, ^{188}Re-MoAb-biotin, ^{188}Re-F(ab´)$_2$ and ^{188}Re-F(ab´)$_2$-biotin preparations were produced for these studies with specific activities of 1.30 ± 0.18 GBq/mg (36 ± 5 mCi/mg). Radiolabelled F(ab´)$_2$ fragment was eluted from the Protein Pak 125 HPLC column as a monomeric peak (retention time of 8.9 ± 0.2 min).

There were no significant differences ($p > 0.05$) between the biodistribution of biotinylated and unbiotinylated ^{188}Re-labelled immunoconjugates. When avidin was injected as a chase after injection of ^{188}Re-MoAb-biotin or ^{188}Re-F(ab´)$_2$-biotin, the blood radioactivity level decreased approximately 50 70% (FIG. 1), the cumulated activity in blood decreased almost 75% (from 191.05 $\pm$ 26.92 to 40.54 $\pm$ 6 MBq·h) and the effective dose diminished 25% (from 0.173 to 0.130 mGy/MBq) respect to that of the radioimmunoconjugates where the "chase effect" was not used.

The ^{188}Re-peptide complex showed that under the procedure reported herein it can be prepared with a radiochemical purity of 90% and a specific activity up to 1.8 GBq/mg without radiolytic degradation of the product.

TABLE II. REACTION CONDITIONS TO LABEL DIFFERENT BIOMOLECULES VIA EHDP

Biomolecule	[$SnCl_2$] (mM)	[EHDP] (mM)	pH	Labeling time (h)	Temperature (°C)	Yield (%)
^{188}Re-IgG	0.88	30	3	18–22	22	98
^{188}Re-IgG	3.52	120	3	0.5	37	97
^{188}Re-IgG	7.04	120	4	2	37	97.5
^{188}Re-IgG	7.04	120	5	18–22	22	97
^{188}Re-MoAb	3.52	120	3	2	22	99
^{188}Re-MoAb	3.52	120	3	0.5	37	98
^{188}Re-MoAb	3.52	120	4	5	37	97
^{188}Re-MoAb-biotin	3.52	120	3	0.5	37	96
^{188}Re-F(ab')$_2$	3.52	120	3	2	37	96
^{188}Re-F(ab')$_2$-biotin	3.52	120	3	2	37	95
^{188}Re-peptide (reduced)	11.76	120	3	1.5	92	90
^{188}Re-peptide (unreduced)	11.76	120	3	1.5	92	90

4. CONCLUSIONS

To obtain ^{188}Re labelled antibodies and peptides in high radiochemical yields (>90%) via EHDP, it was necessary to use acidic conditions and a high concentration of stannous chloride to allow the redox reaction $Re^{+7} \rightarrow Re^{+5}$:$Re^{+4}$ (Table II).

Results showed that the immunoreactivity of the antibodies remains unaffected after the labelling procedure. However, MoAb and its fragments are unstable *in vitro* at neutral pHwhich is in agreement with the results obtained by other workers [1,3]. These investigators have also found that the ^{188}Re-MoAb complex is stable *in vivo*, maybe due to a protective effect of serum proteins against the processes of ^{188}Re reoxidation.

In this work the labelling of MoAb and F(ab')$_2$ with ^{188}Re via EHDP was also evaluated employing a pretargeted technique by avidin-biotin strategy in normal mice, demonstrating that the ^{188}Re-labelled biotinylated antibodies are stable complexes *in vivo*.

The ^{188}Re-peptide complex prepared by this method, was stable for 24 h and no radiolytic degradation was observed. In order to increase the radiochemical purity, a desalting column could also be used. However, this method is limited to labelling with ^{188}Re only those peptides which contain cysteine bridges. The biological properties of the radiopeptides have to be evaluated since reaction condition are not an appropriate environment for their integrity.

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Re-186-BLEOMYCINE: RADIOPHARMACEUTIC FOR DIAGNOSIS AND THERAPY

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Abstract. Bleomycine is an antibiotic used for chemotherapy of several neoplasms. Earlier studies with labelling of bleomycine (BLM) with different radionuclide were done. It was tested for different kind of tumours imaging. Due to previous encouraging imaging results with BLM-Tc-99m , BLM-Co-57 and BLM-In-111 authors decided to check the possibility and methods of labelling BLM with Re-186 to obtain radiopharmaceuticals potentially suitable for diagnosis and treatment of some neoplastic tumours. Different methods of labelling were investigated. The best one are electrolytic and with use of cationic-Sn complex modified by using of gentisic acid and incubation of the reaction mixture at 100^{o}C for 10 min.

1. INTRODUCTION

There is tremendous interest in developing more effective radiopharmaceuticals for diagnosis and treatment of malignant diseases. Last decade development of beta emitting radiopharmaceuticals for palliative treatment of bone metastases is a good example of that [1].

Among other chemical compounds usefulness of BLM was investigated for diagnosis of malignant tumours. BLM is a chemotherapeutic agent used for therapy of neoplastic tumours. It was labelled with Co-57 [2], Tc-99m [3] or In-111[4] and was used for scintigraphic diagnosis of different kind of tumours. There was different biodistribution and stability of radiolabelled BLM depending on which radionuclide it was labelled. Though it was not widely applied very promising results were obtained with Tc-99m labelled BLM. It was rapidly cleared from the blood pool and taken up by neoplastic tumours. There were reports showing its usefulness for diagnosis of eye ball neoplastic diseases, breast cancer , neck tumours [5].

Kairemo and al. [6] performed the study on dosimetric and biokinetic aspects of BLM-In-111 as a potential radiochemotherapeutic agent. Their promising results encouraged authors to undertake the endeavour to check the possibility and methods of labelling BLM with beta emitting radionuclide Re-186.

2. METHODS

The aim of this study was to establish the best method of labelling BLM with Re-186.

Re-186 and Tc-99m belong to the same group in periodic table. Re-186 has a relatively short physical half-life of 90.64 h. It has both beta emission suitable for therapy (Emax = 1,07 MeV) and gamma emission suitable for external imaging (E_γ = 137 KeV). Chemical properties of Tc-99m and Re-186 are similar so one can assume that probably methods of labelling and biodistribution of BLM-Re-186 and BLM-Tc-99m could be comparable as well. If labelling of BLM with Re-186 is successful it will further allow to investigate its imaging and radiochemotherapeutic properties in neoplastic tumours.

Natriumperr (Re186) Mallinckrodt Medical, $SnCl_2$ (Fluka), Dowex1x8, Dowex 50x8, Dowex 50Wx4 in sodium or hydrogen form, 100–200 mesh Serva were used for labelling.

Different kind of labelling methods were employed. Methods which were previously used for labelling BLM with Tc-99m [5] were investigated:
(a) conventional one with $SnCl_2$,
(b) electrolytic,
(c) with use cationic-Sn complex.

The products were analysed by thin layer chromatography (see Table 1 and Fig. 1).

TABLE I. CHROMATOGRAPHIC ANALYSIS OF BLM-Re-186

Chromatographic system		R_f of BLM-Re-186 species		
Support	Solvent	BLM A2	BLM B2	BLM IVF
DC silica gel 60 F_{254}	10%CH_3COONH_4:CH_3OH (1:1 v/v)	0,4	0,68	0,84

The yield of labelling by these three methods was very unsatisfactory.

Methods of HEDP-Re-186 labelling were taken into consideration [7, 8] and gentisic acid (2,5–dihydroxybenzoic acid) was used during the labelling and the reaction mixture was incubated at 100°C for 10 min.

3. RESULTS

In spite of modification in labelling procedure the yield of labelling by conventional method was still very pore. But by cationic-Sn complex method yield of labelling was 95%, by electrolytic method was 98%.

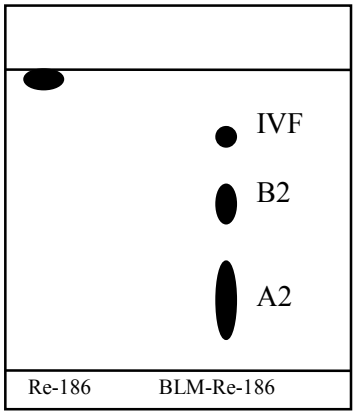

FIG. 1. Typical chromatogram obtained for BLM-Re-186.

Obtained results are very encouraging. Authors showed that it is possible to label BLM with Re-186. BLM-Re-186 can be potentially a suitable agent for diagnosis and treatment of malignant tumours. Further studies on stability of the complex are necessary.

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RHENIUM-186 DIRECT LABELLED HIgG

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Abstract. The aim of this study is to develop and improve existing radiolabelling techniques of peptides and monoclonal antibodies with ^{186}Re for achievement of potential agents for cancer targeted radiotherapy. There were selected methods and techniques for the direct labelling of intact HIgG by studding chemical and radiochemical processes of —S—S— bridges prereduction, reduction of $^{186}ReO_4^-$ and coupling reaction of rhenium with HIgG. The —S—S— bridges prereduction of HIgG to sulfhydryls was effected using different reducing agents: ascorbic acid, 2,3 dimercaptopropanol, cysteine, active hydrogen. The prereduction reactions are controlled by masic ratios of HIgG/reduction agent, pH, temperature and time of incubation. A pH= 4.5 and a 24 hours incubation time are in the advantage of the prereduction yield. The labelling with ^{186}Re of prereduced HIgG with ascorbic acid or active hydrogen and 37^0C incubation in 22 hours releases 92% radiochemical purity.

1. INTRODUCTION

Rhenium-186 has been considered as an ideal radionuclide for radioimmunotherapy because its physical half-life of 90 hours and beta emission of 1.07 MeV. The 137 KeV (9%) gamma emission also allows simultaneous scintigraphic imaging. The protocols regarding the labelling of specific biomolecules with ^{186,188}Re allowed the formulation of an instant kit and the clinical and laboratory testing for both radioimmunotherapy and radiodiagnostic in the same time [1,2,3].

In our laboratory HIgG (G human immunoglobulin) has been labelled with ^{186}Re by direct method in three distinct steps: i) the —S—S— bridges prereduction to —SH groups; ii) the reduction of $^{186}ReO_4^-$ and iii) the coupling reaction of reduced ^{186}Re to —SH groups. The following reducing agents were used: ascorbic acid, cysteine, active hydrogen and 2,3 dimercaptopropanol. $^{186}ReO_4^-$ has been reduced with stannous chloride. The coupling reaction of ^{186}Re with HIgG calls incubation of mixture HIgG in thyol form with stannous chloride and $Na^{186}ReO_4$ at 37^0C during 22 hours.

2. EXPERIMENTAL

2.1. Materials and methods

The materials used in this study were: HIgG (from Cantacuzino Biological Institute, Bucharest, Romania), $SnCl_2 \times 2H_2O$, ascorbic acid and citric acid (from Sigma), all with high chemical purity. ^{186}Re was obtained by irradiation (n, γ) on TRIGA Reactor, Pitesti, Romania, 5×10^{13}n/cm^2.s flux by the nuclear reaction:

$$^{185}\mathrm{Re} \xrightarrow[\sigma = 104b]{(n,\gamma)} {}^{186}\mathrm{Re} \xrightarrow[t = 90h]{\beta^-} {}^{186}\mathrm{Os}$$

2.2. $Na^{186}ReO_4$ preparation

The preparation of $Na^{186}ReO_4$ was been effected by the Eisenhut method [4]. An amount of 10 mg of metallic Re in powder with rhenium 185, 37.7% enrichment, was irradiated in reactor for a week at a 5×10^{13}n/cm^{2}s neutron flux (see FIG. 1). After 3 days cooling, the probe was transferred in a 10 mL vessel and 2 mL of H_2O_2 10% for the $H^{186}ReO_4$ formation were added. The reaction time for the complete oxidation was 2 hours. The vessel was covered during the reaction to avoid contamination. The pHwas adjusted to 5 with NaOH 0.1 N after $H^{186}ReO_4$ setting up. We obtained a solution with 62 mCi/mL (2.3 GBq) radioactive concentration and 98–99% radiochemical purity. The results of quality control effected by Watman 1 paper radiocromatography are presented in TABLE I.

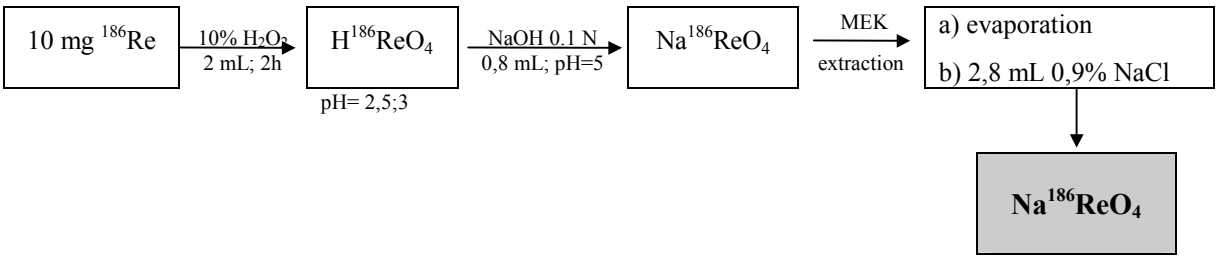

FIG. 1. The irradiated sample processing.

TABLE I. THE DETERMINATION OF RADIOCHEMICAL PURITY OF $Na^{186}ReO_4$

Solvent	R_f (ReO_4^-)	R_f (impurities)	% Radiochemical purity
Acetone	0.87–1.00	0.00–0.08	99.43
Ethanol	0.39–0.53	0.00	99.00
Ethanol: NH_3: H_20 (2:1:5)	0.77	0.00	99.15
0.9% NaCl	0.60–0.84	0.00	98.00

2.3. $Na^{186}ReO_4$ reduction

The stannous chloride was used as reducing agent for $Na^{186}ReO_4$. 200 μL solution containing 2 mg/mL $SnCl_2 \times 2H_2O$ and 20 mg/mL citric acid concentrations, were added to 100 μL $Na^{186}ReO_4$ (≈500 μCi). After 24 h of incubation time the reducing yield was 92%–96%, determinated by Watman 1 paper chromatography in acetone solvent (TABLE II).

TABLE II. REDUCTION SYSTEMS OF STANNOUS CHLORIDE

Reduction system	$SnCl_2 \times 2H_2O$:$^{186}ReO_4$	Reducing yield after 24 h incubation
$SnCl_2$:0,05 N HCl	200 μL:74 MBq/100 μL	96,35
$SnCl_2$:20 mg/mL citric acid	200 μL:74 MBq/100 μL	92,08

2.4. Prereduction of the bounds —S—S— of HIgG

For the prereduction of the bounds —S—S— to —SH groups we used different reduction agents: ascorbic acid, 2,3 dimercaptopropanol, cysteine and active hydrogen. The HIgG solution had been prepared as follows: 1 mg of HIgG was dissolved in 1 mL bidistillated water and divided in 100 μl portions. The reduction agent was added to each HIgG sample (TABLE III). The sample has been purged with pure N_2 for the elimination of air from the system.

After incubation the pHof samples (no 1,2,3,4) was adjusted to 5 by addition of sodium citrate buffer; than, in each sample were added 200 μL of $SnCl_2$ in citric acid solution, 20 mg/mL. After 30 minute of slow purged N_2 the samples were lyophilised.

TABLE III. THE REDUCTION OF THE DISULPHIDE GROUPS TO SULFHYDRYLS

Reducing agent (RA)	(HIgG:RA)/vol	pH	Incubation	
			t^0C	Time
Ascorbic acid	*sample no 1* 100 μg:500 μg/200 μL	4	37	21 h
2,3 Dimercaptopropanol	*sample no 2* 100 μg:64 μg/200 μL	3,5	22	22 h
Cysteine	*sample no 3* 100 μg:120 μg/300 μL	4,5	37	22 h
Sn + citric acid →(AH)*	*sample no 4* 100μg:/300μl	3,5	37	24 h

* *Active hydrogen.*

2.5. The labelling of HIgG lyophilised samples

The lyophilised samples were reconstituted in 1 mL $Na^{186}ReO_4$ (1.2 mCi, pH= 5 for the final solution) and incubated at 37^0C. The pHprobes was always raised to 7 with 0.1 M sodium bicarbonate buffer. The best results of radiolabelling are presented in TABLE IV.

TABLE IV. THE RESULTS OF RADIOCHEMICAL PURITY OF HIGG-^{186}RE, OBTAINED BY INCUBATION FOR 1 HOUR AND RESPECTIVELY 22 HOURS.

HIgG samples	^{186}Re	Incubation time (h)	% Radiochemical purity	
			ethanol	ethanol:NH_3:H_2O (2:1:5)
AA* — HIgG	74 MBq ^{186}Re (red)	1	83	75
AH — HIgG	74 MBq ^{186}Re (red)	1	92	84
AA — HIgG + $SnCl_2$	74 MBq $^{186}ReO_4^-$	22	93	87
AH** — HIgG + $SnCl_2$	74 MBq $^{186}ReO_4^-$	22	95	96

**AA = ascorbic acid*
***AH = active hydrogen.*

2.6. Quality Control

The samples of HIgG-^{186}Re were analysed by Whatman 1 paper radiochromatography and colour reveal test (in 2% ninhydrine ethanol solution). The used solvents and the R_f values are inserted in TABLE V.

TABLE V. THE VALUES OF R_F (RADIOACTIVE AND NINHYDRINE SPOT TEST)

Sample	Solvent	
	ethanol	ethanol:NH_3:H_2O (2:1:5)
HIgG — ^{186}Re	0.00 -0.08	0.84–1.00
$^{186}ReO_4^-$	0.30–0.50	0.77–0.84
Re (red)	0.00	0.00–0.08

The labelled immunoglobulin was analysed by the UV gel chromatography method, too. The Sephadex G-25 columns (0.3 × 15 cm) and elution buffer 0.1 M $NaHCO_3$ in 0.15 M NaCl [2] were used. 100 μl HIgG-^{186}Re solution were loaded on each gel column and eluted with buffer. Fractions of 200 μl in volume were collected and monitored spectrophotometrically at 280 nm. Concomitant

radioactive measurements were effected to a gamma counter. FIG. 2 represents the counting speed (——B curve) and the extinction (—•—C curve) for 14 HIgG-^{186}Re samples.

We see that for the same sample fraction (no 8 in FIG. 2) there are a spectrophotometrical absorption maximum and a radioactivity distribution maximum. The determined radioactive purity was 90–92% for HIgG prereduced samples (no 1 and no 4, TABLE III) with ascorbic acid and native hydrogen (active).

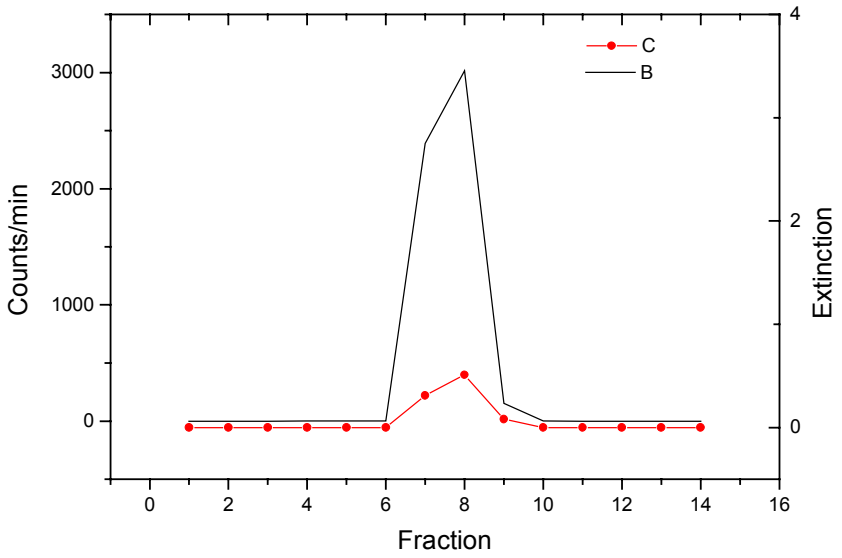

FIG. 2. The radioactive (——B) and UV absorbtion (—•—C) measurements of gel chromatography fractions.

3. DISCUSSION

The reducing agents used in HIgG prereduction are specific for the electronic transfer reactions —S—S— + $2H^+$ (low acid) → —SH + —SH. The parameters which have drastic influence on the labelling yield are: temperature and incubation time, masic ratio HIgG/reducing agent, self radiolyse effect.

For all the studied probes a difficulty in the obtaining of HIgG-^{186}Re was observed. The reducing of perrhenate to inferior oxidation states [5] necessities the increasing of Sn^{2+} quantity. The low acid pHencourages both the prereduction reaction of HIgG and the labelling reaction of ^{186}Re. After the forming and stabilisation of the HIgG-^{186}Re molecule, the increasing of pHto 7 doesn't influence the stability of labelled molecule.

4. CONCLUSIONS

HIgG can be labelled with ^{186}Re by direct method in prereduction conditions with ascorbic acid or active hydrogen. The molar ratio 1.6:1of ^{186}Re:HIgG used in labelling process is favourable for a high labelling yield. In all this study it doesn't observe aggregation phenomena of HIgG-^{186}Re.

That research work was accomplished with the intention of obtaining a kit for labelling with ^{186}Re. The direct labelling technique encourages the achievement of this kit.

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TRIALS TO OPTIMIZE DOSIMETRY FOR ^{153}Sm-EDTMP THERAPY TO IMPROVE THERAPEUTIC EFFECTS

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Abstract. In a trial to improve results of therapy with ^{153}Sm-EDTMP for pain control in patients with disseminated bone metastases dosimetric studies were performed. Out of 30 treated patients 8 were selected for the study at random (5 breast Ca., 3 prostate Ca.). Whole body retention (WBR) of ^{99m}Tc-DPD and ^{99m}Tc-EDTMP was compared with WBR of ^{153}Sm-EDTMP. Volume of metastases and regional ^{99m}Tc-phosphonate uptake were assessed by SPECT and conjugated whole body scan data after phantom studies. Effective half-life was estimated also. Clinically results of pain control, side effects and changes of in vitro parameters were followed after therapy for up to 8 months. Therapy was performed in these patients with 55,5 MBq/kg body weight. Results showed an identical pattern of radioactivity distribution on ^{99m}Tc-phosphonate and ^{153}Sm-EDTMP posttherapy scans, WBR of tracers and therapeutic agent was similar. Tumour volumes were 151–652 mL, count ratios metastases/normal bone 1,72–2,41, so that 6–50% of applied ^{153}Sm-EDTMP were concentrated in bone lesions. This gave dose estimates of 2,8–13,7 Gy in metastases. Evaluation of clinical results showed that the majority of very good results were observed in patients receiving > 10 Gy (n = 3) while with lower doses only 1/4 responded very well. 1 patient was lost to follow-up due to death in the first month after therapy. Moderate and transient myelodepression (platelets) was seen in 3/7 patients without relation to Gy applied. As obviously ^{153}Sm concentration is not homogenous in bone metastases it can be assumed, that in border zones between tumour and bone 30–40 Gy can be delivered when 10 Gy are calculated for the whole lesion, which would explain the satisfactory therapeutic effect in our study. The dosimetric approach to ^{153}Sm-EDTMP therapy could necessitate the application of higher amounts of ^{153}Sm-EDTMP to reach adequate radiation doses in lesions without necessarily increasing risk of myelodepression and with even better clinical results.

1. INTRODUCTION

^{153}Sm-EDTMP is used since years for pain reduction in patients with disseminated bone metastases (1), recently it has also been applied for therapy of inflammatory joint diseases (2). Satisfactory results were reported in 66–80% of treated cases, the radiopharmaceutical was applied in „doses“ of MBq/kg body weight. Considering the different extent of bone lesions and varying uptake patterns the differing and not optimal response rates could be possibly improved by an individually performed dosimetry before therapy.

2. MATERIAL AND METHODS

Overall 30 patients were treated with ^{153}Sm-EDTMP since 1996 (Table I).

Pain and mobility situations were scored before and after treatment (Table II). Diagnosis was assessed by bone scans (^{99m}Tc-DPD, 555 MBq), bone x-ray films, histology of the primary tumour and tumour marker assays (CEA, CA 15–3, SCCA, PSA). Blood counts, liver enzymes (especially alkaline phosphatase), electrolytes, creatinine and ESR were registered before and after therapy according to a well-defined follow-up program (Table III). ^{153}Sm-EDTMP (Quadramet CIS-BIO) was still applied in amounts of 28,0 MBq to 67,5 MBq/kg body weight, with a mean total activity of 2971 MBq (Table IV). Whole body retention of ^{99m}Tc-DPD and ^{153}Sm-EDTMP was indirectly assessed by measuring

24 h urine activity. 8 of the patients were selected for detailed studies to define tumour volume, uptake of either ^{99m}Tc-DPD or ^{99m}Tc-EDTMP in bone lesions and biological half-life of the tracers in metastases receiving 55 MBq ^{153}Sm-EDTMP/kg. For this purpose they had SPECT of relevant body regions with a dual head y-camera (Helix Elscint) with LEAP collimators, where lesion volumes were estimated by comparison with pixel numbers from studies with a Jaszak-phantom using different target volumes and comparable count rates (Fig. 1).

TABLE I. PATIENT MATERIAL

Prostate Ca.	10	males 13/females 17	
Breast Ca.	15	age $\overline{X}$ 67,0 yr. (23–81)	
NSCLC	2	weight 48–84 kg	
Carcinoid	1	bone scan index $\overline{X}$ 56,4	
CUP	1	Mo.	18
Lymphangiosarc.	1	Mo. + NSA	8
		NSA	4

NSCLC = non small lung cancer *CUP = cancer unknown primary*
Mo. = continuous morphine medication *NSA = non steroidal analgesics.*

TABLE II. SCALE FOR CLINICAL EVALUATION OF EFFECT OF NUCLEAR MEDICINE THERAPY OF DISSEMINATED BONE METASTASES

Degree of pain

I. needs morphine or similar alkaloids 3 x/day
II. needs morphine or similar alkaloid 1 x/day in addition Diclofenac or similar preparation
III. needs Diclofenac or similar preparation 2–3 x/day
IV. no analgesics necessary

Degree of mobility

A. bed-ridden
B. can walk with assistance
C. can walk without assistance, yet mobility somewhat impaired
D. can move freely

TABLE III. FOLLOW-UP PROGRAM

- 3, 6, 12 weeks after therapy, then 5, 8, 12 months after therapy.
- Parameters obtained: history (pain/mobility scale, pain diary, additional therapy).
- Blood count, ESR, tumour markers, UN, Creatinine, Ca, P, liver parameters.
- Bone scans 3 months, 6 months, 12 months after therapy.
- bone radiology 5, 12 months after therapy.

TABLE IV. "DOSAGE" AND WHOLE BODY RETENTION

"Dosage"	Applied activity	Retained activity
37 MBq/kg (8)	$\overline{X}$ 2.971,2	$\overline{X}$ 68,6%
55 MBq/kg (20)	SD 898,0	SD 0,13%
28 MBq/kg (1)		
67 MBq/kg (1)		

To assess regional uptake also these SPECT data were used but also data on relative retention of activity in the whole body, in the normal skeleton and in kidneys using conjugated views of whole-body scans (Fig. 2). These data allowed to estimate radiation dose to bone metastases according to the standard MIRD-formula:

$$Gy = \frac{\text{activity(MBq)}}{\text{volume(ml)}} \times \text{residence time (t)} \times \text{s (mGy/MBq/s.)}$$

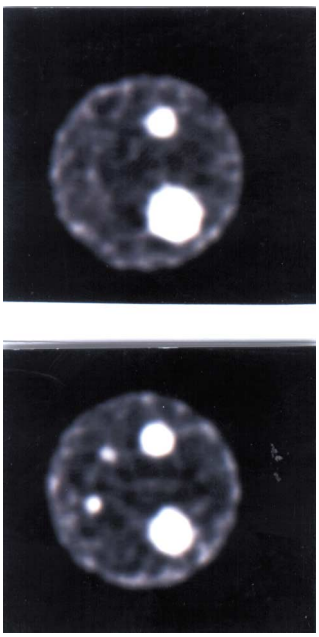

FIG. 1. SPECT-slices of Jaszak-Phantom with ^{99m}Tc "hot spots" 125 mL, 20 mL, 6 mL, 2 mL. Count rate lesion/background= 3/1, background activity 37 kBq/mL, lesion 111kBq/mL.

TABLE V. ESTIMATED TUMOUR VOLUMES, ACTIVITY IN TUMOUR AND RELATION TO WHOLE BODY RETENTION (WBR).

Retained activity in tumour of WBR		**Retained activity in skeleton of WBR**	**Retained activity /10 mL Tu. (MBq)**	**Tu.-Volume (mL)**
$\overline{X}$	22,2%	84,7%	13,8	363
Range SD	15–55%	76,7–88,0%	5,1–25,4	151–652

TABLE VI. RESULTS DOSIMETRIC THERAPY TRIAL WITH ^{153}SM-EDTMP

Pat.	Ca.type	MBq applied	Tu. volume (mL)	Gy	Effect	Side effects
S.B.	Breast	4.958	410	4,0	++	(+)
B.R.	Prostate	3.811	226	9,2	?	?
D.A.	Prostate	3.441	503	5,1	+	0
S.C.	Prostate	3.885	652	13,7	++	0
P.E.	Breast	3.380	421	10,0	++	(+)
H.D.	Breast	2.793	350	11,0	++	0
A.G.	Breast	2.960	302	6,0	+	0
H.O.	Breast	2.479	151	2,8	+	(+)

++ = good + = satisfactory ? = no follow up (+) moderate, transient thrombopenia

TABLE VII. IN VITRO CHANGES AFTER THERAPY

Thrombocytopenia	<70 000	= 0/8	< 100 000 = 3/8
Leukopenia	<2 000	= 1/8	
alkaline Phosphatase ↓		= 6/8	
Tu.-markers ↓		= 3/8	
ESR ↓		= 1/8	

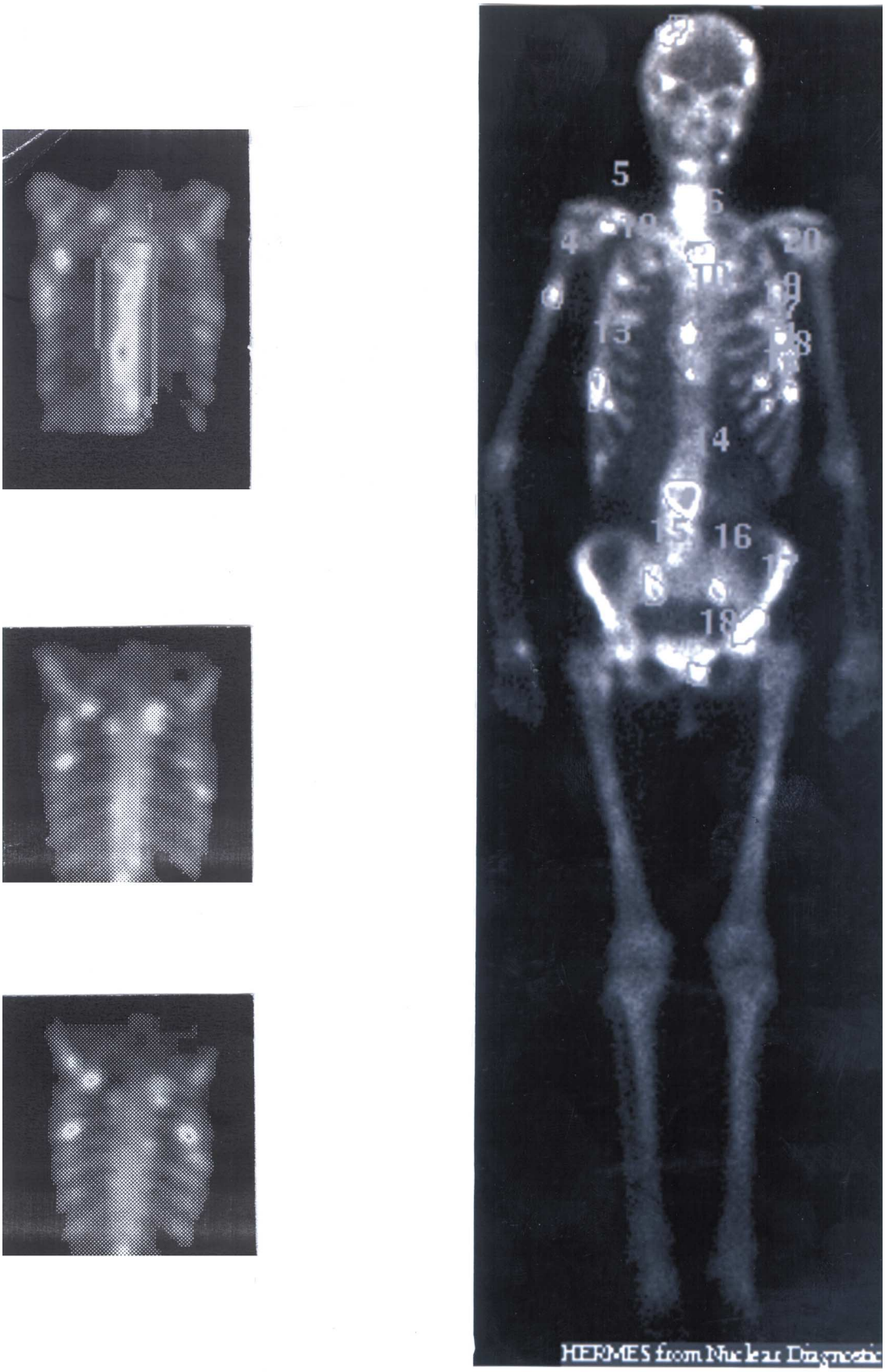

FIG. 2. ***Left****: SPECT-slices after* ^{99m}*Tc-DPD with ROI over spinal and rib metastases permitting by addition estimate of tumour volume.* ***Right****: Whole body scan with ROI over whole body and metastases permitting estimate of radioactivity concentration in tumour.*

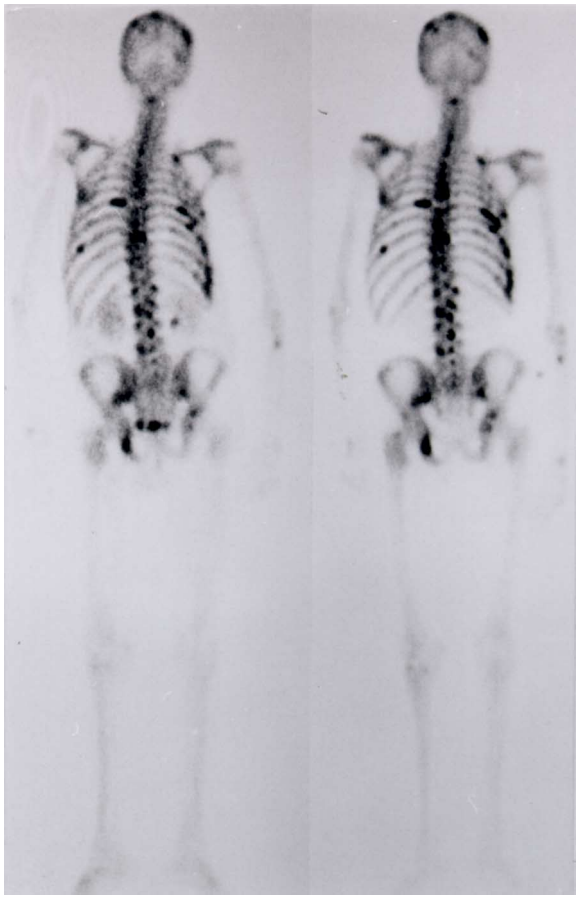

FIG. 3. Identical uptake of ^{153}Sm- and ^{99m}Tc-phosphanate (^{153}Sm left, ^{99m}Tc right) in disseminated bone metastases of breast cancer.

Results of therapy were classified as “very good = ++”, meaning in improvement of > 2 points of the pain/mobility score, “good = +” when improvement of the score was 1–2 points, “negative = - ” when no improvement was registered. “No follow-up possible = ?” refers to patients which died before 1 month after therapy. These deaths were never due to complications of ^{153}Sm-therapy. Side effects (thrombocytopenia, leukopenia) were also noted as well as changes in tumour marker values, alkaline phosphatase and calcemia.

3. RESULTS

Twenty-four h urine excretion and therefore whole body retention was similar for ^{99m}Tc-phosphonates and ^{153}Sm-EDTMP (70,5 ± 0,2%, vs. 68,2 ± 0,1%). Estimated tumour volumes, however, varied considerably as well as count rates over tumour and normal bone which also gave differing values for uptake of the radionuclides in tumour metastases (Table V). Post-therapy scans showed identical uptake patterns for ^{99m}Tc-phosphonates and ^{153}Sm-EDTMP (Fig. 3). Analyzing the data from dose estimates it appears that patients receiving > 10 Gy to the tumour had in general a better result than those with lower doses (Table VI). Bone marrow toxicity was moderate, never required specific therapy, was transient and obviously not related to radiation dose in the tumour. Changes of other in vitro parameters are shown in Table VII.

4. DISCUSSION

The results of our dosimetric approach to ^{153}Sm-EDTMP therapy in 8 patients should obviously be considered as preliminary. They justify, however, more detailed studies concerning dosimetry of ^{153}Sm-EDTMP therapy (3). Obviously it is incorrect to assume a homogenous uptake of ^{153}Sm-EDTMP in bone metastases (4). There is evidence that labelled phosphonates accumulate in border zones between tumour tissue and bone, where an intense activity of osteoblasts can be observed. This could mean that the 640–810 keV of the ß$^-$- radiation of ^{153}Sm affects especially these zones and scarcely the center of a large metastasis. On the other hand the radiation dose to the border zone must be obviously much higher than a dose estimate involving the total volume of a metastasis. It can be assumed that in these border zones with high ^{153}Sm-accumulation local doses of at least 30–40 Gy can be achieved with an average dose to the whole lesion of 10 Gy. It would seem logical to use the high energy ß$^-$-radiation of ^{90}Y-EDTMP (5) for the treatment of metastases with a large volume, as a therapeutic effect could be expected also in deeper layers of the tumour tissue. We know that 40 Gy can achieve remissions in radiotherapy of cancer (6) and we should therefore try to reach at least 15 Gy in the total tumour volume. Whether such increased doses requiring larger amounts of ^{153}Sm-EDTMP can be applied safely should be assessed by prospective studies. Applied activities of 74 MBq/kg body weight ^{153}Sm-EDTMP and more were reported but the somewhat increased myelotoxicity of such strategies was not analysed concerning it's relationship to tumour dose (7). We hope therefore that adequate dosimetry of ^{153}Sm therapy can further improve it's results and that similar approaches will become possible also for other forms of radionuclide therapy.

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RADIOPHARMACEUTICALS OF DTPA, DMSA AND EDTA LABELLED WITH HOLMIUM-166

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Abstract. DTPA, DMSA and EDTA were labelled with ^{166}Ho of low specific activity, 250–275mCi/mg of Ho, produced from holmium oxide by the ^{165}Ho(n, g) ^{166}Ho reaction at a neutron flux of about 10^{14} n.cm^{-2}.s^{-1}. Three pHranges were selected in the study, viz. 2–3, 3–3.5 and 5–6. The labelling reactions were studied as chloride and nitrate solutions in aqueous and saline media at 30 min and 20–24 h of reaction. DTPA was labelled over 99, DMSA at about 90 and EDTA at 100.0% with ^{166}Ho. The complexes were found stable at all times of investigation. A beta chromatogram scanner was used to study by TLC the labelling reactions of DTPA and DMSA with the nuclide and by PC those of EDTA. A biodistribution study in three rats injected intravenous with a saline solution of $^{166}HoCl_3$[DTPA] at pH5.1 showed an initial uptake in blood, kidney and lung after 30 minutes. After four hours the complex was found to have cleared from blood and lung, and localized 100% in kidney. It was stable *in vivo* in the kidney after 24 hours. The g spectrum analysis did not show the formation of any impurity except the four characteristic g energies of ^{166}Ho.

1. INTRODUCTION

^{166}Ho is used in nuclear medicine for the therapy of arthritis by radiation synovectomy, for bone marrow ablation, and in the study of immunospecific radiopharmaceuticals. Its g energy of 80.5 keV (6.71%) and 1379.40keV (0.39%) is suitable for imaging by a gamma camera. ^{166}Ho has a half-life of 26.4 h and decays to a stable daughter, ^{166}Er. The high b^- energy of 1854.5keV (50%), 1773.93keV (48.7%), 394.57keV (0.95%) and 192.26keV (0.30%) holds potential for its application as a therapeutic reagent.

Dadachova *et al.* (1997, 1994) investigated the production and *in vivo* localization in tissue of ^{166}Ho through the b^- decay of the ^{166}Dy/^{166}Ho generator. Smith *et al.* (1995, 1994) found that the [^{166}Dy]Dy/^{166}Ho-DTPA complex formed by the b^- decay of ^{166}Dy did not show any *in vitro* or *in vivo* translocation of the daughter nucleus following localization of the parent at the target site.

Park *et al.* (1996) prepared macro-aggregates of ^{165}Dy and ^{166}Ho by the neutron irradiation of ^{164}Dy-MA. Injection of the macro-aggregates in the knee joint of rabbits showed high *in vivo* retention (>99.5%) at 24 h and 10 days, respectively.

^{166}Ho-EDTMP (ethylene-diamine-tetramethylene phosphonic acid) has proven to be a useful palliative in the therapeutic treatment of bone cancer of human beings (Achando *et al.* 1995). Biodistribution in rats and mice showed a high skeletal uptake, a fast blood clearance and a low soft-tissue uptake.

Turner *et al.* (1994) used the 81keV-gamma emission of ^{166}Ho to determine by SPECT imaging the beta dose absorbed by normal liver of pigs administered with ^{166}Ho micro-spheres in intra-hepatic artery. The study demonstrated the feasibility by SPECT dosimetry of controlling the radiation dose absorbed by critical normal organs from a tracer dose of ^{166}Ho micro-spheres.

Dadachova *et al.* (1994) utilized the reversed-phase LC and HPLC ion exchange chromatography for the separation of carrier-free ^{166}Ho from neutron-irradiated $^{164}Dy_2O_3$ target that produced ^{164}Dy[n, g] ^{165}Dy[n, g] ^{166}Dy by the double neutron capture reaction. The radiochemical yield of carrier-free ^{166}Ho was 95% with a dysprosium breakthrough of <0.1%.

Nijsen *et al.* (1994) produced polylactic acid micro-spheres (PLA-MS) containing neutron-irradiated ^{166}Ho for the therapy of hepatic malignancy. Irradiation of samples in a high-flux reactor did not show any measurable release of ^{166}Ho from the micro-spheres after 144 h.

Shortkroff *et al.* (1994) administered non-radioactive holmium-hydroxyapatite (Ho-HA) particles and non-labelled HA to the stifles of normal rabbits (n = 18) to determine any adverse effect of the particle on the joint and cartilage. Gross inspection of the synovial pouch in the joints of all samples, dissected 4,6,9 and 13 weeks post-injection, indicated a very slight inflammatory response to the agent. Subsequent analyses confirmed this observation with the additional finding that most of the particles in all samples were absent from the joint after six weeks.

In the present study DTPA, DMSA and EDTA were labelled in the acid range with ^{166}Ho of low specific activity, 250–275mCi/mg of Ho, produced from holmium oxide by the $^{165}Ho(n, g)\ ^{166}Ho$ reaction. The organic precursors were labelled with $^{166}HoCl_3$ at pH2–3, 5–6 and with $^{166}Ho(NO_3)_3$ at 3–3.5.

2. MATERIALS AND METHODS

Materials

Holmium oxide, Ho_2O_3 > 99.9% (Fluka)
DTPA (Dotite)
DMSA (Sigma)
EDTA, disodium salt dihydrate (Dotite)
HCl (20%), GR
NaOH, GR
$NaNO_3$, GR
Chelex A 100 resin, Na^+ form, 100–200 mesh, GR (Bio Rad)
Pyridine (Merck)
Ethanol, GR (Merck)
0.9% NaCl (normal saline)
Cellulose plate (Merck)
Whatman no.1 paper
Membrane filter, 0.22 mm
Chromatograph scanner, AMBIS 100, Perkin Elmer
Freeze drier

Ultra pure water was used in the preparation of solutions and in the rinsing of glassware

The target was irradiated in the hydraulic rabbit (position 2) of the JRR-3 reactor at Tokai-Mura.

Method

A. Preparation of complex

1 mg of holmium oxide was sealed in quartz by flame, welded into aluminium container and irradiated for 30 min at 8.87–9.14 × 10^{13} $n.cm^{-2}.s^{-1}$. After irradiation the target was cooled for 25–48 h and transferred to a hot cell where it was de-canned and taken out of the quartz for processing.

The irradiated holmium oxide was dissolved with 2 mL of HCl (20%) in a beaker and evaporated to dryness on a hot plate. The dried mass was cooled to room temperature and re-dissolved by the addition of 10 mL of water/saline. The $^{166}HoCl_3$ solution thus prepared was now removed to a fume cupboard shielded temporarily with lead bricks. It was the mother stock for all labelling experiments.

I. Chloride complex: A lambda-pipette was used to transfer to the beaker aliquots of the stock followed by the addition of a little water/saline. DTPA and DMSA were added as solid, 20 times molar excess of $^{166}HoCl_3$, and EDTA as an aqueous solution, 12 times excess. The solutions containing DTPA and DMSA were made up to a volume of 10 mL, warmed a little on a hot plate till dissolution of the precursor.

II. Nitrate complex: An aliquot of the stock solution was treated with a slight excess of sodium nitrate solution to convert $^{166}HoCl_3$ to $^{166}Ho(NO_3)_3$. The nitrate complex was then processed with the precursors as stated earlier using water/saline to make up the volume. III. Blank: An aliquot from the mother stock was made up to volume with water/saline to prepare blank of $^{166}HoCl_3$. Likewise, to another aliquot of $^{166}HoCl_3$ a slight excess of sodium nitrate solution was added and made up to volume with water or saline to prepare blank of $^{166}Ho(NO_3)_3$. No precursor was added to any blank.

The pHof the clear, colourless labelled solutions and that of the blanks was now adjusted with HCl and NaOH and measured on a pHmeter. The complexes prepared at pH2–3 and 5–6 were those of chloride, and at 3–3.5 those of nitrate. The blanks were prepared at pH3–3.5 and 5–6, none at 2–3. A residence time of 30 min was allowed to the complexes and blanks after pHadjustment.

In the first experiment with DTPA half of the labelled complexes was eluted through Chelex A 100 cationic resin, Na^+ form, 100–200 mesh, in order to separate the labelled and unlabelled ^{166}Ho. The columns (2.5 cm × 1 cm) were equilibrated with ultra pure water before elution. In the succeeding experiments blanks were used instead of column elution to compare percent labelling of the complexes with that of the blanks.

B. Radiochemical purity

After elapse of the residence time, the chloride and nitrate complexes of DTPA and DMSA, 10 mL, along with their blanks, were spotted on cellulose plates (2.5 cm × 20 cm) for thin-layer chromatography, and those of EDTA, after 40–50 min, on Whatman no.1 for ascending paper chromatography. The spots were developed in a solvent of pyridine-ethanol-water (1:2:4) for about two and half h, dried in the air and analyzed by a chromatogram scanner, AMBIS 100, to determine percent labelling and thus radiochemical purity of the complexes. Scanning was followed up 20–24 h later to find stability of the solutions. The complexes were not studied any further. The typical radiochromatogram is shown in Fig. 1. (^{166}Ho DTPA in chloride medium) and in table 1.

C. Biodistribution

All glassware including serum bottles, beakers, pipettes, volumetric flasks, stirring rods, etc. were cleansed in an ultrasonic cleaner, washed under a tap and rinsed with ultra pure water. After cleansing, the glassware was wrapped in aluminium foil, dried in an oven at 220^o C for four hours and stored at -20^o C in a deep freeze. Rubber stoppers were sterilized by gamma radiation at a dose of 3.5krad × 1 h.

Assuming a 1:1 $HoCl_3$: DTPA complex 8 mL of a saline solution of $HoCl_3$ [DTPA] of molar strength 0.006226m mole/mL was prepared in a non-irradiated state. The pHof the solution was adjusted at 5.1, and after 30 min of reaction it was dispensed in sterilized serum bottles by filtering through 0.22-mm filters.

An hour after its pHadjustment 200 mL of the apyrogenic solution/kg body weight was injected into the tail vein of three SD rats, six weeks old, from Charles River, supplied by the Daiichi Chemical Company. The rats were sampled 30 min, 4 h and 24 h, respectively, post injection. The target organs were freeze-dried in a lyophilizer at the end of sampling. Weighed amounts of the target organs were irradiated for 10 min at a flux of 5.8×10^{13} $n.cm^{-2}.s^{-1}$. Fig. 2 shows the bio-distribution results.

TABLE I

Name of complex/ plate number	*pH*	*% of total labelled 30 min*	*R_f 30 min*	*CPM,% 30 min*
^{166}Ho[DTPA]$^{(a)}$		**RAH-5**	**RAH-5**	**RAH-5**
$^{166}HoCl_3$ blank/(1)	5.75	98.5	0.02	33.78
$^{166}HoCl_3$[DTPA]/(2)	5.7	98.8	0.87	32.58
$^{166}HoCl_3$[DTPA]/(3)	2.3	99.4	0.90	33.64
^{166}Ho[DTPA]		**RAH-6**	**RAH-6**	**RAH-6**
$^{166}HoCl_3$ blank	5.75	97.8	0.02	32.87
$^{166}Ho(NO_3)_3$ blank	3.5	99.3	0.01	30.70
$^{166}Ho(NO_3)_3$[DTPA]	3.1	99.3	0.90	36.43
^{166}Ho[DMSA] and ^{166}Ho[DTPA]		**RAH-14**	**RAH-14**	**RAH-14**
$^{166}HoCl_3$[DMSA]	5.7	15.1, 84.5	0.01, 0.91	29.98
$^{166}HoCl_3$[DMSA]	2.1	9.9, 43.6, 46.6	0.00, 0.81, 0.91	27.35
$^{166}Ho(NO_3)_3$[DMSA]	3.1	9.5, 90.1	N/A, 0.91	24.43
$^{166}Ho(NO_3)_3$ blank	3.0	47.5, 26.6, 25.9	0.06, 0.81, 0.93	18.24
^{166}Ho[DMSA] and ^{166}Ho[DTPA]		**RAH-15**	**RAH-15**	**RAH-15**
$^{166}HoCl_3$[DTPA]*	5.3	82.6, 17.1	0.73, 0.90	31.20
$^{166}HoCl_3$ blank*	5.5	60.5, 39.5	0.16, 0.70	36.73
$^{166}HoCl_3$ blank	5.5	54.2, 45.4	0.18, 0.86	32.07
^{166}Ho[EDTA]		**RAH-32**	**RAH-32**	**RAH-32**
$^{166}HoCl_3$[EDTA]	5.5	99.9	0.78	34.99
$^{166}HoCl_3$[EDTA]	2.9	99.7	0.79	21.92
$^{166}HoCl_3$ blank	5.5	97.0	0.00	20.98
$^{166}Ho(NO_3)_3$[EDTA]	3.5	99.2	0.77	22.11
^{166}Ho[EDTA]		**RAH-33**	**RAH-33**	**RAH-33**
$^{166}Ho(NO_3)_3$ blank	3.5	97.8	0.00	35.90
$^{166}HoCl_3$[EDTA]*	5.3	100.0	0.77	40.35
$^{166}HoCl_3$ blank*	5.3	96.6	0.02	23.75

Note: (a) Plate, shown in Fig. 1, has been marked (1), (2), (3) from left to right.
** Saline medium*

D. Radionuclidic purity

To determine its radionuclidic quality a 5-mL aliquot of an aqueous solution of $^{166}HoCl_3$ was analyzed by an HPGe detector. Spectral analysis of the sample showed four characteristic gamma energies of ^{166}Ho. The results did not show any other nuclide impurity.

3. RESULTS AND DISCUSSION

The table and the figures presented herewith show a few TLC and PC chromatograms/plates out of a large number of experiments. The percent labelled at 20–24 h of reaction was about the same as at 30 min, which were over 99% for DTPA, about 90% for DMSA and 100.0% for EDTA.

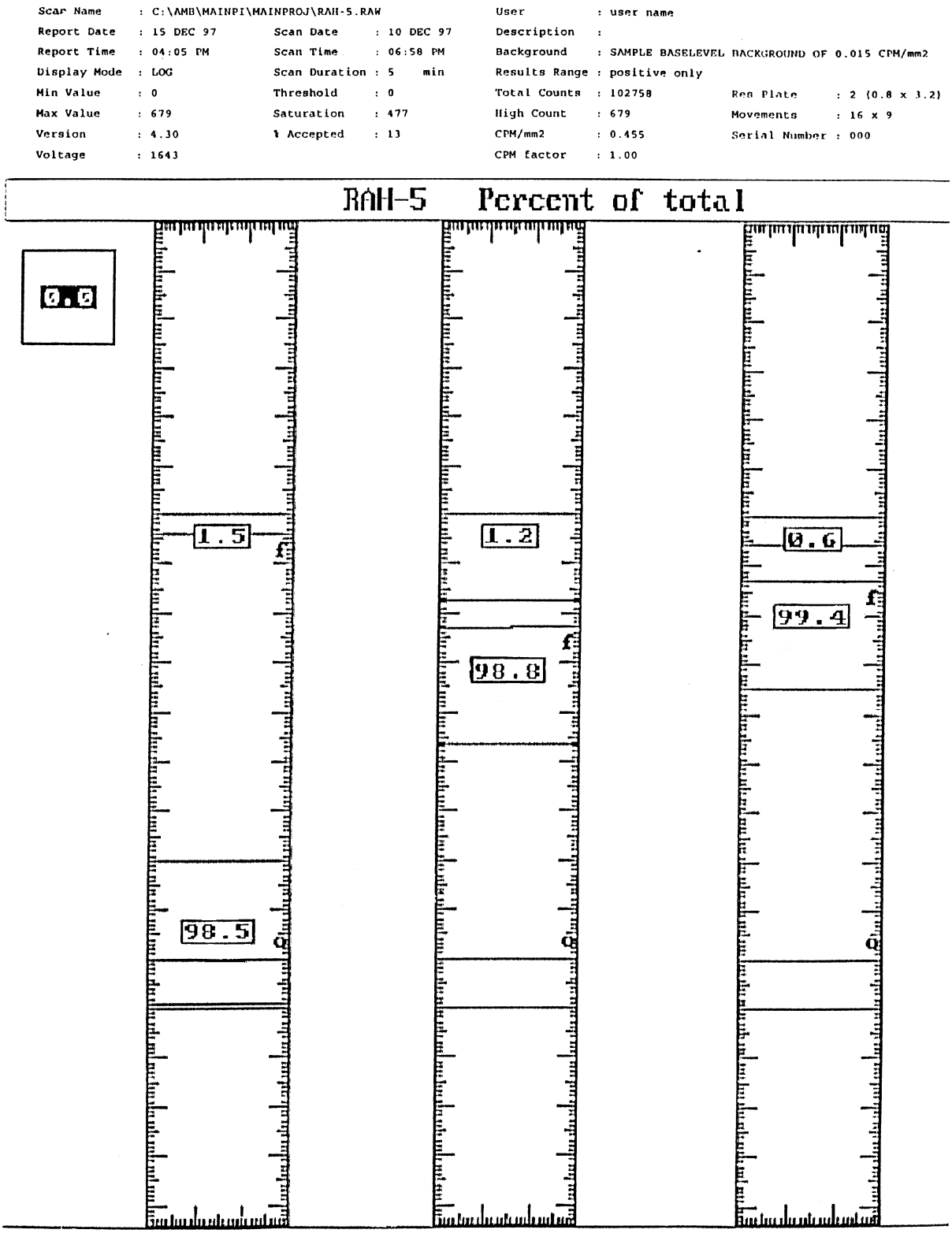

FIG. 1. ^{166}Ho[DTPA] in the chloride medium at 30 m of reaction. The plates (l or r) are at pH5.75 ($^{166}HoCl_3$ blank), 5.7 ($^{166}HoCl_3$[DTPA]) and 2.3 ($^{166}HoCl_3$[DTPA]), respectively.

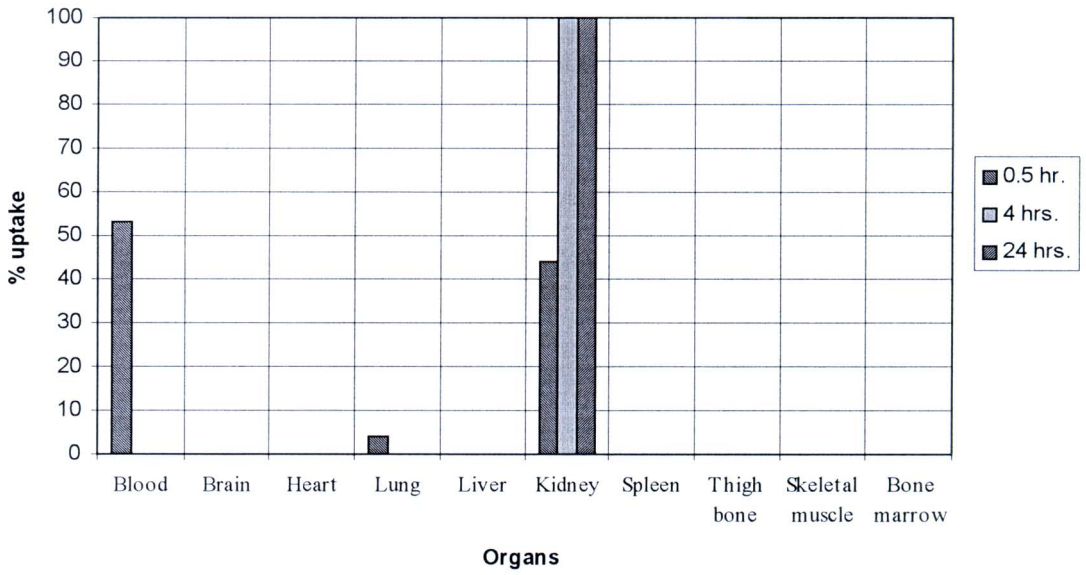

FIG. 2. Biodistribution in rats of $^{166}HoCl_3$[DTPA] at pH5.1

In addition to percent labelling the AMBIS 100 scanner yielded as output a two-dimensional quantification of statistical data such as R_f, CPM, etc. of the plates. The table shows these values. CPM has been expressed as a percentage after processing of data.

It is seen from the figures that DTPA and EDTA were labelled with ^{166}Ho at a high efficiency. Stability of the complexes after a residence time of 20–24 h was good. The table shows the R_f to be high, as also the *inter se* distribution of CPM is good.

In the first experiment with DTPA the stock and the solution obtained by elution through Chelex A 100 resin showed near identical labelling indicating that DTPA had formed complex with almost the entire ^{166}Ho available, and, therefore, there was no unbound ^{166}Ho. In the latter experiments blanks rather than eluted solutions were used for inter-comparison of the labelling efficiency. Dadachova *et al.* (1997) experimented with Chelex 100 resin, NH_4^+ form, for separating Ca and Fe impurities from a-HIBA they used for elution of ^{166}Ho. Applebaum *et al.* (1988) separated labelled and unlabelled ^{153}Sm with Sephadex C25 resin.

DMSA formed more than one complex with ^{166}Ho none of which was characterized. The labelling efficiency of DMSA varied widely going to a maximum of 90% with $^{166}Ho(NO_3)_3$[DTPA] at pH3.1 after 30 min of residence time. At this stage of investigation no explanation is offered for the irreproducible behaviour although some of the complexes had high R_f (partly shown in the table).

The blanks, which are potential radiopharmaceuticals, showed indifferent behaviour. The TLC and PC analyses revealed that the blanks in the DMSA experiments had greater movement up the solvent front than the blanks of DTPA or EDTA. The percent labelled was higher at 20–24 h of reaction than at 30 min.

Fig. 2 shows that after an initial localization in blood, kidney and lung, the uptake after 60 min of residence time of $^{166}HoCl_3$[DTPA] at pH5.1 was 100% in kidney at 4 h and 24 h of sampling thereby showing good *in vivo* retention of the complex. The solution was too dilute to be detected in other organs. Faeces and urine of the rats were not collected which imposed restriction on detecting how soon the complex was cleared from the body.

The gamma spectrum shows the nuclide to be of high purity.

4. CONCLUSION

All the complexes need to be characterized. Investigation has to be made on localization of the $^{166}HoCl_3$[DTPA] complex in organs other than those shown in the study. Both *in vitro* and *in vivo* stability of the complexes beyond 24 h is to be studied. The EDTA complex of ^{166}Ho holds promise for further investigation. It is obvious that radiochemical and radionuclidic purity of the complexes, particularly those of DTPA and EDTA is high. A relatively simple process technology together with high labelling efficiency seems to make further study of the complexes attractive.

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STUDIES ON THE PREPARATION AND EVALUATION OF COLLOIDAL CHROMIC PHOSPHATE — ^{32}P FOR POSSIBLE THERAPEUTIC USE

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Abstract. Radionuclide therapy has become the focus of recent attention in nuclear medicine, thanks to the emergence of new therapeutic radionuclides as well as the known prospects of local instillation approach and the exciting promise of targeted therapy concept. This has naturally led to a revived interest in the use of established products of earlier generation also, for example ^{32}P compounds. In response to such a demand of nuclear medicine physicians in India, ^{32}P labelled colloidal chromic phosphate suspension (CCPS) was prepared by suitable modifications to a reported procedure. ^{51}Cr was used as tracer for initial studies of standardisation, in order to avail the benefits of relatively greater ease and higher efficiency of assay of gamma activity at low levels. Recovery of the colloid and purification were accomplished by dialysis leading to about 60% radiochemical (RC) yield. The RC purity of the CCPS formulated in 30% dextrose solution was over 98% as assessed by paper chromatography. The particle size was below 5μM, with nearly 99% of the particles present in the size range of 0.6–2.5 μM. The stability of the colloid was found to be not less than 7 days, in terms of soluble phosphate content of the CCPS. The consistency of biological behaviour of CCPS was attempted to be studied by i.v. administration in test animals, although the envisaged end use is only by local instillation. The animal studies revealed prominent lung uptake (~70%) indicating the presence of >10μM particles formed in vivo, most probably due to agglomeration in serum. The easy reliable preparation of CCPS in acceptable yield, purity and particle size distribution demonstrated in the present study, considered along with the added advantages of abundant, economic availability and convenient production logistics of no-carrier-added ^{32}P, would merit further investigations on CCPS and similar *M(III)-phosphate colloids for possible therapeutic applications.

1. INTRODUCTION

Radionuclide therapy has become the focus of recent attention in nuclear medicine [1,2]. This has been mainly due to the emergence of new therapeutic radionuclides in conjunction with the known prospects of local instillation approach and the exciting promise of targeted therapy concept. This has naturally led to a revived interest in the use of established products of earlier generation also, for example ^{32}P compounds [3]. There are a number of articles discussing the requirements of therapeutic radionuclides for different applications [4,5]. Mostly pure beta emitters such as ^{32}P, ^{89}Sr, ^{90}Y (Table-I) and ß,γ emitters such as ^{153}Sm, ^{166}Ho, $^{186/188}$Re are being used. The latter group of radionuclides have rightly attracted more attention, in view of suitable energy of beta emissions combined with imaging capability using the gamma emissions accompanying the beta particles. However, if it is required to use radionuclides bereft of accompanying gammas for any therapy, the choice would be confined to the nuclides shown in Table-I. Amongst these, ^{32}P offers the attractive features of easy abundant availability at relatively very low cost, convenience of production logistics and reasonably satisfactory radiation characteristics (Table-I). ^{90}Y has been the focus of much attention, while ^{143}Pr has good potential for use.

^{32}P is a proven therapeutic radionuclide used extensively in the past, especially as a radiocolloid for treatment of pleural and peritoneal effusions secondary to primary malignancy [1,6,7]. There is a good amount of literature on utilisation of colloidal chromic phosphate–^{32}P (CCPS) for such applications. CCPS has been suggested for use in oncology, for example, as an effective adjuvant treatment in ovarian malignancy [8], as treatment of choice for malignant pericardial effusions [9] and intraarterial use for prevention of post-operative liver metastases in high risk colorectal cancer patients [10]. CCPS has also been suggested for use in radiation synoviorthesis, for hemophilic arthropathy [11].

TABLE I. PURE BETA EMITTER RADIONUCLIDES FOR THERAPY

Radionuclide & Half-life	E_β /MeV		Tissue range/mm		Route of production & Production logistics	Chemical Characteristics
	Max.	Ave.	Max.	Ave.		
^{32}P, 14.3 d	1.71	0.7	9	3	$^{32}S(n,p)^{32}P$	anionic, soluble; colloidal solution; organic phosphorus compounds — phosphonate as salt/metal chelate
^{89}Sr, 50.5 d	1.46	0.58	8	2.4	$^{88}Sr(n,\gamma)^{89}Sr$ σ (n,γ) = 0.006 b High neutron flux essential	M^{2+}; limited to use as Sr^{++}
^{90}Y, 2.67 d	2.28	0.93	12	~3.5	Decay of $^{90}Sr(T_{1/2}$ 30 y) $^{235}U(n,f)^{90}Sr$; Radioisotope generator provision; Special precautions for ^{90}Y purity.	M^{3+}; versatality, complexes and particulates
^{143}Pr, 13.6 d	1	0.31	~5	~1.1	$^{142}Ce(n,\gamma)\ ^{143}Ce \xrightarrow{\beta^-} {}^{143}Pr$ σ = 0.95b 33h 'NCA'	M^{3+}; versatality, complexes and particulates

Note: ^{89}Sr: 910 keV gamma of very low abundance of ~0.01% has been ignored.

In recent times, the major benefits of radionuclide therapy have been harnessed through palliative treatment of metastatic bone pain in patients suffering from cancer of prostate, lungs and breast [12]. The magnitude of improvement in the quality of life of such cancer patients following radionuclide therapy, has been reported to be phenomenal. The demonstration of large scale success of this approach has emanated from the use of $^{89}SrCl_2$ (Metastron^{TM}). In view of the very high cost of the commercial product Metastron as well as the poor production prospects of ^{89}Sr in medium flux reactors available in developing nations (Table-I), recourse to exploring the possibility of using ^{32}P-phosphate for this purpose was taken up by some investigators, mainly from India. This was later followed by IAEA through a Co-ordinated Research Project (CRP) involving other countries too. Highly promising results were demonstrated in these studies, which, in turn, have led to a resurgence of interest in seeking to use ^{32}P products for other therapeutic applications. The approach involves local instillation, both for proven, long ago established modes like treating pleural/peritoneal lesions, as well as the more recent modes of treating hepatic tumours and for radiation synoviorthesis.

The major drawback of use of ^{32}P reported has been the uptake in bone marrow as phosphate, either directly or from circulating activity following the wash-out/leaching of ^{32}P as a soluble species from the site of instillation of the particulate formulation (e.g. colloidal suspension). The latter also results in irradiation of non-target tissues by the circulating activity. The former criticism has, however, been shown to be much less restrictive, in view of the by now well-proven safety and efficacy of ^{32}P-phosphate treatment of patients of metastatic bone pain. Administration of up to 14mCi ^{32}P in selected group of patients, qualifying in the initial screening for hematopoietic profile criteria, has not shown any unduly high hematopoietic toxicity. The latter criticism of washout of soluble phosphate from colloidal formulations instilled, would by the same argument, become less restrictive than imagined earlier. The levels of radioactivity required for various applications vary significantly; e.g. 1 mCi for radiation synovectomy of knee joint and 0.5 mCi for other joints [11], 10–15 mCi for intrapleural/peritoneal instillation [1] etc. It would be hence useful to generate data

using current techniques in this regard to assess the actual utility of colloidal chromic phosphate-^{32}P suspension at appropriate dose levels and hence suitable investigations are warranted.

In response to such interest recently evinced by nuclear medicine physicians in India for exploring the utility of ^{32}P colloid, a study was undertaken to standardise a method of formulation of ^{32}P labelled colloidal chromic phosphate suspension (CCPS) and its evaluation for possible therapeutic use by local instillation. The method of preparation of CCPS has been described in early literature [13,14]. The method of Anghileri [13] was followed in the present study with suitable modifications to the reported procedure. Our experience is reported in this paper.

2. MATERIALS AND METHODS

^{32}P was produced by fast neutron bombardment by natural sulphur target, followed by dry distillation and purification by ion exchange chromatography and was available as phosphoric acid (no carrier added, specific activity >5000 mCi/mmol) in dilute HCl, ex-stock in our Centre. ^{51}Cr tracer used in our studies was produced by Szilard-Chalmers reaction on K_2CrO_4 target and was available as sodium chromate (~300 μCi/μg) ex-stock in our Centre. Degraded gelatin available as Haemaccel™ (3.5% solution) was used. All the other chemicals used were from standard commercial sources.

Radioactivity measurements were made in a pre-calibrated ionization chamber (NPL, UK) or radioisotope dose calibrators (ECIL, India and CAPINTEC, USA) for samples in μCi/mCi ranges, while a NaI(Tl) scintillation counter was used for assay of activity in chromatographic supports and dialysate samples.

Paper chromatography (PC) with water solvent and Whatman-1 paper support as recommended in USP [15] and dialysis against water (mostly overnight run) were employed for estimation of both RC purity of the radiocolloid and soluble tracer activity.

Preparation of CCPS

The typical procedure for preparation of colloidal chromic phosphate suspension (CCPS) is described below.

As a prelude to the preparation of CCPS-^{32}P, a few batches of CCPS-^{51}Cr were processed and tested. ^{51}Cr as Na_2CrO_4 was doped with 5 mL of carrier H_2CrO_4 (10 mg/mL) and added to 4 mL H_3PO_4 (10mg/mL), followed by 5 mL of water. 1 mL of 3.5% degraded gelatin and 1 mL of Na_2SO_3 (200 mg/mL) were then added. The reaction mixture (RM) was heated to boiling for 2 min and later allowed to cool to ambient temperature. Aliquots were withdrawn for assessment of radiochemical (RC) yield and purity of radiocolloid, as described later under RC purity analysis.

CCPS-^{32}P was prepared using ^{32}P as H_3PO_4 doped with carrier H_3PO_4 and following the same protocol as described above. The cooled RM was divided into 3 aliquots. Pure colloidal chromic phosphate — ^{32}P was recovered by two different processes, viz. dialysis and centrifugation, using two lots. The third lot served as pre-purified reaction mixture for comparative evaluation and stability studies.

1 mL of the RM was placed into the dialysis tubing suspended in a beaker of water and purification processes carried out for about 24 hours. The leaching/wash-out of soluble phosphate from the RM was assessed (as ^{32}P activity) by PC of aliquots taken from the RM in the tube at different time points during the dialysis.

Another aliquot of the RM was first centrifuged, the supernatant removed and its radioactivity estimated. The colloid was washed with water and centrifuged again. The supernatant of the first

washing was removed and an aliquot of radioactivity counted. Washing was repeated four times and the relative efficiency of removal of soluble phosphate-^{32}P was estimated.

The purified product obtained by both the methods was formulated in 30% dextrose as suggested in USP [15]. The product was sterilised by heating in an autoclave after being dispensed under aseptic conditions.

RC purity evaluation

The assessment of RC purity of the RM, dialysed product and the product obtained by centrifugation was carried out by PC with water solvent on Whatman 1 paper support. PC of aliquots of the tracers, $^{51}CrO_4^{--}$ and $^{32}PO_4^{---}$, were run simultaneously as reference. Movement of these along with that of colloidal chromic phosphate - ^{32}P is indicated in Fig. 1.

The stability of the product was checked by drawing aliquots at regular time points and estimating the RC purity of the colloidal suspension.

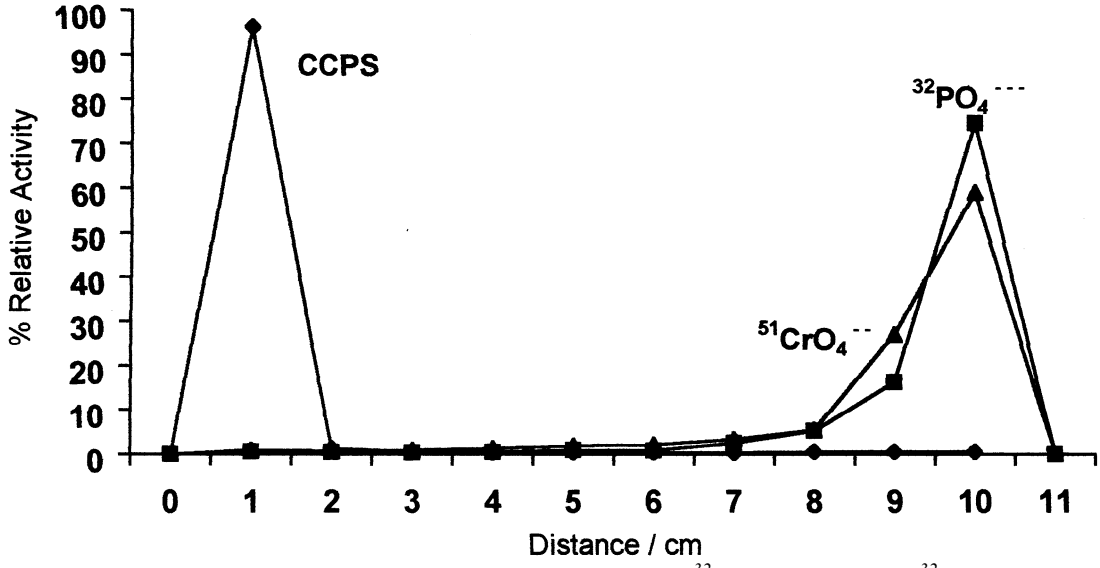

FIG. 1. RC purity analysis of (I) colloidal chromic phosphate -^{32}P (CCPS) (II) $H_3\ ^{32}PO_4$ and (III) $Na_2\ ^{51}CrO_4$ [PC/Support: Whatman 1 Paper/Solvent: Water].

Biodistribution studies

In order to assess the consistency of the biological behaviour of CCPS-^{32}P, limited bio-distribution studies were carried out in rats and mice by i.v. administration of 0.1 mL of CCPS-^{32}P into the tail vein of the animal. The animals were sacrificed by decapitation 30 min p.i. Major tissues of the body were removed and assayed for radioactivity in comparison to a standard source, in turn, relatable to the injected dose (i.d.).% i.d. in the tissues was calculated.

Particle size evaluation

For this purpose, CCPS was formulated as described above, but without any radioactivity. An aliquot of ~10 μL was smeared on a glass slide, air dried and investigated under an electron microscope (Lietz Wetzlar). A positive image of the product particles was recorded. These photo micrographic images (film) were scanned using a computer image scanning facility and stored. These stored computer images were analyzed using CIPS software (comprehensive image processing system) and typical size distribution of the particles was assessed (Fig.2).

3. RESULTS AND DISCUSSION

Initial studies of standardization with ^{51}Cr tracer helped establish the preparation protocol for CCPS and indicated radiochemical yield of around 60%. The results indicated in Table-II show that the RC purity was over 99% and that soluble chromium content was very low, even at 7 days after formulation. There was no notable difference in the quality of product obtained by either of the method of purification.

TABLE II. RC PURITY AND STABILITY OF COLLOIDAL CHROMIC PHOSPHATE ($^{51}CRPO_4$) [PC: SUPPORT — WHATMAN 1 PAPER & SOLVENT — WATER]

		PRODUCT RECOVERED BY			
		Centrifugation		Dialysis	
LOT	AGE OF SAMPLE	% CCPS#	% Soluble ^{51}Cr*	% CCPS	% Soluble ^{51}Cr*
1	Fresh	>99	<1	-	-
2	1 d	99	~ 1	>99	<1
	3 d	99	~ 1	98.2	~ 1
	7 d	99	~ 1	98.9	~ 1

 # ^{51}Cr as colloidal chromic phosphate.
** ^{51}Cr as soluble chromate.*

The product thereafter formulated with ^{32}P also showed high RC purity (Table-III). However, there was significant difference in the quality of product depending upon the method of purification employed. The product formulated after centrifugation showed low stability in terms of continuous wash out of soluble phosphate. This could be due to occlusion of soluble phosphate in the product during centrifugation, which later on gets released into solution with time, leading to poor RC purity and, in turn, poor stability noted (Table-III).

TABLE III. RC PURITY AND STABILITY OF COLLOIDAL CHROMIC PHOSPHATE -^{32}P SUSPENSION (CCPS) [PC: SUPPORT — WHATMAN 1 PAPER & SOLVENT — WATER]

	PRODUCT RECOVERED BY			
	Centrifugation		Dialysis	
AGE OF SAMPLE	CCPS#	Soluble phosphate*	CCPS#	Soluble Phosphate*
Fresh	97.3	2.2	>99	<1
1 d	93.3	6.7	>99	<1
2 d	90.3	8.7	-	-
7 d	-	-	99	1

 # ^{32}P as colloidal chromic phosphate.
** ^{32}P as soluble phosphate.*

Out of the methods of purification i.e. dialysis and centrifugation, the former was found to be more convenient to practice, in particular behind shielding. The duration required was, however, nearly 24 hours (Table-IV). The RC purity of the product formed by dialysis was always superior and stability higher compared to that of the product obtained by centrifugation (Table-III). On the other hand, at least 4 steps of washing and centrifuging were needed for ensuring acceptable purity in the other case, as evident from Table-IV. The product formulation was also not stable, even for one day.

TABLE IV. PURIFICATION OF COLLOIDAL CHROMIC PHOSPHATE -^{32}P (CCP)

(A): By Dialysis

Duration of dialysis/Hours	Soluble $^{32}PO_4^{---}$ in RM$^{\#}$/%$^{@}$	Efficiency of removal of soluble phosphate - $^{32}P^{\$}$
0	57.0	0
0.5	43.5	23.6
1	30.5	46.5
2	21.5	62.3
20	5.2	91.0
24	1.3	>99

(B): By Centrifugation

Order of washings	Soluble $^{32}PO_4^{---}$ in RM$^{\#}$/ Relative %$^{@}$	Efficiency of removal of soluble phosphate - $^{32}P^{\$}$
Supernatant of RM	100	-
1st wash	24	76
2nd wash	3.8	96
3rd wash	1.15	98
4th wash	0.23	99.7

NOTE : # RM: Reaction mixture undergoing purification
@: Value estimated experimentally
$: Calculated as 100 — @, above

The results of particle size distribution analysis (computerised analysis of photomicrographs) revealed that over 99% of the particles were below 2.5 μM (Fig.2). Only 9 particles of 5–10 μM and 3 particles of 10–14 μM were noted out of over 10 000 particles.

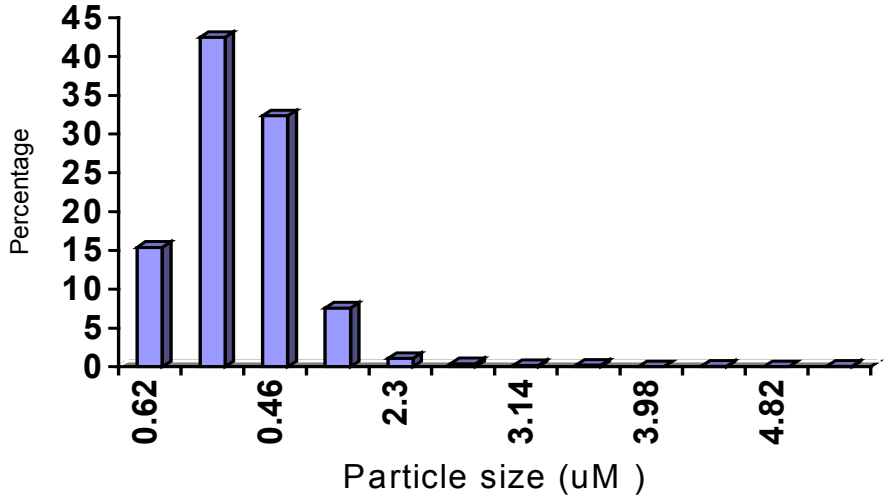

FIG. 2. Particle size distribution of inactive colloidal chromic phosphate — computer analysis of particle size distribution recorded on photomicrograph.

CCPS has been envisaged for therapy by local instillation. As a prelude to such local instillation in test animals, which would require application of relatively more elaborate techniques, biological behaviour following direct i.v. administration was first attempted to be studied. The results shown in Table-V confirmed the trends and consistency of biological behaviour. However, the high uptake in lungs indicated the presence of >10 μM particles in-vivo. This is contrary to the findings of the particle size distribution, analyzed by reliable means, showing that nearly 99% of the particles are present in the size range of 0.6–2.5 μM (Fig.2). This is not, however, altogether abnormal and could

be attributed to agglomeration of suspended colloidal particles occurring in serum in-vivo. The low uptake in liver of 8.2% and 0.2% in spleen would indicate the presence of corresponding amount of un-agglomerated colloidal particles. (Table-V). The particle size requirement for some applications reported are as follows: 0.6–2μM for intraperitoneal use [7], 2–5μM for radiation synoviorthesis [16] and 0.5–1.5nM for intraarterial administration [10].

TABLE V. BIO-DISTRIBUTION DATA OF COLLOIDAL CHROMIC PHOSPHATE -^{32}P SUSPENSION (CCPS) IN MICE.

Organ/Tissue	% inj. dose at 30 min p.i. (Mean of 2 animals)
Lungs	70
Liver	8.2
Spleen	0.2
Heart	0.3
Kidneys	1.2
Femurs	0.9
Blood	<0.1

Based on our results with ^{51}Cr tracer, it is felt that the use of radiotherapeutic M(III) phosphate colloid could be an attractive alternative to the use of colloidal chromic phosphate — ^{32}P. The release of soluble part of CCPS appeared to arise mainly from the anionic phosphate moiety and not from the cationic component. It is hence proposed that ^{143}Pr and ^{153}Sm colloids could be investigated for possible therapeutic purpose.

4. CONCLUSION

Thus in the present study, we have demonstrated a simple reliable procedure to formulate CCPS-^{32}P. An improved and easy to adopt procedure of purification by dialysis has been standardised. Attempts to establish protocol for evaluation by local instillation in animal models for possible therapeutic use are warranted and such studies are underway. Investigations on colloidal phosphate suspension of other radiotherapeutic-metal, [*M(III)], would merit attention.

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^{153}Sm COMPLEXES OF PHOSPHONIC ACID LIGANDS

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Abstract. ^{153}Sm was produced by irradiating enriched samarium-152 targets (Sm_2O_3) at a flux of 2.2×10^{13} n/cm^2/s for 7 days. α-Amino methylene phosphonic acid ligands such as propylene diamine tetramethylene phosphonate (PDTMP) and butylene diamine tetramethylene phosphonate, (BDTMP) were synthesised by modifying a method reported for the synthesis of EDTMP. Complexation of these synthesised phosphonate ligands with ^{153}Sm was carried out by varying experimental parameters such as mole ratios of ligand to metal, pH, time and temperature of the reaction in order to maximise the yields. The complexes were characterized for radiochemical purity by paper chromatography. Though quantitative complexation was obtained in the case of PDTMP, the complexation of BDTMP was not quantitative. Extensive studies on the *in vitro* stability, bone mineral uptake and biodistribution were carried out on ^{153}Sm-PDTMP complex. About 30% bone uptake and retention were observed in 24 hours. However, 20% of the injected activity was seen in liver. The uptake in all other organs was insignificant.

1. INTRODUCTION

Radionuclides have been used to treat skeletal diseases since decades [1]. At present an array of radionuclides has been proposed to treat bone pain due to cancer [2]. The most promising among these radionuclides is ^{153}Sm. This radionuclide has favourable radiation characteristics such as a half life of 1.93 d and beta energy {0.81 MeV (20%), 0.71 MeV (49%) and 0.64 MeV (30%)} which are suitable for radiotherapy. It also emits γ rays of 103 keV (30%) which is suitable for imaging purposes during therapy. Among the several phosphonic acid ligands studied for complexing with ^{153}Sm [3] ethylene diamine tetra methylene phosphonic acid [EDTMP] when complexed with ^{153}Sm has been proved to be a good therapeutic agent for the treatment of pain due to skeletal metastases [4,5].

We have in our laboratory synthesised a few more α amino methylene phosphonic acid ligands to investigate their suitability of complexation with ^{153}Sm and the potential usefulness of these complexes as bone seekers. These ligands were synthesised by a modification of the method described for EDTMP [6]. After characterizing the ligands by ^{1}H NMR spectroscopy, these ligands were used for complexation. Experimental parameters were varied to optimize a method to obtain quantitative complexation. The assessment of the complexes for radiochemical purity, stability and biodistribution pattern on Wistar rats was carried out. The data obtained from these studies are presented in this paper.

2. MATERIALS AND METHODS

Enriched samarium (99.7%) target as^{152} Sm_2O_3 provided as a gift from IAEA was used for irradiation studies. Propylene diamine tetramethylene phosphonic acid (PDTMP) and butylene diamine tetramethylene phosphonic acid [BDTMP] were synthesised and purity of these compounds was ascertained by ^{1}H-NMR. Figure 1 shows the structures of these phosphonic acid ligands. Whatman No.1 and Whatman No.3 chromatography papers were used for paper electrophoresis and paper chromatography studies, respectively. All other chemicals used were of GR grade.

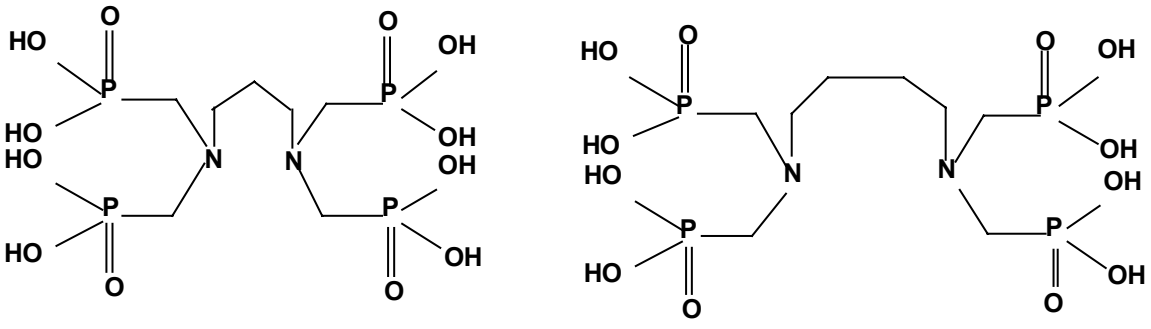

FIG. 1. Structure of synthesised ligands.

2.1. Production of ^{153}Sm

1.5 mg of enriched samarium oxide was sealed in a quartz ampoule. The ampoule was placed in an aluminium can and irradiated in Dhruva reactor at a flux of 2.2×10^{13} n/cm^2/s. The irradiated sample, after cooling for 24 h, was dissolved in 0.1 M HCl.

2.2. Preparation of ^{153}Sm-PDTMP

This was prepared by mixing a known quantity of the ligand, PDTMP (5–20mg/mL) in 2 M NaOH with a known amount of activity as $^{153}SmCl_3$ containing a carrier concentration of $3x10^{-4}$ M as ^{152}Sm. The specific activity of the isotope was adjusted to contain 20 mCi/mg (740 MBq/mg) of Sm for experimental purposes. The pHof the reaction mixture was adjusted to be above neutral and the reaction volume was maintained as 1 mL. Several experiments were carried out to optimise the reaction conditions to obtain quantitative yields.

2.2. Preparation of ^{153}Sm-BDTMP

^{153}Sm-BDTMP was also prepared as mentioned above by mixing the ligands (5–40 mg/mL) with a known amount of activity as $^{153}SmCl_3$, maintaining the reaction mixture at alkaline pHin a reaction volume of 1 mL.

As earlier mentioned the reaction parameters were varied to obtain quantitative yields. The% complexation of both the ligands were determined by paper chromatography in 0.9% saline.

2.4. Assessment of the Complex

2.4.1. Paper Chromatography

This was performed using Whatman No.3 paper. ~5 μL of the reaction mixture was applied at 2 cm from one end of the paper strip. The paper strips were developed in 0.9% saline until the solvent reached the top of the paper strip. The strips were dried and cut into 1-cm sections and the radioactivity was measured.

2.4.2. Paper Electrophoresis

5 μL of the reaction mixture was applied on the centre of a 30 cm whatman 1 paper. Electrophoresis was carried out for 75 minutes at 8 V/cm in 0.025 M Bicarbonate buffer pH~9. The strips were dried and scanned for radio active zones. Separate strips were run for samarium chloride and samarium hydroxide.

2.5. In vitro *bone mineral uptake studies*

This was carried out by incubating the radioactive complex at 37°C in the presence of hydroxyapatite (HA) particles in human serum for 3 h. 0.1 mL of a slurry containing 100 mg/mL of HA in saline was mixed with a known amount of the ^{153}Sm-phosphonate (PDTMP) complex in the presence of 1 mL of human serum. After incubation, the slurry was centrifuged and the radioactivity on the HA particles was measured. The experiment was repeated using $^{153}SmCl_3$ under identical conditions.

2.6. Biodistribution studies

Biodistribution studies were performed in male Wistar adult rats weighing between 300–500 g. The animals were injected 2–3 MBq of ^{153}Sm-phosphonate complex under light anesthesia and sacrificed at 3 h, 24 h and 48 h of post injection. 5–10 mL of blood was collected by cardiac puncture, 30 seconds prior to sacrifice. Various organs were excised, including bone and muscle. The radioactivity was measured in a gamma counter designed for counting animal tissues. The results are expressed as% injected dose/of organ.

2.7. Stability Studies

The stability of the complex stored at 22°C was studied at different intervals of time at the pHoptimized for complexation as well as at neutral pH(physiological).

3. RESULTS AND DISCUSSION

3.1. ^{153}Sm production

The specific activity obtained by irradiating enriched Sm targets at a flux of ~2.2 × 10^{13} n/cm^2/s for 7 d in Dhruva reactor, ranged from ~0.5–1 Ci/mg (18.5 -37 Gbq/mg).

3.2. Radiochemical yield of the complex.

In paper chromatography using 0.9% saline, the R_f of uncomplexed Sm was 0 and of the phosphanate complexes was ~1. In the case of PDTMP it was noted that the complexation yield increased with increasing ligand to metal ratio (Table-1) and reached a maximum at a mole ratio of 132:1.

TABLE I. EFFECT OF L:M MOLE RATIOS ON COMPLEXATION YIELD OF PDTMP WITH ^{153}SM

L:M	Ligand (mg)	% Complexation*
6.6:1	1	-
13:1	2	-
33:1	5	28.3 ± 2.0
66:1	10	94.9 ± 1.4
132:1	20	98.3 ± 0.7

*N = 3, pH12, Reaction time 2 h at 22°C, * ± SD.*

The pH of the reaction mixture also played an important role on the complexation yield. Though there was ~50% complexation at pH8, maximum complexation was observed only at pH12, when the reaction was carried out at room temperature (22°C ambient) in a reaction time of 2 h (Table 2).

TABLE II. EFFECT OF PH ON COMPLEXATION YIELD OF PDTMP WITH ^{153}SM

pH	Ligand (mg)	% complexation*
8	20	49.5 ± 4.8
10	20	53.6 ± 6.3
12	20	97.4 ± 0.8

*L:M 132:1, n = 5, Reaction time and temp:2 h at 22°C, *± SD.*

However, when BDTMP was complexed with ^{153}Sm under similar conditions as that of PDTMP, the complexation was very poor. As the ligand concentration was increased, complexation began to occur and at a ligand to metal ratio of 264:1, significant complexation (~80%) was observed in a reaction time of 16–18 h at room temperature (22°C) (Fig-2). Elevating the temperature of reaction to 60–80°C, on a boiling water bath at a L:M of 132:1, varying the time of heating, also did not improve the complexation (Fig-3). Hence, further studies were carried out only on ^{153}Sm-PDTMP complex.

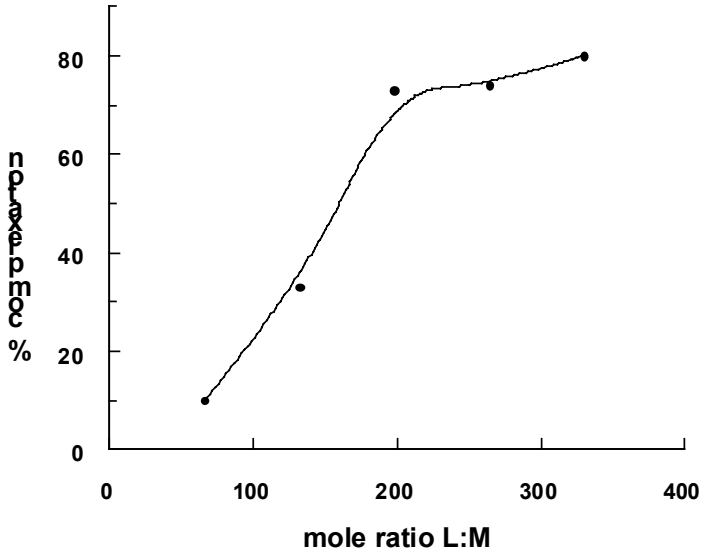

FIG. 2. Effect of mole ratios on the complexation of ^{153}Sm-BDTMP, reaction — ambient temperature. 16–18 h.

3.3. Radiochemical purity studies

Paper chromatography in saline showed a R_f of 1 for the complex prepared at pH12 and the RC purity was 98%. However when the pHof the complex was adjusted to 7, two different R_f values were observed showing the formation of two different species of the complex (Fig 4). The electrophoretic pattern of ^{153}Sm-PDTMP and other radioactive Sm species is shown in (Fig- 5). The complex migrated to the anode showing that the complex is negatively charged while ^{153}Sm as hydroxide remained at the point of application. The $^{153}SmCl_3$ moved towards the cathode. Table 3 shows the results of the stability studies of the ^{153}Sm-PDTMP complex at pH7 and pH12 stored at various intervals of time at room temperature (22°C).The complex showed higher stability at pH12 whereas the radiochemical purity of the complex stored at pH7 decreased to ~ 87% after 6 h.

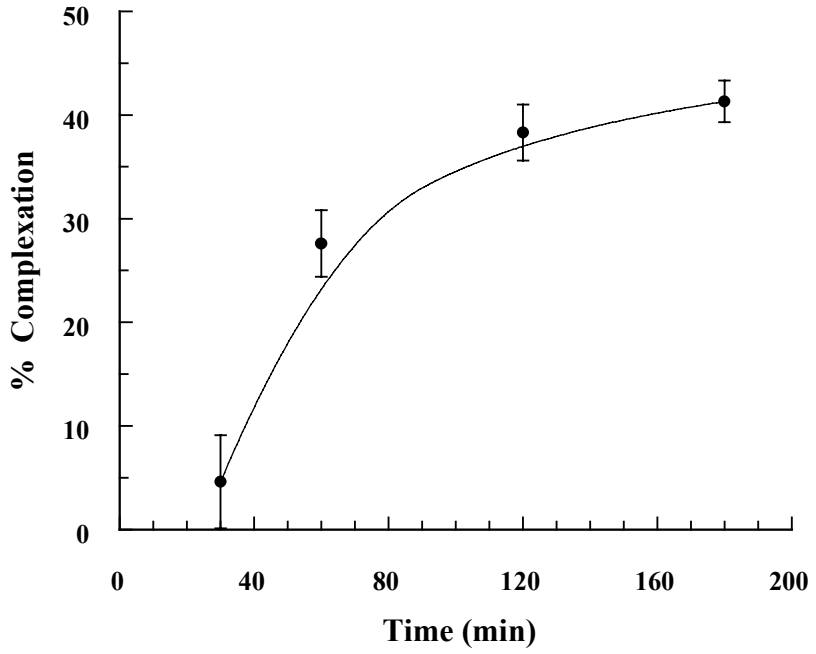

FIG. 3. Effect of reaction time at elevated temp. (60–80°C) on% complexation of ^{153}Sm-BDTMP; L:M 132:1, n = 3.

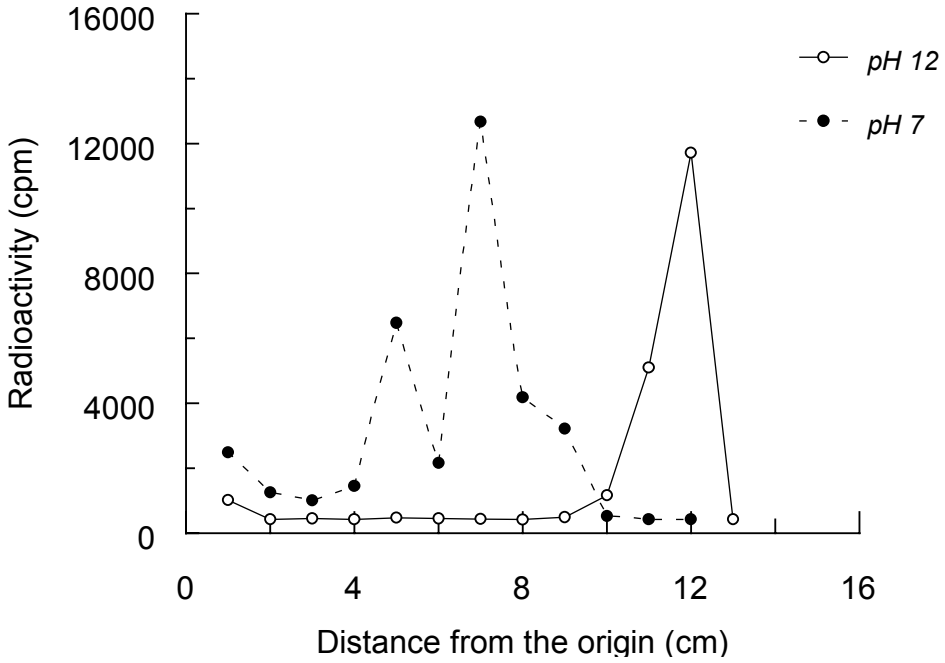

FIG. 4. Radiochemical purity studies of ^{153}Sm-PDTMP by paper chromatography.

TABLE III. STABILITY OF ^{153}SM-PDTMP COMPLEX STORED AT 22°C

pH	Radiochemical Purity					
	0 h	2 h	4 h	6 h	24 h	48 h
12	98	98	98	98	98	97
7	93	93	94	87	88	87

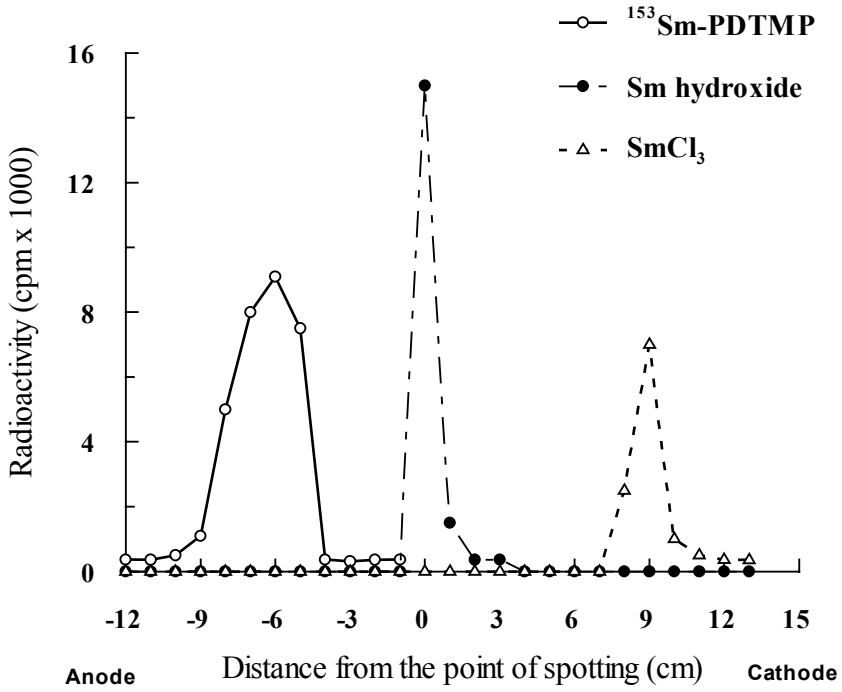

FIG. 5. Paper electrophoretic pattern of various samarium species.

3.4. In vitro bone mineral uptake studies

~20% bone mineral uptake was observed in 3 hour contact time. No leaching of radioactivity was seen for a 3 hour incubation with human serum. Insignificant (<1%) bone mineral uptake was observed when ionic ^{153}Sm was used.

3.5. Biodistribution studies

The results of the biodistribution studies are given in Table 4. The uptake in bone was calculated from the activity observed in tibia and fibula. Thirty-four percent of the injected activity was observed in bone at 3 h post injection. The uptake in other organs was insignificant except in liver which showed ~20% of the injected dose. The residual activity was cleared mainly through the bladder.

TABLE IV. RESULTS OF THE BIODISTRIBUTION STUDIES OF ^{153}SM-PDTMP.

Time (h)	Blood	Muscle	Bone	Femur	Liver	Kidney
3	3.80 ± 0.35	15.3 ± 0.7	33.9±3.4	1.02±0.11	16.1±0.35	3.38±0.39
24	0.12 ± 0.05	5.9 ± 0.82	29.7±1.6	0.92±0.55	20.3±0.57	3.81±0.71
48	0.17 ± 0.07	5.5 ± 0.07	35.0±4.2	1.11±0.46	21.1±0.88	2.71±0.25

Values reported are% injected dose/organ. ± SD; Blood, bone and muscle are taken as 5, 6, 46% of the body weight respectively; n = 3–5

4. CONCLUSION

The present studies indicate that like EDTMP, ^{153}Sm-PDTMP complex also accumulates in bone. The high uptake of the complex in the liver can be attributed to the instability of the complex at physiological pHand subsequently the hydrolysis of free Sm which may be present in colloidal form thus concentrating in the liver. BDTMP could not be complexed quantitatively with ^{153}Sm, possibly, owing to the large ring size (7-membered) formed on complexation.

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MAG_2GABA-BIOCYTIN SYNTHESIZED WITH NEW INTERMEDIATES FOR RADIOLABELLING ^{99m}Tc AND ^{188}Re

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Abstract. ^{188}Re from ^{188}W-^{188}Re generator, is recently introduced in therapeutic nuclear medicine and made it possible to use whenever needed. We synthesized MAG_2GABA-Biocytin (MGB), labelled with ^{188}Re for pretargeted radioimmunotherapy and evaluated biological behavior of ^{188}Re-MGB. N-hydroxysuccinimidyl ester of S-benzoyl mercaptoacetyldiglycine (NHS-MAG_2) was synthesized first, reacted with g-aminobutyric acid(GABA) to give MAG_2GABA and then converted to NHS-MAG_2GABA and conjugated to biocytin to give the MGB. To label MGB with ^{188}Re (50mA ag), 200m)l 1M tartrate pH7, 200m l stannous (10mg/mL) and 180MBq perrhenate were mixed and heated for 30min at 100°C. The reactant was purified with C_{18} Sep-Pak. HPLC analysis of the ^{188}Re-MGB performed on reverse phase C_{18} column with a gradient. To see the stability, ^{188}Re-MGB was added to serum at 37°C. Binding capacity of ^{188}Re-MGB to avidin or streptavidin was determined by size exclusion HPLC system. Biodistribution was studied in ICR normal mice(n=4/group), from 5min to 120min. In Raji cells tumour bearing nude mice(n=3), biotinylated Lym-1(40meg) injection after 48 h, streptavidin(50m)g) was injected. 24 h later, ^{188}Re-MGB(0.5meg) was injected, and biodistribution was observed 2 h later. ^{188}Re-MGB was obtained with labelling yield 98%. Stability in serum was maintained over 70% until 3 h. Binding capacity of ^{188}Re-MGB to streptavidin was greater than avidin. In normal mice, ^{188}Re-MGB was excreted via hepatobiliary pathway,%ID/g of GI tract was 52.1 at 120min. In Raji cells tumour bearing nude mice, liver and colon were higher than those of normal mouse. Tumour uptake at 120min was 0.05%ID/g. ^{188}Re-MGB was effectively labelled and retained binding activity with streptavidin. ^{188}Re-MGB may have a role in pretargeted radioimmunotherapy.

1. INTRODUCTION

Avidin-biotin system is widely used in medical research, especially in pretargeted radioimmuno-imaging and therapy. Biotin can be easily conjugated to antibody and other large molecules. The very high affinity of avidin or streptavidin for biotin makes the avidin-biotin system applicable to multistep targeting of tumours. Pretargeting tumour with biotinylated monoclonal antibody can reduce the radioactivity in normal organs, and protect antibody from radiolysis by beta ray.

^{188}W-^{188}Re generator is recently introduced in therapeutic nuclear medicine and made it possible to use whenever needed. ^{188}Re can be labelled with biotin, peptides and other compounds using bifunctional chelate, such as MAG_3 or MAG_2GABA. We developed a simple route for the facile synthesis of tetradentate bifunctional ligand, MAG_2GABA and conjugated this with biocytin. MAG_2GABA-biocytin was labelled with ^{188}Re, stability in serum, binding capacity with streptavidin and biodistribution were observed.

2. METHODS

The new key compound, N-hydroxysuccinimidyl ester of S-benzoylmercaptoacetyldiglycine (NHS-MAG_2) was synthesized first and reacted with gamma-aminobutyric acid to give MAG_2GABA. This MAG_2GABA N_3S chelator was then converted to NHS-MAG_2GABA and conjugated to biocytin to give the MAG_2GABA-Biocytin (MGB) in Fig 1.

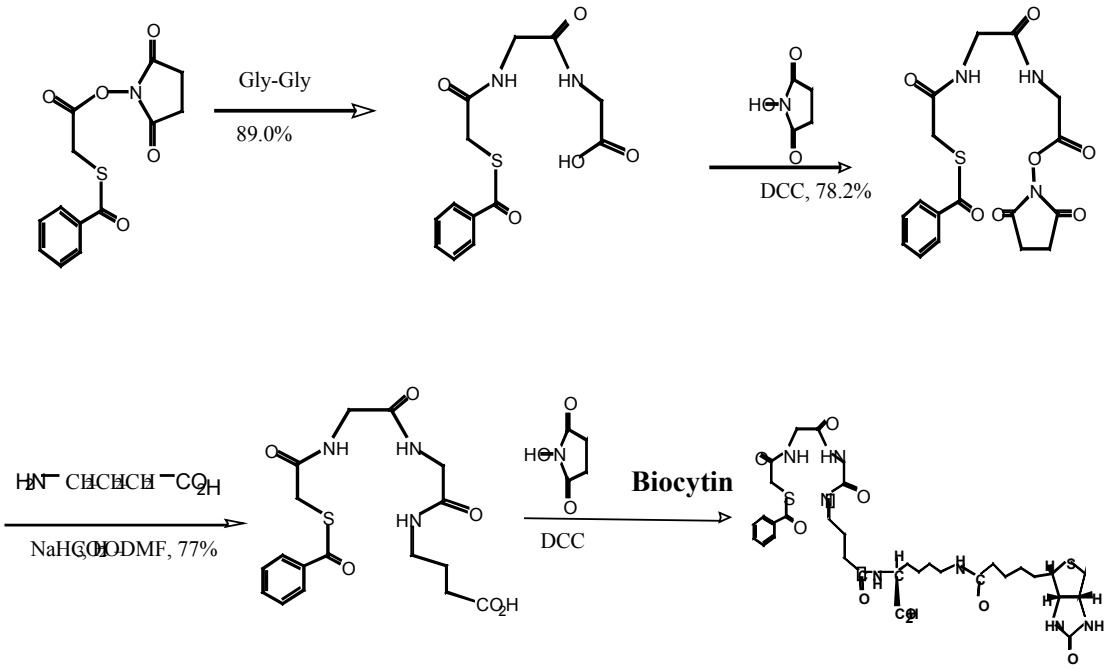

FIG. 1. Synthesis of MAG_2GABA-Biocytin.

To the aqueous solution of gamma aminobutyric acid (1.57g, 0.015moles) and sodium bicarbonate (2.53g, 0.03moles) was added the dimethylforamide (DMF)/ethyleneglycol dimethylether solution of NHS-MAG_2 (6.1g, 0.015moles, synthesized from S-benzoylmercaptoacetyl NHS ester with overall 70% yield) dropwise. The clear reaction mixture stirred for 2 h at room temperature and concentrated under reduced pressure. The residual aqueous solution was adjusted to pH2.0 with concentrated HCl. The white precipitate was filtered and recrystallized from acetonitrile to give the MAG_2GABA with 77.7% yield (m.p. 158–165°C). MAG_2GABA (0.89g, 7.5 mmoles) and N-hydroxysuccinimide was dissolved in DMF and treated with ethyleneglycol dimethylether solution of dicycolhexylcarbodiimide (DCC; 1.56g, 7.5mmole) at room temperature. The reaction mixture was stirred for 15 h, and then filtered to remove the dicyclohexyl urea. The filtrate was evaporated and crystallized from hot isopropanol to give the product with 87.8% yield (m.p. 174–177°C, decom.). The DMF solution of NHS-MAG_2GABA (0.49g, 1mmole) was added to the basic aqueous solution of biocytin(0.37g,1mmole) and reaction mixture was stirred for 14 h. Adjustment of pH2.0 gave the white product (660mg, 88% yield).

For the labelling of home-made MGB ^{188}Re, 10 mel MGB (20mg/mL in DMSO) was added in reaction vial, followed by the addition of 200m l 1M sodium potassium tartrate (pH7), 200m l stannous tartrate (10mg/mL) and 370 MBq ^{188}Re perrhenate. Finally the reaction mixture was heated for 30min at 100 °C. Colloid were determined with ITLC-SG (Gelman) developed with methanol:phosphate buffered saline (1:1). The reactant was purified with a C_{18} Sep-Pak cartridge. First, cartridge was eluted with 0.001N HCl to eliminate impurities. Labelled compound was eluted with ethanol:saline (1:1). HPLC analysis of the ^{188}Re-labelled and ^{99m}Tc-labelled MGB was performed on RP C18 column with a gradient mixture of methanol and 10 mM phosphate buffered saline (pH7.4) at a flow rate of 1mL/min. From 0 min to 10min, 20% methanol was changed to 50% methanol. Until 20 min, the concentration of methanol was maintained to 50%. Binding to streptavidin was confirmed by HPLC with TSK 4000 size-exclusion column. Biodistribution was studied in ICR normal mice (n = 4/group), from 5min to 120min. For in vivo studies 1×10^7 Raji cells were inoculated into the left thigh of nude mice (n=3), biotinylated Lym-1 (40m4g) intravenously injection. After 48 h, streptavidin (50m5g) was injected. 24 h later, ^{188}Re-MGB (0.5m0g) was injected, and biodistribution was observed 2 h later.

3. RESULTS

The desired product MGB was synthesized from S-benzoylmercaptoacetyl NHS ester with overall 41.5% yield. m.p.=194–196°C ^{1}H-NMR (DMSO-d_6), d, 8.49 (t, 1H), 8.12(t,1H), 8.0(d, J=7.73Hz, 1H), 7.95–7.56(m, 5H), 6.38, 6.33(s, 2H), 4.29(m, 1H), 4.13(m, 1H), 3.89(s, 2H), 3.77(d, J=5.63Hz, 2H), 3.67(d, J=5.86Hzs, 2H), 3.10(m, 1H), 3.05(q, J=6.7Hz, 2H), 3.00(q, J=6.6Hz), 2.83(dd, J=12.4, 5.11Hz, 1H), 2.57(d, J=12.4Hz, 1H), 2.12(t, J=7.50Hz, 2H), 2.04(t, J=7.4Hz,2H), 1.2–1.6(m, 14H). MASS (ESI) m/z 750.57(M^{+}+1).

The retention time of ^{188}Re-MGB was about 13min on radiochromatogram, colloid was below 1% and labelling yield was 95% for ^{188}Re. Stability ^{188}Re-MGB at 3 hour were above 70%. In vitro serum stability was maintained over 70% until 3hours(Fig 2).

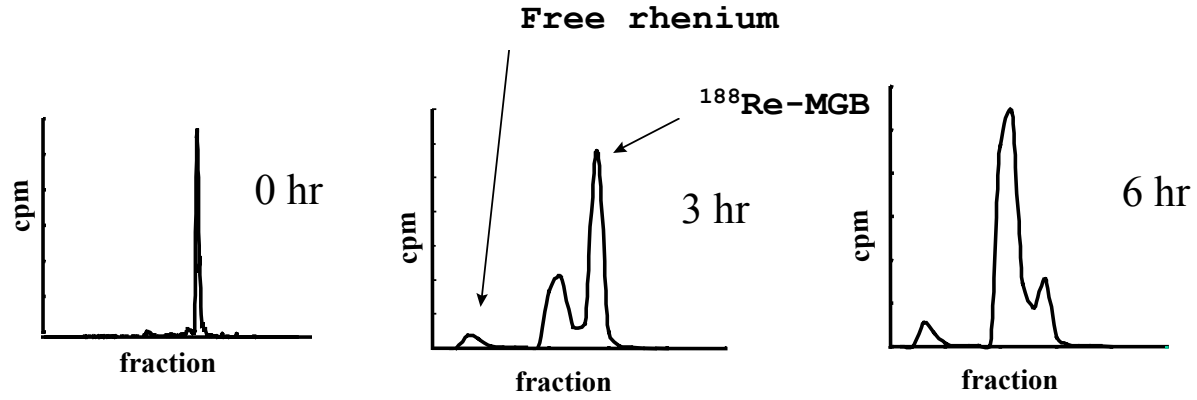

FIG. 2. Reverse phase C_{18} HPLC radiochromatogram of ^{188}Re-MGB in serum.

Binding capacity of ^{188}Re-MGB to streptavidin was greater than avidin, showin in Fig 3. Normal mice, ^{188}Re-MGB was excreted via hepatobiliary pathway, and%ID/g of GI tract was 52.1 at 120min (Fig 4). In Raji cell bearing nude mice, liver and colon were higher than those of normal mice. Tumour uptake at 120min was 0.05%ID/g (Fig 5).

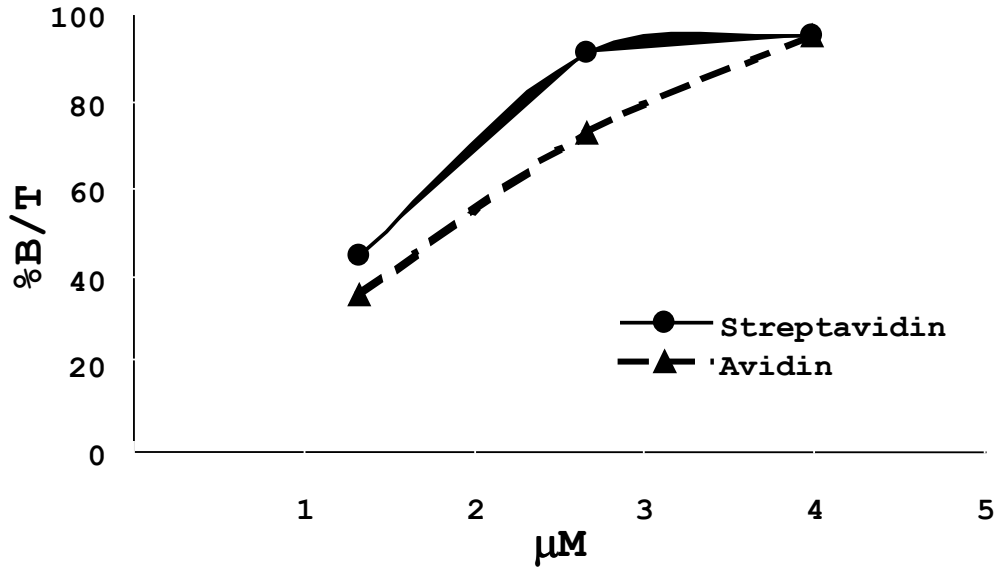

FIG. 3. Binding capacity of ^{188}Re-MGB to avidin, streptavidin.

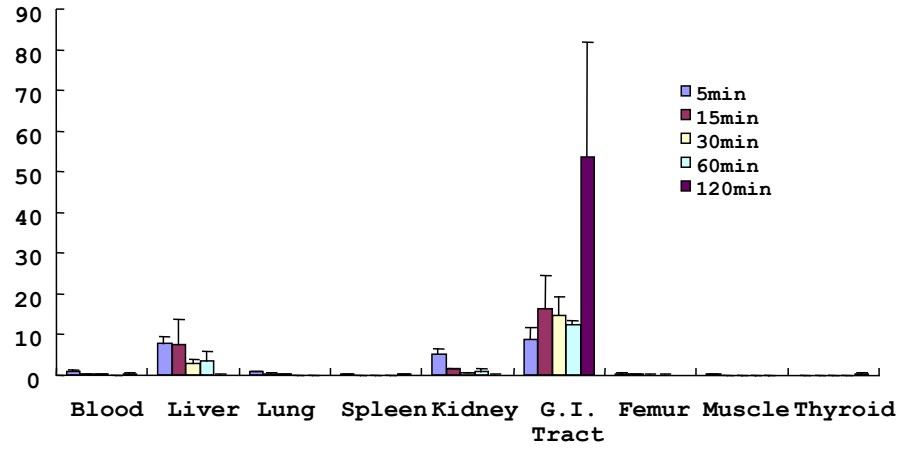

FIG. 4. Biodistribution of ^{188}Re-MGB in normal mice.

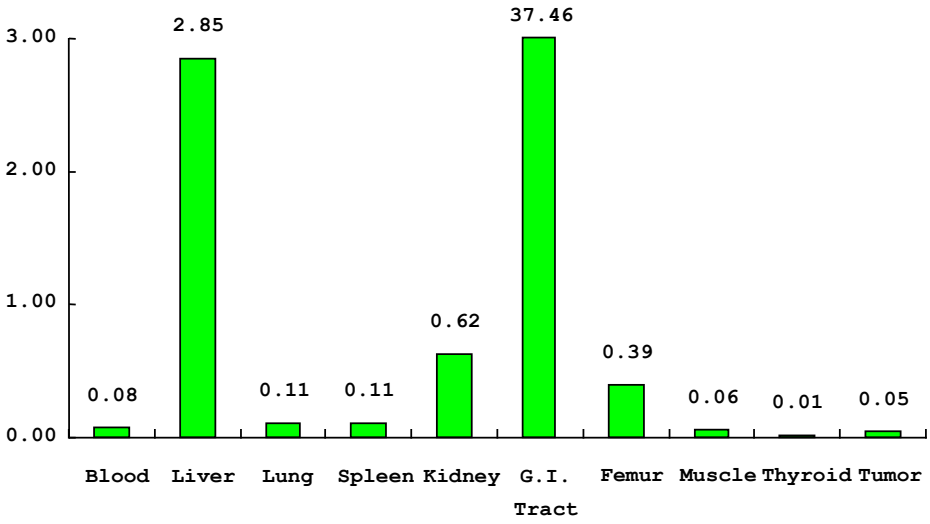

FIG. 5. Biodistribution of ^{188}Re-MGB, pretargeting with biotinylated Lym-1 and streptavidin in Raji cell bearing nude mice at 120 min.

4. DISCUSSION

The synthesis may be applied to preparation of positional isomers of MAG_3-type ligand containing diverse carboxy terminal residue in place of glycine to give a large library of N_3S chelates. So this simple synthesis may be used to prepare new bifunctional chelators with unique properties related to carboxy terminal residue. MGB was labelled with ^{99m}Tc and ^{188}Re for scintigraphy and therapy. Avidin-biotin pretargeting system has been applied to enhance tumour to normal tissue ratios[3]. However, multistep targeting method could be sometimes difficult to apply in a nude mouse tumour model, probably due to higher level of endogenous biotin in mice than in humans. ^{188}Re-MGB could be metabolized by serum biotinidase, but free ^{188}Re is not released from ^{188}Re-MGB. ^{188}Re-MGB seems to be excreted or targeted before metabolism by biotinidase. By adjusting pretargeting protocol and dose escalation of ^{188}Re-MGB, therapeutic effect in tumour bearing mice could be expected. ^{188}Re-MGB may have a role in pretargeted radioimmunotherapy

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3. THYROID CANCER

RADIOIODINE THERAPY IN MANAGEMENT OF THYROID CARCINOMA — A REVIEW OF 138 PATIENTS

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Abstract. Differentiated thyroid carcinomas are being treated by using a widely accepted protocol of surgery and radioiodine therapy followed by supplementation of thyroid hormones in the Nuclear Medicine Centre (NMC), Dhaka Medical College Hospital (DMCH) since 1990. In the present study 138 patients(Male-54, Female-84) with differentiated thyroid cancers received radioiodine therapy for ablation of residual thyroid tissue with a dose of 2.77–3.7 GBq (75–100 mCi), for lymph node metastases 5.55–6.5 GBq(150–175mCi), for lung metastases 5.55 GBq(150 mCi) and for bony metastases 7.4 GBq (200 mCi). Among 138 patients papillary carcinoma was observed in 94 cases (68%; Male-42, Female-52), follicular type was found in 30 cases (22%; Male-8, Female-22) and mixed type in 14 patients (10%, Male-4, Female-10). Single dose of 2.77–3.7 GBq(75–100 mCi) of radioiodine was received by all 138 patients. Among the unablated patients 62 received double doses totalling 9.25 GBq (250 mCi), 44 received three doses 12.95 GBq (350 mCi) and one patient received 8 doses 33.3 GBq (900 mCi). Out of 138 patients single dose ablated 76 cases and 62 remain unablated. Multiple doses ablated 28 patients and 34 still remain unablated and is under follow up. The success and failure in management of patients with differentiated thyroid cancer over 8 years period have been discussed here revealing a satisfactory outcome.

1. INTRODUCTION

Thyroid cancer is the commonest endocrine malignancy, yet management remains controversial [1]. Physicians have long differed over the extent of initial surgical resection, over the proper use of adjuvant radioiodine to ablate residual thyroid remnants and over the best method of treating the recurrent disease. Much of this controversy stems from the long life expectancy associated with differentiated thyroid carcinoma, its low incidence in most population, and its frustrating tendency to recur often even many years after the initial therapy. Thus the efficacy of any management protocol can be justified only after a large number of patients have been treated and after long follow-up periods which could provide sound statistical basis for comparisons [2].

In spite of this sparked controversy, differentiated thyroid carcinomas are being treated by using a widely accepted protocol of surgery and radioiodine therapy followed by supplementation of thyroid hormones. The efficacy of this approach has been well documented. In the Nuclear Medicine Centre of Dhaka Medical College Hospital, this method has been successfully practiced in collaboration with surgeons and is being considered a major part of the management of differentiated thyroid carcinoma since 1990.

The avidity of differentiated thyroid carcinoma for iodine is the basis for the use of radioiodine [3] both for the detection and treatment in primary, recurrent and metastatic tumour.

This retrospective review was performed to evaluate the effectiveness of radioiodine in the ablation of residual thyroid tissue after surgery in differentiated thyroid cancer, metastases and recurrence of disease.

2. MATERIAL AND METHODS

In the present study a total of one hundred & thirty eight patients (Male-54, Female-84) were reviewed. Some of these patients were reported to NMC, Dhaka in presurgical state for initial diagnosis and they were evaluated by Ultrasonography, in vivo and in vitro nuclear medicine

techniques and final diagnosis was documented by histopathology during or after operation. In most cases a total or near total thyroidectomy was performed at the time of 1st operation or as a 2nd procedure. Following the total thyroidectomy, a period of 4 weeks was allowed to elapse to permit serum TSH to rise, before a large dose scan was performed using oral administration of I^{131}, dose 111–185 MBq (3–5 mCi) 72 hours later using rectilinear scanner [4].

Patients subjected to post operative evaluation by I^{131} scanning for residual thyroid mass and metastases, were initially administered with 3.7 MBq (100 μCi) of I^{131} orally for screening at 24 hours. Specially in cases of hemithyroidectomy or lobectomy it has been observed that significant amount of residual thyroid tissue remained intact and in these cases I^{131} ablation therapy was initiated immediately [5] with an average dose of 2.77–3.7 GBq (75–100mCi).

In our protocol a first ablation dose for residual thyroid tissue of 2.77–3.7 GBq (75–100 mCi) is generally used although most clinicians prefer a fixed dose of 3.7–7.4GBq (100–200 mCi) [6–9]. Patients are usually admitted into a designated room with ensuite bathroom facilities; visiting time being restricted to not more than 10 minutes a day. Daily monitoring was done until the patient was discharged with radiation levels acceptable under national regulation.

In cases of evaluation of recurrences and metastases, patients taking thyroxine undergo a minimum of 4 weeks withdrawal of treatment and in cases of T_3, a 2 week cessation of therapy was followed before large dose scan was done. In cases of cervical node metastases 5.55–6.5 GBq (150–175 mCi) was applied. In lung metastases 5.55 GBq(150 mCi) and in bony metastases average 7.4 GBq (200 mCi) doses were given.

In subsequent follow ups patients were evaluated clinically, biochemically, by I^{131} large dose scan and serum thyroglobulin estimation. Follow up was usually performed at six monthly or yearly intervals with repeated I^{131} therapy until tumour ablation was attained.

3. RESULTS

The results of radioiodine treatment for differentiated thyroid carcinoma in the present study are presented here.

Total study population included 138 patients, age ranging from 15–65 years (mean 33.7 years). Occurrence of the disease was common in 30–40 years age group. Number of reported patients were gradually increasing as represented by histogram (Figure 1).

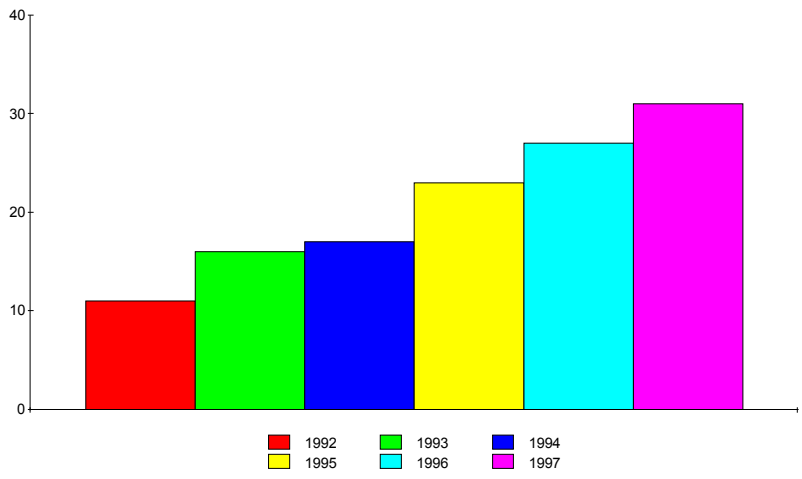

FIG. 1. Graphical presentation of patients accumulation.

In sex distribution along with the histopathological variety of thyroid cancer it was observed that female patients were predominant, female 84 (60.86%) and male-54 (39.14%). Common variety was observed to be papillary type, 94 (68%), follicular type was 30 (22%) and mixed variety was found in 14 cases (10%). (Table I).

TABLE I. TYPE OF CANCER WITH SEX DISTRIBUTION

TYPE OF PATIENT	MALE	FEMALE	TOTAL	PERCENTAGE
PAPILLARY	42	52	94	68%
FOLLICULAR	08	22	30	22%
FOLLICULAR VARIANT OF PAPILLARY (MIXED)	04	19	14	10%
TOTAL =	54	84	138	

Out of 138 patients, total thyroidectomy was done in 18 cases (13.04%), near total thyroidectomy in 34 cases (24.6%), hemithyroidectomy in 38 cases (27.5%), sub total thyroidectomy in 4 cases (2.8%) and extended thyroidectomy in 10 cases (7.24%). In case of extended thyroidectomy all 10 patients needed single dose of radioiodine while all 4 cases of sub total thyroidectomy required multiple doses. Among the hemithyroidectomy group all 36 patients needed multiple doses of radioiodine and only 2 cases received single dose. 14 cases of total thyroidectomy needed single dose while 4 needed multiple doses. In case of near total thyroidectomy 26 patients were given single dose of radioiodine while only 8 patients were applied multiple doses (Table II).

TABLE II. TYPES OF SURGERY AND DOSE NEEDED FOR ABLATION

Number of doses	Total thyroidectomy	Near total thyroidectomy	Hemi-thyroidectomy	Sub-total thyroidectomy	Extended thyroidectomy
Single	14	26	02	0	10
Multiple	4	8	36	4	0

All 138 patients initially received single dose of I^{131} 2.7–3.7 GBq (75–100mCi) consequently 62 received two doses 9.25 GBq (250 mCi), 44 received three doses–12.95 GBq (350 mCi) and only one patient received exceptionally large number of dose, 33.3 GBq (900 mCi). Regarding ablative treatment it has been observed that single dose in 138 cases yielded complete ablation in 76 (55%) cases while 62 (45%) remained unablated. All unablated patients were given multiple doses and finally 28(20%) more patients were ablated and 34(25%) are still unablated and are under follow up. (Table III)

TABLE III. OUTCOME OF 138 THYROID CARCINOMA PATIENTS RECEIVING RADIOIODINE THERAPY FOR ABLATION

No. of Dose	No. of cases	Ablated	Unablated	Total Dose
One	138	76	62	2.77–3.7 GBq
Two	62	18	44	9.25 GBq
Three	44	10	34	12.95 GBq
More than three	1	-	1	33.3 GBq

In table IV variety of metastases were depicted. Commonest type of metastases was observed in lymph nodes, next common type is bony metastases followed by lung and surrounding fibrofatty tissue respectively.

In table V side effects after radioiodine therapy were described. All these complications were temporary i.e. short term effects. No long term effect was observed in any of the patients.

TABLE IV. TYPE AND NUMBER OF METASTASES

TOTAL NO. OF CASES	TYPE OF METASTES	NUMBER OF CASES
138	Lymph node metastasis	69 (80.23%)
	Bone metastases	09 (10.47%)
	Lung metastases	04 (4.65%)
	Infiltration into surrounding fibro fatty tissue	04 (4.65%)
		Total No. of metastases-86

TABLE V. SIDE EFFECTS OBSERVED AFTER RADIOIODINE THERAPY AMONG STUDY POPULATION

SHORT TERM	
COMMON	RARE
Nausea	Sialadenitis
Vomiting	Transient bone marrow depression
Gastritis	Vocal cord paralysis
Acute Radiation Sickness:	
a) Fatigue	
b) Headache	
Radiation Thyroiditis	
LONG TERM COMPLICATION — NOT OBSERVED	

4. DISCUSSION

The term cancer induces an apparent fear in the affected patients as fate of many of the cancers are still a matter of despair. Although thyroid cancer constitutes <1% of all cancers, differentiated thyroid cancer covers 80% of all the cancers of thyroid and if managed properly yields an excellent result [10]. In the sense of outcome of the treatment of cancerous patients, differentiated thyroid cancer has emerged as one of the cancers having better prognosis and there is almost no threat to longevity.

In the past, this form of cancers were being treated by surgery and/or thyroid hormone administration for TSH suppression [11]. Following surgical removal of the tumour with a total thyroidectomy and extirpation of any evident nodal disease, radioiodine proved its major role in achieving a complete cure [12, 13]. Despite many confusions, an internationally accepted protocol regarding the management of these cases has been established.

In our study patient accumulation is gradually increasing every year which indicates awareness of the patients about the disease and more acceptance of radioiodine therapy by the referring physicians.

Adequate surgical debulking of the thyroid and removal of nodes, if any, is a primary requisite for radioiodine therapy. In cases of recurrence localized to the neck, the use of surgery before radioiodine therapy should be considered to debulk the tumour and optimize the efficacy of radioiodine. The combination of surgery and radioiodine has a better outcome than the use of surgery alone [14]. In our study it is evidenced that amount of radioiodine required for thyroid cancer therapy is inversely related to the removal of thyroid gland.

Papillary type of thyroid cancer is most common in our population, which is in good agreement with the literature [15]. Lymph node metastases is the commonest type in our study which is well correlated with papillary predominance. Among the post radiation complications short term side affects are only found instead of any long term complications. Surely the duration of follow up is not enough to comment about the long term complications.

In our centre one patient with medullary carcinoma was applied radioiodine therapy after surgery recognized that these tumours themselves do not take up radioiodine, the rationale for this is the multicentricity of the tumours in patient with familial form of the disease [15]. Six months after the therapy the patient's condition is still uneventful except for high serum calcium level and without any evidence of metastases.

Two patients with differentiated thyroid carcinoma got external beam radiation, one had extensive neck nodes involvement and the other had involvement of the shoulder joint with a big swelling. No significant improvement of disease was observed after beam therapy. In our centre this method of radiation is not encouraged as supported by literature [16].

I^{131} radioiodine remains the most frequently used form of radionuclide therapy with its clearly defined role in both benign and malignant conditions, 40 years experience of its use has been shown to be safe, effective, cheap and the theoretical risks of tumour induction and chromosomal damage have not been demonstrated in practice. Many of the lessons learnt from the experience with I^{131} radioiodine are now proving useful as new radioiodine therapies are developed and integrated into routine management. An essential part in achieving this success is the patients good appraisal of the steps of the treatment. Instead of general cancer phobia, well acceptance to this treatment and undergoing regular follow up can provide patients with well differentiated thyroid cancer an almost normal and active life. Further to this it is worthwhile to state that with persistent modern surgery and I^{-131} therapy rarely should a patient die of well differentiated thyroid carcinoma [17].

This study identifies several short comings from what the optimum management of thyroid cancer might be considered. In practice a good interdisciplinary communication between surgeons and nuclear medicine specialists and a locally agreed and implemented protocol will improve the care of thyroid cancer patients.

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ROLE OF HIGH DOSE I-131 IN TREATMENT OF DIFFERENTIATED THYROID CARCINOMA: AN EXPERIENCE OF 354 PATIENTS AT INMOL

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Abstract. A total of 354 patients were registered at INMOL from 1985 to 1997. A predominant majority were comprised of females (72%) while only 27% were males. Maximum incidence was in the 5th decade of life followed by the 4th, 6th and 3rd & 7th. Below 20 years of age, the incidence was only 3.95% and it became sparse again beyond the age of 70 years. Histologically, papillary carcinoma was the commonest subtype. Follicular was the 2nd in prevalence followed by anaplastic medullary and undifferentiated (2.8%). Mixed follicular and papillary variety comprised 1.9%. Majority of the patients come to us after surgery which was subtotal thyroidectomy in 33%, near total thyroidectomy in 22% of cases, radical thyroidectomy in 3.1% cases, and 20% after partial lobectomy. Twenty-seventy point four percent came after FNA, excision biopsy, lymph node biopsy, or cold nodule excision. Ninety-two percent of our patients were from Central Punjab, 6.2% from South of Punjab and 0.5% from the Potohar Region. Patients from the Northern areas only comprised 1.4%. Fifty-two percent were in stage II, 24.5% in stage III, 14% in stage IV and only a small percentage of 4.77 in stage I. Commonest site of metastasis in stage IV cases was bone, followed by lungs, and lymph nodes. In 0.5% metastasis was seen in the liver. For primary treatment, 100 mCi was given once to 41% patients. In 17.5% patients 1131 100 mCi was given more than once. In 12.4% patients 100 mCi was followed by a high dose. One hundred mCi was given once to 1.4% while 100 mCi dose had to be repeated more than once in 9.8%. 100 mCi was given once in 8.2%. Radiotherapy was given to patients with anaplastic and medullary carcinoma or for palliative reasons to 9.3% patients. In our study 20.3% patients of anaplastic and medullary CA received radiotherapy. Role of chemotherapy is limited in Ca. Thyroid. Single agent Adriamycin was given in stage IV patients of anaplastic and medullary carcinoma that is 1.4%, and 55.3%. patients continued to follow us up for 1 year, 24.8% were followed for 2 years, 3.1% of patients expired. Longest follow-up seen was for 7 years in 0.8% of our patients, and 15.8% were lost to follow-up.

1. INTRODUCTION

Thyroid carcinoma is relatively uncommon. In most instances, the cause of thyroid carcinoma is unknown, although experimentally prolonged stimulation by thyroid stimulating hormone may lead to the development of thyroid carcinoma. Some can appear to related to a dose dependent phenomenon on involving radiation to neck during childhood. Thyroid malignancy has been observed 20–25 years after the exposure in atomic bomb survivors. Thyroid cancers that are related to radiation are well differentiated papillary and follicular carcinomas, derived from thyroglobulin producing follicular cells.

Thyroid carcinoma is three times more common in women than in men. It is also evident from our study. Well-differentiated tumours are slow growing and effectively managed by the use of radioactive iodine therapy with doses 100 mCi to 200 mCi (3.7 MBq–7.4 MBq) after various surgical procedures to ablate the residual or metastatic thyroid cancer. In this study the role of high dose I-131 is evaluated.

2. MATERIAL AND METHODS

The period of this study ranged from 1984 to 1997, encompassing the entire period of the existence of this Institute. During this period a total of 354 patients of Ca. thyroid were registered with us. Out of them 257 (72%) were females and 97 (27%) were males. Ca. Thyroid was seen to be predominantly an ailment of females.

Most of our patients fell in the age group between 41 to 50 years (22.3%), closely followed by the 4th decade with an incidence of 20.9%. Subsequently the incidence was seen in the order of the 6th decade (17.7%), 3rd (17.5%) and 7th decade (1 5.2%), after which there was a sharp fall off in the 2nd decade (3.95%). Beyond 70 years of age the incidence again was 2.27%.

Histologically papillary carcinoma was by far the commonest 184 cases (51.9%) followed by follicular carcinoma 112 cases (31.6%). Mixed follicular and papillary pattern was seen in 7 patients comprising 1.9%. 21 patients (5.9%) presented with anaplastic carcinoma, 10 patients (2.8%) with undifferentiated carcinoma and 12 patients (33%) with medullary carcinoma. In a small number of patients (8) only 2.2%, the histology could not be ascertained.

Mostly patients came to us after having undergone some kind of surgical resection, which most commonly was subtotal thyroidectomy in 117 patients (33%). Near total thyroidectomy was done in 79 patients (22.3%). Eleven patients came to us after radical thyroidectomy with nodal dissection (3.1%). Partial lobectomy in 1, and isthmectomy was done in 74 patients (20%). FNA proved malignant in 11 (13.1%). Debulking surgery for huge goitre was done in 4 patients (1.1%). Excision biopsy in 44 (12.4%). Lymph node biopsy showed metastatic Ca. Thyroid in 11 patients (3.1%). Cold nodule resected turned out to be malignant in 3 patients (0.8%).

Majority of our patients, 185 (52%), were in stage II on presentation. Eighty-seven (24.5%) in stage III, 51 (14.4%) were in stage IV and only a meagre 31 patients (4.7%) were in stage I on presentation.

The commonest site of metastasis in stage IV was bone, seen in 21 (5.9%) patients, followed by pulmonary, 19 cases 5.3% Lymph nodes involved in 6 (17%) cases, 3 patients (0.8%) presented with involvement of both lungs and bones. Liver was involved in 2 cases (0.5%).

3. RESULTS

For treatment purposes, the foremost choice for follicular and papillary carcinoma was high dose I-131 100 mCi (3.7 GBq), given once to 145 patients (40.9%). In 62 patients (17.5%), the presence of functional residual thyroid tissue necessitated the dose to be repeated more than once, while in 44 patients (12.4%) 100 mCi (3.7 GBq) was followed by a high dose 150 mCi (7.4 GBq) for complete ablation of thyroid remnants. A high dose of 150 mCi (7.4 GBq) was given once in 8 (2.2%) patients with metastatic disease and complete ablation was achieved after single dose. Less than 100 mCi (3.7 GBq) was given once in 5 patients (1.4%). Thirty-five patients (9.8%) of the total cases were given less than 100 mCi (3.7 GBq) more than once on the criteria of finding residual functional thyroid tissue.

According to the data, 259 patients out of 354 total patients were treated with 100 to 150 mCi (3.7 GBq to 7.4 GBq) and complete ablation was obtained with high dose rate I-131, while only 40 patients were treated with less than 100 mCi of I-131 on outdoor basis when we did not have indoor facilities at INMOL. Radiotherapy was given to 72 patients (20.3%) only for anaplastic and medullary carcinoma. 21 patients were of anaplastic carcinoma and 12 patients were of medullary carcinoma. In 33 patients (9.3%) it was given for palliative purpose in wide spread metastasis.

Chemotherapy was given to 8 patients (2.25%) only for undifferentiated and anaplastic carcinoma 5 patients (1.4%) medullary carcinoma one patient.

4. DISCUSSION

Radioactive iodine I-131 has been used as adjunctive therapy in the management of differentiated thyroid carcinomas for more than 40 years. The aim of the treatment of thyroid cancer with radioactive iodine is to destroy all functioning thyroid cancer after surgery. Prior to radioiodine

therapy, the ability of residual thyroid or metastatic thyroid cancer to concentrate radioactive iodine I-131 is evaluated by whole body scan with I-131. Ablation of thyroid remnants is usually carried out four to six weeks after thyroidectomy. Ablation of residual thyroid tissue may be obtained by the administration of 3.7 GB to 7.4 GB because the efficiency of radioiodine therapy is directly related to tumour uptake and retention effective tumour uptake is approximately 0.5% radioiodine dose per gram with biological half life approximately 4 days, when 3.7 GBq I-131 administered to the patient. In this way a tumour may receive 25000 Gy or 5 times the absorbed dose that can be delivered by external radiation. Two to 3 months after ablation whole body radioiodine imaging is performed to assess the complete ablation of thyroid remnants. Subsequent management is based on the results of the imaging procedure. Repeated treatments are carried out in cases of persisting thyroid remnants.

In our study, 259 patients were treated with high dose of radioiodine ranging from 3.7 GBq to 5.6 GBq. Complete ablation was seen in the majority of patients with high dose radioactive iodine without any serious side effects, while less than 3.7 GBq of radioiodine was given more than once in 35 patients. Multiple doses were given for complete ablation.

5. CONCLUSION

According to our retrospective study, results of high dose Iodine-131 are encouraging and without any serious side effects. Nowadays all the patients with differentiated thyroid cancers are being treated with high dose of radioiodine I-131.

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RADIOIODINE THERAPY FOR PEDIATRIC PATIENTS WITH THYROID CANCER

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Abstract. From 1986 to 1998, 753 patients under the age of 16 were operated on for thyroid cancer. A metastatic disease was diagnosed in 110 (14.6%) cases. In 108 patients (14.3%), there were lung metastases, and in 2 lung and bone metastatic lesions. In 22 of 110 patients (20%), metastases were detected by routine X-ray before therapy. Two patients died without treatment and 108 were selected for radioiodine therapy. Tumor histology was as follows: papillary carcinomas — 104, follicular — 3 and medullary — 1. Sex ratio was 1.2f/1m. Most of the patients had an extended disease. Neck lymph nodes were positive in 103 (95.4%) of cases and in 76 (70.4%) neck metastases were bilateral (pN1b). In 86 patients tumour involved the thyroid capsule and surrounding extrathyroid tissues (pT4). All the patients underwent thyroidectomy with either unilateral or bilateral radical neck dissection. Diffuse lung metastases were diagnosed in 88 cases. A single dose activity of sodium-iodine-131 varied from 3 to 5 GBq. In several advanced cases, the activity of radioiodine in following courses was enhanced up to 7 GBq. The total delivered activity for patients varied from 1.25 up to 43.7 GBq. Response was noted in 107 patients. There were 79 complete responders and in 28 patients partial response was reached. Cancer progression was seen only in one patient with medullary carcinoma, after three courses of radioiodine therapy. All the patients were alive from 6 to 56 months after surgery.

1. INTRODUCTION

Thyroid cancer in Belarus has become an actual problem since 1990 when high incidence of this disease in children was first recognized [1]. It was shown that childhood carcinomas were caused by radionuclides exposure at the time of Chernobyl Power Plant disaster in 1986 [2,3,4].

The peak of incidence for thyroid cancer in children was observed in 1995. After that date the total number of pediatric patients gradually decreasing. We explain this by the fact that exposed children are getting older and spontaneous carcinomas are very rare. The incidence in adults is permanently growing.

Thyroid cancer in children has no specific clinical manifestations and may be diagnosed as a nodule with or without lymph nodes enlargement. According to our previous experience this carcinomas frequently spread into lungs but exact risk for such metastases is not clear.

The purpose of this study is to demonstrate the results of radioiodine therapy as well as to clarify the risk factors for lung metastases in children exposed to radiation.

From 1986 to 1998, 753 patients under 16 were followed up for thyroid cancer in Thyroid Cancer Center of the Research Institute of Radiation Medicine and Endocrinology (Minsk, Belarus). Of them lung metastases were diagnosed in 110 (14.6%) cases. Two patients with a metastatic disease died without any therapy and 108 were selected for radioiodine therapy (Table I). Two patients had both lung and bone metastatic lesions.

The most common tumour type was papillary cancer (96.3%). Tumor histology was as follows: papillary carcinomas — 104, follicular — 3 and medullary — 1.

At the time of Chernobyl disaster 88 (81.5%) patients were under 5 years old and the rest 18.5% were at the age of 5 to 12. There is every reason to believe that thyroid carcinomas in early age group (under 5) have a high potential for metastatic formation (Table II).

2. RESULTS

TABLE I. PATIENTS' DATA

Variables		Children (age under 15)	Teenagers * (age 15–16)	Total
Total number of patients		618	135	753
Patients with lung metastases		97 (15.8%)	11 (8.1%)	108 (14.3%)
Patients' sex:				
	-males	43	6	49
	-females	54	5	59

Sex ratio was 1.2f/1m.

TABLE II. AGE OF PATIENTS AT THE TIME CHERNOBYL DISASTER

	Age, years old													
Sex	*0*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*Total*
Boys	6	11	6	4	7	3	2	4	3	0	2	0	1	49
Girls	15	12	7	5	8	4	4	2	2	0	0	0	0	59
Total	21	23	13	9	15	7	6	6	5	0	2	0	1	108
%	*19.4*	*21.3*	*12.0*	*8.3*	*13.9*	*6.5*	*5.6*	*5.6*	*4.5*	*0.0*	*1.9*	*0.0*	*0.9*	*100*

The probability of lung metastases depended on tumour (pTN) stage. The highest risk was observed for neck lymph nodes involvement and tumours spreading into surrounding tissues. In our series most of the patients had a locally extended disease. Neck lymph nodes were positive in 103 (95.4%) cases including 76 (70.4%) with bilateral involvement of lymph nodes (pN1b). In 86 patients a tumour invasion of surrounding tissues was diagnosed (pT4). Of them multifocal thyroid lesions (pT4b) were diagnosed in 39 patients. Only two patients had a small carcinoma without metastases in lymph nodes (Table III).

TABLE III. STAGING DATA

pT/pN	N0	N1a	N1b	Total
pT1a	1	0	0	*1 (0.9%)*
pT1b	1	0	0	*1 (0.9%)*
pT2a	0	8	2	*10 (9.3%)*
pT2b	1	0	8	*9 (8.3%)*
pT3b	0	1	0	*1 (0.9%)*
pT4a	2	13	32	*47 (43.5%)*
pT4b	0	5	34	*39 (36.1%)*
Total	5 (4.6%)	27 (25.0%)	76 (70.4%)	108 (100%)

The important fact is that in 20 patients(18.5%), lung metastases were detected by routine X-ray before any therapy. In these patients metastases were detected as bilateral small nodules. In some cases these nodules tended to merge and to form lesion bigger than 3 cm in the largest measurement. In the majority of patients, metastases were diffuse and undetectable by primary X-ray. These lesions were proved by isotope investigations after surgery.

In all cases thyroid tumours and regional metastases were removed by thyroidectomy with a simultaneous unilateral or bilateral radical neck dissection.

Radioiodine therapy implied the oral usage of sodium-iodine-131. A single dose activity varied from 3 to 5 GBq and in some advanced cases activity was enhanced up to 7 GBq. The total delivered activity per patient varied from 1.25 up to 43.7 GBq. A number of courses for cure ranged from 1 to 14.

A response was received in 107 patients. There were 79 (73.1%) complete responders. In 28 (26.0%) patients a partial response was reached. In this group the patients continue therapy. Tumor progression was in only one patient with medullary carcinoma after three courses of radioiodine therapy. Most of the patients received from 3 to 5 courses of radioiodine therapy. All the patients are alive from 6 to 56 months after surgery (Table IV, V and VI).

TABLE IV. THE RESPONSE RATES

Effect	Children (age under 15)	Teenagers (age 15–16)	Total
Complete response	74	5	79
Partial response	22	6	28
Cancer progression	1	0	1
Total	97	11	108

TABLE V. DURATION OF FOLLOW UP FOR COMPLETE RESPONDERS

Duration of follow up	Children (age under 15)	Teenagers (age 15–16)	Total
Less than 12 months	13	1	14
12–24 months	34	3	37
24–36 months	20	1	21
36–48 months	6	0	6
48–60 months	1	0	1

TABLE VI. NUMBER OF COURSES AND RESPONSE RATES

Effect	Number of courses														
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*Total*
Complete response	1	4	16	12	10	13	8	5	3	3	1	0	2	1	79
Partial response	2	5	4	6	5	1	3	1	0	1	0	0	0	0	28
Cancer progression	0	0	1	0	0	0	0	0	0	0	0	0	0	0	108

CONCLUSIONS

1. Radioiodine therapy is a highly efficient therapy for pediatric patients with lung metastases of thyroid cancer.
2. The exposure to radionuclides in early age group enhances the risk of lung metastases of childhood thyroid carcinoma.
3. The probability of lung metastases depends on tumour stage. The highest risk was observed for neck lymph nodes involvement (95%) and T4 tumours (80%).

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ABLATION RATE IN 410 PATIENTS WITH DIFFERENTIATED THYROID CANCER

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Abstract. This study covers the results of radioactive iodine treatment given to 410 (306 female and 104 male, mean age 45.4 ± 8.9 yr.) patients operated for differentiated thyroid cancer in the period of 1985–1996. Mean follow up period was 5.4 ± 1.2 yrs. All the patients had residual thyroid tissue lower than 2 gr. TSH was above 30 IU/mL. Doses following the first one were either the same as or 30–50% higher than the previous one. The patients were grouped in three dose levels and percentage success was calculated for each group. None of the patients in group I and II had metastases before the I-131 treatment All the patients in the group 3 had metastases. With the first treatment percentage success was 42.3 in group I, 81.5 in group II and 74.1 in group III. A total of 96.2 in group I, 96.3 in group II and 90.2 in group III was achieved after the second dose. It is concluded that giving a standard dose of 2775–4625 MBq radioactive iodine must be the method of choice for treatment of differentiated thyroid cancer.

1. INTRODUCTION

Differentiated thyroid carcinoma is a tumour derived from thyroid follicular cells and follicular cancer cells may function like normal thyroid tissue [1]. Since only follicular cells can hold and accumulate iodine. Radioactive iodine has a major role for these tumours for treatment and diagnosis[2].

The main pathway followed in the treatment of thyroid cancers is the surgical removal of primary tumour, hormone replacement, ablation with I-131 and treatment of the recurrence of metastatic tissue by I-131. There are different protocols as low dose or high dose I-131 treatment.

In this study the effectiveness of different doses of I-131 was retrospectively evaluated in 410 patients who have been diagnosed with differentiated thyroid cancer and been operated on.

2. MATERIAL-METHOD

410 pts (306 female, 104 male) who had visited Ankara University Medical Faculty, Nuclear Medicine Department in the years 1985–1996 were included in this study. The mean age was 45.4 ± 8.9 and all had had thyroidectomy operation. None of them had >2 grams of thyroid tissue and TSH levels were always above 30 IU/mL. Radioactive iodine treatment was given 4–6 weeks after the operation.

Primary histopathologic diagnosis of the patients were as follows:

325	Papillary carcinoma
63	Follicular carcinoma
4	Medullary carcinoma
7	Papillofollicular carcinoma
6	Hurthle-cell carcinoma
1	Mixed type carcinoma
4	Unknown diagnosis

Evaluation of results are made in three groups: 1.Less than 2775 MBq receiving group, 2.2775–4625 MBq receiving group, 3.More than 4625 MBq receiving group.

3. RESULTS

26 patients (group 1) were treated with 2775 MBq or lower doses of I-131. Of these 26 Pts 11 (42%) had been ablated after the first dose (between two doses), 14 (53.9%) were treated after the second dose. Only one needed a third dose for treatment whereas none of them needed a fourth dose.

303 patients didn't show any invasion except the residual tissue in the neck region in their first I-131 whole body scanning which was 4–6 weeks after the operation. They were treated with 2775–4625 MBq doses. In the 2775–4625 MBq receiving group to (303 pts) ablation was achieved with the first dose in 247 pts (81.5%), the second dose in 45 pts , the third dose in 3 pts, fourth dose in 5 pts and the fifth dose in 1 pt.

Those who had metastases (81) received 4625–7400 MBq I-131 (group 2). Complete ablation was reached in 60 of more than 4625 MBq I-131 receiving patients with metastases (74.1%) where as 13 Pts received a second dose, 5 pts received a third dose and 2 pts received a fourth dose. 1 patient needed a fifth dose for treatment.

All the doses following the first one were 30–50% higher than each previous dose and replacement therapy was planned so as to keep the TSH level below 0.1 IU/mL. HTG levels were measured 3 months after and I-131 whole body scanning was performed 6 months after each dose.

TABLE I. RESULTS OF DIFFERENT DOSES

	Group I (<2775 MBq)		Group II (2775–4625 MBq)		Group III (>4625 MBq)	
		%		%		%
First dose	11	42.3	247	81.5	60	74.1
Second dose	14	53.9	45	14.8	13	16.1
Third dose	1	3.8	5	1.7	5	6.2
Fourth dose	-	-	5	1.7	2	2.4
Fifth dose	-	-	1	0.3	1	1.2
Total # of pts	**26**	**100**	**303**	**100**	**81**	**100**

When ablation rates were evaluated according to the type of primary tumour it is observed that 20/325 with papillary carcinoma received 2775 MBq or less, 253/325 received 2775–4625 MBq, 52/325 with close metastases received more than 4625 MBq. 45/253 who received 2775–4625 MBq needed a second dose, 15/253 a third dose and 5 a fourth dose for treatment.

Eight of 14, whose first dose was less than 2775 MBq, received a second dose whereas 2 needed a third dose.

Twelve of 47 received a second, 5/47 a third and 2/47 received a fourth dose after the first dose which was above 4625 MBq.

Papillary Carcinoma

325 Pts

20 Pts < 2775 MBq-12 pts ablated after the first dose
253 Pts 2775–4625 MBq-207 pts ablated after the first dose
52 Pts > 4625 MBq-40 pts ablated after the first dose

Sixty-three patients who had been diagnosed with follicular Ca were followed 6 received 2775 MBq or less, 40 received 2775–4625 MBq, 17 received above 4625 MBq. Two of 6 needed a second, 10 of the group receiving 2775–4625 MBq needed a second, 4 needed a third and 1 needed a fourth dose. Five of 17 who received more than 4625 MBq needed a second dose for treatment whereas 2 needed a third and 4 needed a fifth dose.

Follicular Carcinoma

63 Pts

16 patients receiving <2775 MBq–4 patients ablated after the first dose
40 patients receiving 2775–4624 MBq–30 patients ablated after the first dose
17 patients receiving >4625 MBq–12 patients ablated after the first dose

4. DISCUSSION

Radioactive I-131 treatment after total thyroidectomy plays a great role in the achievement of complete cure. The first step in treatment should be ablation of residual tissue and then treatment of metastases. Thyroid cancers have been treated with radioactive I-131 for 40 years and this is the most effective way of treatment so far [1, 2].

Presence of residual tissue and metastases have been evaluated with I-131 whole body scanning 4–6 weeks after the removal of thyroid tissue with operation, when the TSH level is equal to or above 30IU/mL. Some researchers report that using higher doses for scanning (1110–1850 MBq) may help in better visualisation of metastases. But this may increase the possibility of stunned thyroid [3, 4]. For this reason use of Tc-99m pertechnetate thyroid scintigraphy is thought to be more proper. In this retrospective study 185 MBq I-131 has been used for whole body scanning and no evaluation has been made for stunning effect.

The reasons why residual thyroid tissue must be ablated can be summarised as follows: 1) Thyroid cancers can be multifocal and malignant cells may exist in residual tissue. Studies show that multiple tumour foci may exist in one lobe (50%) or in both lobes (32%) in thyroid malignancies [5]; 2) Occult metastases may also exist which may be removed by ablation therapy [6, 7]; 3) Differentiated tumours may show anaplastic changes in 2% of cases. Ablation of functional tissue may help in this case; 4) Normal thyroid tissue has a greater affinity to I-131 than metastatic tissue. When normal tissue is ablated, metastases may be better visualized and treated; 5) When whole thyroid tissue is removed, HTG can be taken as a dependable criteria in the follow up; 6) Normal thyroid hormone reduces secretion of endogenous TSH and effect I-131 uptake.

Different doses are tried for the ablation of tissue. A study reports a high ablation rate with 1110 MBq [8] but there are other reports agreeing or conflicting with their results [9, 10].

Maxon et al [11] claimed that there is no significant difference between low dose and high dose therapy in terms of ablation rates achieved (they have obtained 81% ablation with low dose).

When residual tissue is more than 2 grams 69% ablation rate is achieved with high doses where as only 37% ablation was reached with low doses [12,13]. Some reports give the results obtained by giving 1110 MBq the first day and 555 MBq the following day. The aim of this type of therapy is to avoid the need of hospitalization of the patient [14]. Another study comparing the results obtained with 1110 MBq, 3700 MBq and 5550 MBq didn't report any significant differences [15, 16]. When all these results are evaluated, it is observed that there are differences in choosing the patient like the mass of thyroid remnant tissue or presence and localization of metastases.

It is usually believed that high dose therapy must be the method of choice for treatment. But late side effects and its relation with survival rate has not been well evaluated. Our study covers the retrospective results obtained in 410 patients. According to our results we obtained 81.2% ablation rate with 2775–4625 MBq I-131, 42.3% with less than 2775 MBq I-131 and 74.1% with more than 4625 MBq I-131.

As a conclusion, we observed that the best ablation was achieved in the 2775–4625 MBq receiving group. This finding is in agreement with previous experience. It is concluded that the standard dose of 2775–4625MBq gives the highest ablation rate for the treatment of differentiated thyroid cancer patients.

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4. HYPERTHYROIDISM

I-131 THERAPY FOR THYROID DISEASES: DOSES, NEW REGULATIONS AND PATIENT ADVICE

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Abstract. I-131 therapy has been widely used in the past 50 years. Its main applications are hyperthyroidism and functioning thyroid cancer. The indications, doses, regulations, precautions and guidelines differ in various centers. The following are recommended: 1. I-131 should be indicated in agreement of the endocrinologist and the nuclear physician with the patient consent; 2. Pre-treatment I-131 thyroid uptake must be performed; 3. The only contraindication for treatment is pregnancy, in children it might be used with caution; 4. For thyrotoxicosis both a calculated or an ablative dose (555 MBq) criteria are acceptable In this case secondary hypothyroidism must be considered an objective rather than a complication; 5. In uninodular toxic goiter a 1110 MBq dose is recommended; 6. Iodine free diet is indicated only for cancer patients; 7. Propylthiouracil (PTU) must be discontinued 5 days before treatment, it should be reinitiated 5 days later; 8. Prophylactic use of corticoid in Graves' disease still require more clinical data to support its use; 9. In treatment failure, wait six months for a new dose; 10. In intrathyroid cancer disease an ablative dose of 3700 MBq should be administered 4 weeks post-thyroidectomy or with a TSH level above 30 μUI/mL; 11. A whole body scan should be done one week later; 12. Follow-up whole body scan should be used only if there is clinical suspicion of metastasis. Thyroid hormone replacement must be discontinued for 30 days or with TSH value above 30μUI/mL. For follow-up scan 185 MBq of I-131 are recommended to ovoid thyroid tissue stunning; 13. For metastases, 5700 to 7400 MBq dose is recommended if there are cervical lymphatic nodes or distant metastases. We recommended to adopt the criteria proposed by the United States Nuclear Regulatory Commission (NRC) published as 10 CFR 35.75 and the Regulatory Guide 8.39 for patients release after I-131 administration.

1. INTRODUCTION

For the last fifty years, I^{131} has been widely used in thyrotoxicosis treatment [1]. Initially, due to its potential risk it was restricted to males older than 60 years of age but, later its use was generalized to women and children. This paper is partially based on recommendations from a panel of experts organized by the Chilean Society of Endocrinology and Metabolism [2]. The decision between the use of either I^{131} or other therapies is out of the scope of this paper.

A. HYPERTHYROIDISM

1. In the choice of treatment with I^{131} participate: the patient, the endocrinologist and/or the nuclear medicine physician.

With this, we intend to consider the opinion of all participants. In some situations, the criterion of the physician indicating the dose is different than that of the professional who administers it. This issue has been a source of concern for the patient. Treatment should be the product of a teamwork sharing common criteria.

2. IODINE UPTAKE: a) Previous iodine uptake should be done to be sure that the thyroid will receive the desired dose and also to rule out a silent thyroiditis; b) this measurements should be done as close as possible to the therapeutic dose.

Iodine uptake may be altered by elevated intakes of this element [3]. In addition, when the diagnosis of Basedow-Graves' disease is not clear, the differential diagnosis with the thyrotoxicosis stage of silent thyroiditis should be ruled out [4, 5].

3. CONTRAINDICATIONS: The only accepted contraindication is pregnancy, it should always be ruled out by an accurate method, hopefully 24 h before administering the dose and avoiding subsequent exposure to risk.

Although there are reports of women who received I^{131} while being pregnant without mayor problems, there is no doubt that the exposure of the embryo to radiation should be avoided. The administration of I^{131} after the tenth week of gestation may cause fetal hypothyroidism.

4. DOSE CALCULATION: a) Calculate the dose in order to end up with an euthyroid patient; b) Administer an ablative dose that should be more than 15 millicurie (mCi).

Undoubtedly this is one of the most controversial issues. In this respect, is relevant to discuss the proposed alternatives.

Those who favor a calculated dose seek to prevent hypothyroidism. Even though, the overall aim of a therapy is to cure the disease and not to replace it by another, in Basedow Graves' we are not treating the disease but rather curing thyrotoxicosis. There are many alternative formulas to calculate the optimal dose of I^{131} [6–15].

Those in favor of administering an ablative dose, seek to quickly suppress hyperthyroidism and prevent recurrences [16,17]. This is important because of the impact of hyperthyroidism in the quality of life and working capabilities of the patients. Also, in elderly people or in patients with a heart disease, arrhythmia or heart failure may develop. Moreover, the permanent reduction in bone density during periods of thyrotoxicosis should be kept in mind [18]. Although I^{131} may normalize thyroid function, it does not mean that Basedow Graves' disease is controlled; for this reason, the ablative dose has the advantage of preventing both recurrences and the eventual growth of the residual parenchyma, which may occur with low doses. Because ablative doses quickly induce hypothyroidism, this may be diagnosed early and treatment with levo-thyroxin may be instituted before the development of symptoms.

For the above reasons, some of us prefer the use of an ablative dose, starting early with levo-thyroxin substitution, which is a simple treatment, with no contraindications and low cost. The following facts favor this approach: a) with any calculation of the dose there is a sizeable percentage of patients with hypothyroidism; b) hypothyroidism may develop later; c) sometimes a second dose of I^{131} is required for the control of thyrotoxicosis and this is also associated to a high percentage of hypothyroidism and d) thyrotoxicosis is not innocuous.

5. THYROTOXIC UNINODULAR GOITER: a dose of 30 mCi should be administered.

Thyrotoxic uninodular goiters are more resistant to I^{131} treatment because they are composed of hyper-functioning autonomous cells. In this case, the risk of subsequent hypothyroidism is very low because iodine is not uptaken by the rest of the gland due to TSH suppression [19].

6. DIETARY RECOMMENDATIONS: it not necessary to restrict foods or iodinated salt during treatment of hyperthyroidism.

Iodine intake increases the circulating iodine pool; therefore, iodine uptake may be altered. However, in hyperthyroidism, the overstimulation of the thyroid gland makes the eventual amount of iodine in the diet irrelevant. Nevertheless, the intake of some iodinated drugs like amiodarone or other iodinated compounds may have an influence because they contain large quantities of the element [20]. In these cases, I^{131} uptake is very low.

7. PREVIOUS TREATMENT WITH PROPILTHIOURACIL (PTU). a) in elderly patients or those with heart disease, euthyroidism with PTU should always be attempted previously, b) treatment with PTU must be stopped 5 days before the administration of I131.

The treatment with I^{131} elicits a sudden release of hormones stored in the thyroid into the circulation. Therefore, previous depletion with PTU is necessary in patients for whom a sudden increase of circulating thyroid hormones poses a risk. There are documented cases of thyroid storms after treatment with I^{131} [21, 22]. Some authors have postulated that previous PTU administration, renders the thyroid gland more resistant to I^{131} and therefore it could be necessary to increase the I^{131} dose [23–25]. The mechanism is not well known but it has been suggested that iodine depletion could alter the clearance of this element by the thyroid gland [26]. A residual effect of PTU is unlikely because it has been shown that 2 to 4 days after stopping PTU, there is a rapid increase in circulating thyroid hormone levels.

8. TREATMENT WITH PTU FOLLOWING I^{131} ADMINISTRATION a) it should be considered in patients with underlying diseases in whom an exacerbation of thyrotoxicosis following iodine treatment will be detrimental; b) treatment with PTU should resume 5 days after I^{131} administration.

As it was mentioned earlier, thyrotoxicosis may worsen following treatment with I^{131}. Although this has been linked to hormone release due to radiation thyroiditis, it rather seems to be a consequence of PTU discontinuation [27]. Moreover, following treatment with I^{131}, there is an increase of TSH stimulating factor (TRAb). McGregor in 1979 and Atkinson in 1982 showed increased levels of this antibody 3 months after the administration of I^{131} [28, 29]. TRAb levels return to baseline values within a year and at that point, they start to progressively decrease for periods of up to 10 years [30]. This could explain the exacerbation of hyperthyroidism and the delay in the return to euthyroidism in some patients who did not received an ablative dose.

9. PROPHYLACTIC STEROIDAL TREATMENT IN PATIENTS WITH OPHTHALMOPATHY. The information is scarce and non-conclusive. However, some authors suggest that the use of steroids together with I^{131} could be beneficial.

Undoubtedly, the relationship of Graves' ophthalmopathy with the treatment of hyperthyroidism is a controversial problem. There are opposing views, from those who contraindicate the use of I^{131} in patients with ophthalmopathy to those who consider the use of ablative doses in the severe form of the disease [31, 32]. Either if we accept that I^{131} may favor the development of ophthalmopathy or just worsen a pre-existent condition, the use of prophylactic steroids in doses of 20–40 mg of prednisone per day during a month, lowering the dose after that for the next 3 months has been recommended. This treatment would lower or delay the development of ophthalmopathy following I^{131} treatment [33–36]. It has also been suggested that post-treatment hypothyroidism could have a role in the development of the ophthalmopathy and thus the early use of thyroxin would be advisable [37]. More studies are necessary to clarify the eventual relationship between I^{131} treatment and ophthalmopathy, as well as, the validity of prophylactic therapies.

10. POST-TREATMENT CONTROL. Clinical criteria should prevail and thyroid hormone levels should be monitored not before 30 days of the administration of I^{131}. Keep in mind that TSH may be low for prolonged periods of time.

The best laboratory tests for therapeutic monitoring are T3 and T4. These tests should be requested when clinical improvement of the patient is seen; this generally happens after 30 days in patients receiving ablative doses and after a longer period in patients treated with calculated doses. It should be noticed that TSH measurements may be erroneous because this hormone may remain suppressed for weeks or months even if the patient has normal thyroid function [38–40]. In euthyroid patients, TSH levels should be measured at 6 to 12 month intervals in order to rule out the

development of hypothyroidism. Some euthyroid patients treated with calculated doses may present transient hypothyroidism within the first year. However, a significant percentage of them (70%) develop permanent hypothyroidism from 2 to 11 years afterwards [41].

11. TREATMENT FAILURE, a) treatment should not be deemed as failure before 6 months of I^{131} administration, b) if there is persistence of thyrotoxicosis after this period and the second dose is considered, this one should be higher than the first dose.

When treatment with a calculated dose is successful, most patients achieve normal thyroid function within 8 weeks. Nevertheless, some patients normalize their thyroid function within 6 to 12 months because the biologic effects of radiation go beyond the initial radiation thyroiditis that destroys a significant number of follicular cells. Other cell populations undergo genetic damage, which alter cell division with the subsequent slow but progressive loss of thyroid tissue. This is the reason for the delay in the achievement of euthyroidism after I^{131} administration and is the basis for not attempting new doses before 6 to 12 months [42]. When a new dose is administered, it is usually higher than the first one and causes an elevated percentage of hypothyroidism [19].

12. TREATMENT IN CHILDREN AND ADOLESCENTS. There is no formal contraindication for its use; some authors use it as the treatment of choice. Nevertheless, as long as insufficient experience is available, caution is recommended.

One of the main problems with anti thyroid drug treatment in childhood hyperthyroidism is that only 25% of children achieve remission at 2 years, in part due to a lack compliance [43]. On the other hand, surgery may have more severe complications in children than in adults. This has led to a steady increase in the use of I^{131} in this population. In 1985, Hamburger presented his results in patients between 3 and 18 years of age and concluded that: "I^{131} is a safe, simple and low cost therapy and is currently considered the initial treatment of choice for these patients"[44]. The group from the Cleveland Clinic also favors this type of treatment with a high ablative dose [45]. Even though, no complications from I^{131} treatment have been demonstrated so far [46], and as a result of the Chernobyl accident, other authors suggest caution [47].

13. RADIATION RISKS FOR THE PATIENT. There are no reports of an increased risk of neoplasias, genetic damage or infertility with the doses used in hyperthyroidism.

Since 1946, millions of adults have been treated with I^{131}, without an increase in cases of leukemia or other forms of cancer. In addition, no increased risks of congenital malformations in children of treated parents have been reported [48].

14. MEASURES FOR RADIATION PROTECTION: An hyperthyroid patient treated with I^{131} may irradiate more than some cases of cancer.

The use of therapeutic doses of I^{131} is a potential radiation risk for family members, individuals close to the patient, health personnel and the environment. The responsible for radiation protection is the professional who administers the iodine. Regulations should be established by technical organisms.

15. RECOMMENDATIONS ABOUT FERTILITY. Pregnancy should be avoided for 6 months to one year after I^{131}.

Even though the available information shows no increased risk of genetic damage [49], a waiting period post-therapy of 10 half lives (around 3 months), has been suggested. The National Council on Radiation Protection and Measurements states that: "It is prudent to postpone pregnancy for at least several months after I^{131} therapy to repair any genetic damage which may have occurred" [50]. In general, the recommendation is to postpone pregnancy for a minimum of 6 months to a year.

B. THYROID CANCER

1. ABLATION OF REMNANT LOBE: at least 50 mCi should be administered.

If after a subtotal thyroidectomy the definite pathology report shows thyroid cancer, in exceptional cases it is suggested to eliminate the other lobe with iodine rather than surgery. In these instances, we suggest to use at least 50 mCi because in contrast with hyperthyroidism, this is normal thyroid tissue. Iodine uptake, size of the lobe, etc., must be considered.

2. ABLATIVE I^{131} AFTER A TOTAL OR NEAR TOTAL THYROIDECTOMY IN PATIENTS WITH DISEASE CONFINED TO THE THYROID. It is suggested to wait 4 weeks after surgery, unless TSH values increase above 30 mUI/mL. At that time, a therapeutic ablative dose of 100 mCi should be administered. 7 days afterwards a whole body scan is performed.

The recommended dose is controversial. Good results have been reported with lower doses in series like that of Beierwaltes of 511 patients treated between 1947 and 1984. However, with the dose of 100 mCi, there are higher percentages of success and there may be even an effect on undetected metastases [51].

3. WHOLE BODY SCAN IN THE FOLLOW-UP OF THE DISEASE: a) it should be done only when metastases are suspected or there are bad prognostic indices, b) treatment with thyroxine should be stopped for 30 days or TSH above 30 mUI/mL, c) a 5 mCi dose of I^{131} should be used.

This should not be considered a routine procedure to detect metastases since thyroglobulin may increase before a metastasis is visible on an iodine scan [52]. Discontinuation of thyroxin replacement is usually problematic due to the development of hypothyroidism [53]. If there is no contraindication (heart disease patients, advanced age, etc.), an alternative to shorten this period is to change to liothyronine, 25 mg bid and to stop for 2 weeks. With this approach, an adequate elevation of TSH is generally achieved [54]. The use of recombinant TSH seems to be a promising alternative [55]. Optimal doses between 5 and 10 mCi and even lower doses have been suggested. Although some authors report a higher detection rate for metastases with the use of higher doses, functional tissue "stunning" may occur [56] and the tumor cell loses its ability to uptake iodine. Because there is some delay in the occurrence of this "stunning", if the decision is to administer a therapeutic dose, it is advisable to do it as soon as possible [51].

4. SINGLE THERAPEUTIC DOSES IN METASTASES. a) the dose should be between 150 and 200 mCi, b) the dose should not be repeated before 6 months.

Bierwalters reported good results with doses no <100 mCi when there is uptake only in the thyroid bed; no <150 mCi if there are enlarged lymph nodes and at least 175 mCi if there are distant metastases. No effectivity has been proven for doses above 200 mCi [51].

5. DIET RECOMMENDATIONS: A low iodine diet is recommended for 7 days before treatment.

In contrast to hyperthyroidism, in cancer iodine uptake is usually low. A decreased urinary excretion of iodine following a week with iodine-free diet has been shown [51].

The following types of food should be excluded: iodinated salt, milk and derivatives, eggs, fish and seafood, fast food and any food seasoned with iodinated salt. In our country, salt has a high iodine content [57–59].

6. RADIATION RISKS FOR THE PATIENT, PREGNANCY AND FERTILITY: The same as in hyperthyroidism.

Some studies like that of Smith who presented 32 adolescents less than 20 years of age who received therapeutic doses above 250 mCi for thyroid cancer reported 69 subsequent pregnancies. There was no increased risk of infertility compared to the general population and there were only 2 congenital malformations in the offspring of mothers treated during pregnancy or 6 months before conception. This reinforces what has been previously stated for hyperthyroidism in relation to postpone pregnancy for at least a year after treatment [60]. In males treated with I^{131} some cases of infertility have been reported, however, low sperm counts have recovered after a few months. This risk is higher in patients with large pelvic metastases [51].

C. RADIATION SAFETY, PRECAUTIONS AND PATIENT ADVICE:

Therapeutic doses of ^{131}I may be a potential radiation risk both for family members and individuals close to the patient, as well as, health workers and the environment. Therefore, it must be used according to strict safety measures, precautions and special instructions in order to avoid unnecessary exposure to radiation.

The administration of ^{131}I must be done under the responsibility of a physician who must hold a license to manipulate radioactive materials. The reception, use and storage of radioactive material must be done at a medical institution which holds a radioactive installation license.

The physician administering the ^{131}I dose shall be responsible for taking all the precautions to avoid unnecessary radiation to people close to the patient, health personnel and the general public. This professional must keep a logbook with all the radioactive quantities administered to each patient. As a rule, all reasonably acceptable measures should be taken to decrease radiation exposure to a minimum (criteria known as "ALARA" = As Low As Reasonably Achievable). In places where there is no written rules to release patients submitted to radioactive treatment, we recommend the adoption of the U.S.Nuclear Regulatory Commission (NRC) established in rule 10 CFR35.75 [61], which was revised and came into effect on May 29, 1997. This rule is in agreement with the dispositions of the International Commission for Radiation Protection (ICRP 60, 1990) and the National Council for Radiation Protection and Measurements of the USA (NCRP).

In summary, the new rule number 10 CFR 35.75 establishes the following:

- Any patient who may expose other individuals to an equivalent effective dose above 1 mSv (100 mrem) must receive written instructions from the treating physician that fulfil the "ALARA" criteria.

- In order to send home a patient who has undergone radioactive treatment, he or she should not expose any other individual to a radiation dose above 5 mSv (0.5 rem in a year).

This new rule allows for the establishment of criteria based on each individual and his or her own environment [62]. Thus, more flexibility is allowed for a person who lives alone in a solid house and more strict measures must be taken when the patient lives with the family in a small room in a less solid construction. This new rule replaces the former that only allowed to send home individuals with dosages lower than 1110 MBq (30 mCi) or when radiation measured at 1 meter from the patient did not go over 0.05 mSv (5 mrem) per hour.

D. SUGGESTIONS FOR PATIENTS' WRITTEN INSTRUCTIONS SHEET

Why are you going to receive radioactive treatment?

You are going to receive radioactive iodine treatment because together with your doctor has been decided that this is the best option for your disease. Most of the radiation emitted by the iodine will be absorbed by your thyroid gland, which is located in the anterior part of the neck. This radiation interferes with the function of your gland producing a desired and beneficial effect for your disease. However, small quantities of the radiation present in your body may reach people close to you exposing them to this radiation unnecessarily. Although there is no evidence that this radiation exposure has damaged other individuals, people should avoid exposure to any unnecessary radiation.

How is radioactive iodine administered and what sort of preparation is required?

Radioactive iodine is given orally in variable quantities according to the type of your disease. Your treating doctor together with the physician who will actually administer the treatment determined the dose. According to the administered dose and your condition, it is possible that you should remain hospitalized for some days. Women must be completely sure that they are not pregnant at the time they receive the treatment. Food should not be ingested in the 2 hours before receiving the treatment and in some cases, an iodine-low diet will be recommended for a few days. You should talk to your doctor to clarify all your doubts in order to organize the activities of you and your family.

For how long does iodine remain in my body?

Radioactive iodine remains in your body just for a few days. Mainly the urine eliminates most of the iodine not retained in your thyroid, within 48 hours. A small quantity will be present in the saliva, sweat and stools. The radioactive iodine that remains in your thyroid gland also decreases quickly. This means that the possibility of unnecessary radiation exposure to other people also decreases in a matter of days.

In which way other people may be exposed to my body's radiation?

Radiation emitted by the radioactive iodine in your body is very similar to the x-rays used in radiological exams. For this reason, people who remain close to you and for prolonged times may be exposed to an unnecessary and avoidable radiation.

Besides the above mentioned radiation, there is the possibility that other people close to you may directly ingest small quantities of radioactive iodine eliminated by your body in the urine, saliva or sweat.

In which way can I reduce the risk of radiation exposure to other people?

Even though the amount of radioactive iodine present in your body is small, and there is no evidence that the radiation emitted by it may cause problems, anyway it is advisable to decrease the opportunities to exposure as much as possible. The three basic principles to avoid unnecessary radiation exposure are:

- **Distance**: do not get too close to any other person. Radiation decreases significantly with increasing distance.
- **Time**: Radiation exposure to other people depends on how long they remain near you. Therefore, avoid prolonged contact with other people.
- **Hygiene**: Good hygiene minimizes the possibilities of direct contamination with radioactive iodine. Because most of the iodine is excreted by the urine, it is very important that you wash your hands thoroughly after going to the toilet.

PRACTICAL ADVICE

- Ask your doctor to give you all the necessary recommendations in detail to avoid unnecessary radiation to people who are close to you and other individuals. Clarify all your doubts and do not be afraid to ask.
- Sleep alone during the first days after the treatment. During this period, avoid kissing and sexual intercourse. Avoid close and prolonged contacts with other people, especially children and pregnant women because they are more sensitive to radiation than the rest of the population.
- If you have a small child or you are in charge of one, request especial instructions from your doctor. Do not hold him or her on your lap; do not feed him or her, change diapers, etc. If you are breast-feeding, you must stop because the iodine is excreted into breast milk. You must switch to other types of milk.
- You must wash your hands thoroughly after going to the toilet. Use more toilet paper than the usual amount. Flush the toilet 2 or 3 times after using it. Men are advised to urinate sitting down to avoid splashing urine outside the toilet bowl or in its borders.
- Drink large amounts of fluid to eliminate as much urine as possible. Eat tart candy or lemon juice to produce more saliva and in this way prevent iodine retention within salivary glands. Keep your toothbrush separated from those belonging to the rest of the family.
- Put aside for your use a set of silverware (spoon and fork) and wash them separately with abundant water. Do not bite your nails or put objects in your mouth like pencils, necklaces, etc.
- Separate a towel for your exclusive use. Wash your underwear and bed linens separate from the rest and rinse it several times.

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RADIOIODINE THERAPY FOR HYRERTHYROIDISM

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Abstract. A ten year study (1988–98) was done at the Veterans Memorial Medical Center on radioiodine (RAI) therapy for hyperthyroidism. A total of 162 patients received 131-I after careful selection hence was included in this study. A predominantly female population was seen (81%) compared to only (19%) males. The most frequent age group were in the third and fourth decades of life. Those included had clinical manifestations of thyrotoxicosis aside from the abnormal thyroid function tests and elevated RAI uptake. Almost all were given antithyroid drugs and beta-blockers prior to RAI ablation. Doses ranged from 7 to 12 mCi depending on gland size and degree of toxicity. Success rate of treatment was 92% which meant that the symptoms were abated and there was shrinkage of the thyroid gland after a single dose of iodine. The most common short term complications were sialitis and local neck tenderness while hypothyroidism was the commonest long term complication.

1. INTRODUCTION

The use of I-131 in 1946 for the treatment of hyperthyroidism marked a historic event. It ushered in a new era of radionuclides in medicine and led to the birth of nuclear medicine. Today I-131 has become one of the most commonly used agent for the treatment of hyperthyroidism.

Ninety percent (90%) of its effect is due to beta radiation and ten percent (10%) is due to gamma radiation. The mechanism of action is production of radiation thyroiditis (3–10days) and chronic gland atrophy (over a period of 3 years).

To achieve the necessary dosage levels, four considerations are needed: maximal amount of I_{131} taken by the thyroid gland, size of the tissue to be irradiated, effective half life of the isotope in the thyroid gland and relative sensitivity of the thyroid to I-131.

There are two kinds of dosing–the preferred dose where 160 uCi/gram of tissue is given (15–20 uCi) or the usual dose of 80 uCi/gram (2–15 uCi). In giving these dosages four basic approaches are utilized: administer the same dose in mCi according to gland size to every one (rarely used); vary dose in mCi according to gland size depending on severity of hyperthyroidism; administer a dose calculated to deliver a predetermined number of microcuries per gram of estimated thyroid weight (based on RAI scan), and lastly, estimate dose in terms of rads delivered based on half life of I_{131} in the gland, thyroid weight and 24 hour RAI uptake.

The two major principles are: give a dose to produce hypothyroidism in most recipients by giving 200 uCi/gram or more (10–12mCi) then give thyroid hormone replacement ; give a calculated dose to produce a cure with lowest incidence of hypothyroidism that is 50 –80 uCi/gram (3–5 mCi).

However, precision in the calculation of I_{131} dose makes very little difference in the outcome in any individual patient. The inherent sensitivity of the thyroid seems to vary widely for unknown reasons.

The success in treatment is high with incidence of cure as follows:70–86% in single doses; 10–20% using two doses and less than 5% required 3 doses or more.

Adjunctive therapy in the form of antithyroid drugs, beta blockers and steroids may be needed.

There are short and long term complications where hypothyroidism is the most important complication that must be treated. Long term follow-up is advocated by FT4 and TSH determinations. There is an unknown risk for malignancy and genetic damage.

Finally, I_{131} has been a choice of treatment for hyperthyroidism with some considerations in the United States, Europe and Asia. It is rapidly effective, predictable and inexpensive.

2. METHODOLOGY

Patients coming from the Outpatient Thyroid Clinic as well as admitted patients from the VMMC and private referrals with clinical manifestations of hyperthyroidism were included in this study. RAI uptake and RAI scan FT3, FT4 and serum TSH by RIA were determined. A few patients had ultrasonography of the thyroid gland and were already on antithyroids that a scan cannot be done. Excluded from the study were pregnant and lactating women, children below 18 years of age as well as those with large and bulky neck masses.

The patients were given three options of either continuous antithyroids (1–2 years), RAI ablation, or surgery.

3. RESULTS

After proper patient selection, one hundred sixty two (162) patients were treated with I_{131} hence were included in this study.

There was a predominantly female population of 81% and only 19% of males. The most frequent age group affected were those of the third and fourth decade of life . (See Figure 1)

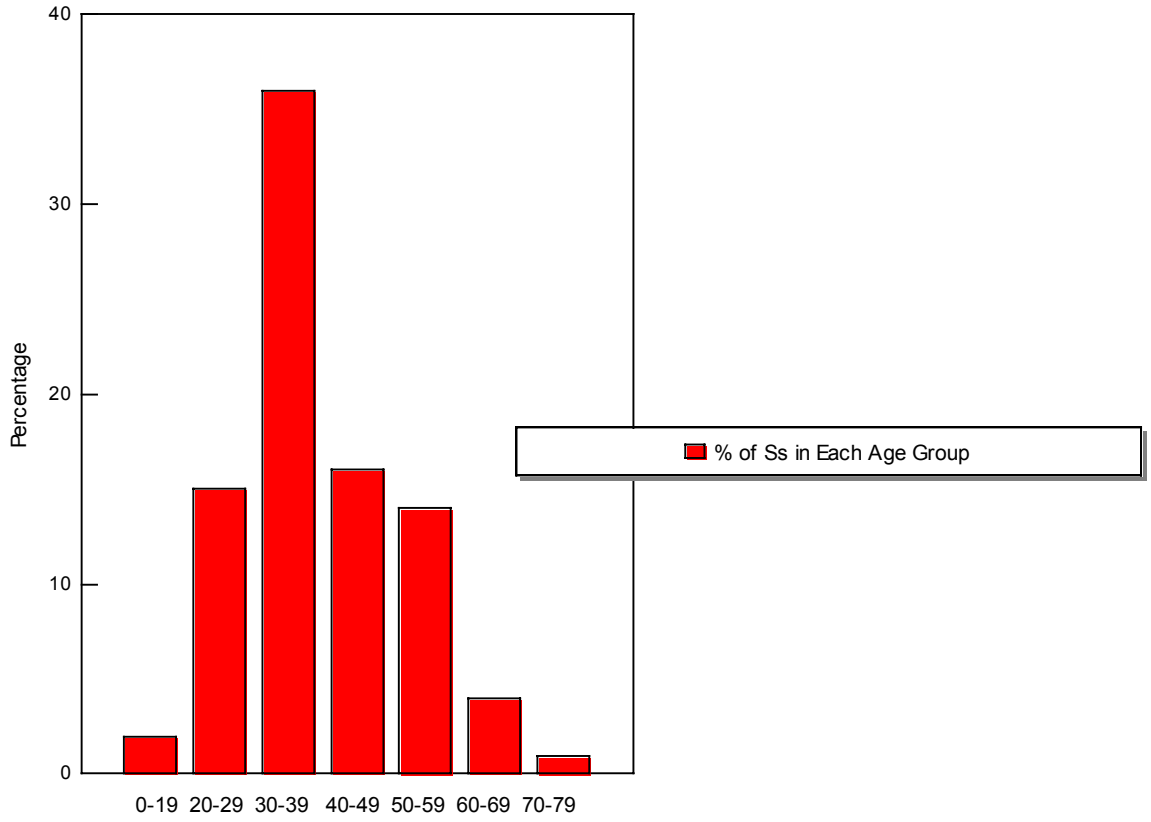

FIG. 1. Age group frequencies.

Ninety (90%) percent of the total population had history of emotional stressful event and only ten percent (10%) had positive family history.

The most frequent clinical manifestations were unexplained weight loss in spite of good appetite, palpitations, profuse sweating, goiter, fine tremors, exophthalmos and muscle weakness. (see Figure 2)

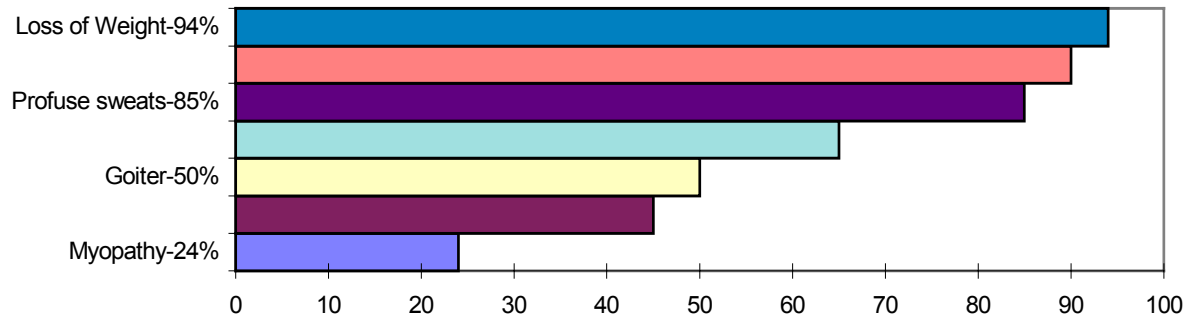

FIG. 2. Most frequent clinical signs and symptoms.

Biochemical parameters included elevated 4 and 24-hour RAI uptakes, elevated FT3 and FT4 as well as decreased serum TSH by radioimmunoassay. We started the patient on antithyroid for two to four weeks prior to the ablation therapy I-131. We had six patients who were allergic to the antithyroids so that they were given I-131 at once. Most patients were also given long acting beta blockers and around ten percent of the population were given steroids for ophthalmopathy.

The antithyroids were stopped 3–5 days prior to treatment . They were also given an iodine - free diet for the same length of time.

Since our earlier experience of solving the dose and using five mCi or less resulted in recurrence of hyperthyroidism, we started the patient on 7 mCi up to 12 mCi depending on the degree of toxicity and thyroid mass or volume. We also computed the preferred dose occasionally.

A total of 102 (63%) received 10 mCi and 42 patients (26%) received 7 mCi while nine patients each received 8 and 12 mCi consisting of 5%. (See Figure 3)

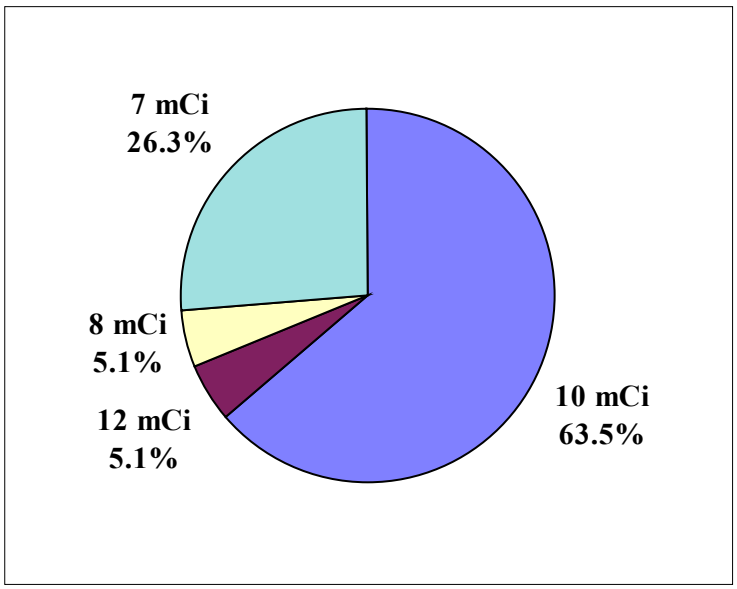

FIG. 3. RAI Dose/Frequency.

Post I_{131} therapy were uneventful except for minor complaints of sialitis-10% and mild neck pains-12%. The most common long term complication was hypothyroidism which occurred in 9.2% of the population. As soon as this was recognized, thyroid hormone replacement was given.

Success of treatment was 92% which meant symptoms of the disease were abated and shrinkage of the gland was achieved in 2–4 months with just a single dose while 7% required a second dose. Two patients were to be on continuous antithyroid treatment.

4. DISCUSSION

The aim of I-131 administration is the production of radiation thyroiditis just enough to reduce thyroid function to normal, without causing hypothyroidism. It seems though that the autoimmune mechanism of each individual vary from one person to another [1] hence the variation in the treatment response. Also certain life events [2] as emotional stress may affect the body's immune processes as seen in the majority of our patients causing Graves' disease. In some patients they had positive family history.

Our study also showed that fixed dosage is just as effective as a dosage calculated at great expense. Fixed dosage is less time consuming and more cost effective [3] Estimating the dose in terms of rads delivered based on half life of I-131 in the gland, thyroid weight and 24-hour uptake seems to be more theoretically valid but has been proven inaccurate because half life of the therapeutic dose is often different from the tracer dose.

Precision in the calculation makes very little difference in the outcome in any individual patient, so that we can say that in large group of patients, I-131 is a function of dose and similarly the persistence of hyperthyroidism is inversely proportional to dose.

Finally, our study is similar to that of other studies as of H Peters [4] wherein there was more success in giving a standard dose based in thyroid size rather than calculating the dose (71%) against (58%).

5. CONCLUSIONS

Radioactive iodine is the treatment of choice for hyperthyroidism in properly selected patients. Treatment must be individualized. The delivery target remains to be 4000 to 5000 rads to destroy overactive follicular cells. Precise calculation makes very little difference in the outcome in the individual person due to inherent sensitivity. Low dose decrease the incidence of hypothyroidism but decreases effectiveness. Complications such as sialitis and local neck tenderness are short tern complications and hypothyroidism is the commonest long tern complication. Serum T4 is a better gauge than serum TSH for the first post-treatment months while TSH becomes sensitive later. Long term studies (40 years follow-up) shows that radiation does not induce genetic damage leukemia, or thyroid carcinoma, All attempts at dosimetry have thus far failed to reliably deliver a dose to the thyroid that avoids recurrence and does not ultimately lead to hypothyroidism and the reason is complex.

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EVALUATION OF RESULTS OF MORE THAN 20 YEARS TREATING HYPERTHYROIDISM BY I-131

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Abstract. The authors have summarized their works of more than 20 years using I-131 for treatment and close observation of 723 patients with hyperthyroidism in 1000 ones in the Nuclear Medicine Department, Bach Mai University Hospital in Hanoi to collect data and draw experience for the report. Patient selection for the treatment is based on clinical features and laboratory tests results by the Nuclear Medicine Department such as thyroid uptake, scintigraphy and RIA determinations of thyroid hormones. I-131 dose is determined in compliance with a prevailing formula. The average dose is 6.2 ± 1.1 mCi (that is 233.1 ± 40.7 MBq). The average number of times is 1.3 time for one patient. The results are as follows:

- Euthyroid status after 4- year following- up from date of I-131 dose administration: 72.3%
- Persistent or recurrent hyperthyroidism: 20.0%
- Hypothyroid complications:
  - appear 4 to 12 months after date of I-131 administration: 3.0%
  - appear 4 years after date of I-131 administration: 7.7%
  - appear 6 years after date of I-131 administration. 14.0%
  - so the cumulative hypothyroid rate is 2.3% per year.
  - No occurrence of other serious complications by all the observed patients.

This is therefore a safe, efficient treatment method to be applied on a large scale including adolescents and children. However, much more study has still to be made on the dose due to high rate of recurrence of the therapeutic method although the hypothyroid complications cases are not serious. Hyperthyroidism is a common health problem in Viet Nam [1]. Previously, only anti-thyroid drugs and surgery were used. Use of I-131 was firstly introduced to Viet Nam in the Nuclear Medicine Department in Bach Mai in 1974 and afterwards applied larger nationwide. Initial therapeutic results have been published in national medical magazines. This is a general study aiming at analyzing the way to carry out the work and get experience and recommendation from gained results for further work in the future.

1. MATERIALS AND METHODS

1.1. Patients

The number of hyperthyroid patients under treatment in our Department amounts to more than 1,000. But only 723 cases are treated and closely observed. A majority is affected by Graves-Basedow disease: 521/723 patients (accounting for 72%). The remaining 202/723 (accounting for 28%) have toxic nodular goiter. They come to the hospital by themselves or are introduced by lower-level medical units of the outskirt of Hanoi. Their personal particulars are shown in Table I

TABLE I. PERSONAL PARTICULARS OF PATIENTS.

Patients	Health problem		Age			Sex	
	Basedow (1)	Nodular (2)	<30	30–50	>50	Male	Female
Number	521	202	36	487	203	94	629
%	72	28	5	67	28	13	87

Notes: (1) Graves Basedow's disease
(2) Toxic Nodular Goiter.

As the intention is not to treat for the time being too young patients, therefore the youngest patient here is 24 and the oldest, 74.

Detailed records show that the patients are under following categories (Table II):

TABLE II. CLASSIFICATION OF PATIENTS BY PREVIOUS TREATMENT

Patients	Under previous anti-thyroid drugs treatment	Having bad side effects with anti thyroid drugs	Recurrence after thyroidectomy	Not under any treatment whatsoever
Number	535	22	14	152
%	74	3	2	21

- Patients already treated by anti-thyroid drugs but either the results are nil or not stable: 535 patients accounting for 74%.
- Patients getting bad side effects such as itching, having pimples, feeling uneasiness and vomiting, leukopenia, with anti-thyroid drugs: 22 patients accounting for 3%.
- Patients with recurrence of the disease after thyroidectomy: 14 patients accounting for 2%.
- Patients going directly to our Department without any previous treatment 152 patients, accounting for 21%.

1.2. Treatment process

As to patients under clinical examination, attention is paid to overall physical status, characteristics of the thyroid goiter, ophthalmopathy, fingers' trembling, cardio-vascular, digestive, neurotic troubles etc. [2]. Necessary laboratory tests are also made such as electro-cardiography, hemo/biochemical tests. Patients also get radioiodine thyroid uptake, scintigram. Since 1982, the determination of such concentration T3, T4, TSH, FT3, FT4 in plasma by RIA and IRMA method has been also applied. Results are gained through automatic sample changing gamma counter MAG-312 manufactured by Berthold- Germany [3, 4, 5].

Those are the indicators to determine the disease, to evaluate the seriousness of hyperthyroidism as well as to evaluate the treatment efficiency.

Table III shows the appearance of main clinical symptoms of observed patients affected by hyperthyroidism.

TABLE III. APPEARANCE OF MAIN CLINICAL SYMPTOMS OF OBSERVED PATIENTS AFFECTED BY HYPERTHYROIDISM

Patients	Big goiter (1)	Proptosis (2)	Cardio-vascular troubles (3)	Digestive troubles (4)
Number	231	152	348	101
%	32	21	48	14

Notes: *(1) The weight of the thyroid gland is over 50 grams.*
(2) May be accompanied with other opthalmopatic symptoms.
(3) ECG clearly changed and pulses over 90 per minute.
(4) Loose stool or mild diarrhea.

1.3. Dose of I-131 used in treatment

We have calculated the dose used in treatment in compliance with the common formula [1, 6]:

$$D = (C.W)/U$$

in which:

D: (in μCi) is the dose used by the patient

C: the radioactivity of I-131 necessary in 1 gram of the thyroid tissue.

This value is often determined from 80 μCi to 120 μCi (that is from 2.96 to 4.44 MBq) depending on the characteristics of goiter, concentration of thyroid related hormones in plasma and the over physical status of the patient.

W: the weight (in gram) of thyroid gland. Its value is determined by palpation. It is cross checked with scintigram and with ultrasonic pictures of the thyroid gland later.

U: 24-hour thyroid uptake after dose administration (in%).

The average dose for each patient is 6.3 ± 1.1 mCi (i.e. 233.1 ± 40.7 MBq). In fact, there are 24 patients (0.3% of total patients) using total doses exceeding 20 mCi (740 MBq). A majority of patients (578 patients accounting for 80%) uses only a single dose. As many as 109 patients (15%) use double dose every 3 month due to lack of efficiency of the 1st dose. And 36 patients (5%) even use triple dose due to recurrence. Those who can not recover or are recurred the disease amount to 145 (20% of the total patients under treatment). Thus, the average number of drug administration time k is:

$$k = [(578 \times 1) + (109 \times 2) + (36 \times 3)]/723 = 1.3$$

All patients stay in the hospital as interns for a duration of 3–5 days and then get their medical check after the 1st three months. Then periodically after 6 months or 1 year, we send invitation letters to them for medical check but they can come to us whenever they feel the presence of some abnormal symptoms.

The medical check consists of both clinical examination and determination of thyroid hormones. The criteria for evaluation are:

- *Euthyroidism:* Normally getting body weight, no fingers' trembling, relieving of tiredness, feeling of easiness, normal pulsation (about 80 beats per minute). Hormones are determined at normal level.
- *Persistent hyperthyroidism:* Presence of hyperthyroid symptoms–though lessening and hormone value determined at abnormal rate. The majority of the cases can be determined in the 1st three months after radioiodine administration.
- *Recurrent hyperthyroidism*: Recurrence may occur after 6 months, one or two years feeling better since the 1st radioiodine dose administration.

2. RESULTS

2.1. Clinical symptoms

Table IV shows the clinical therapeutic results after treatment.

TABLE IV. THE CHANGES OF CLINICAL FEATURES AFTER TREATMENT

Patients	Putting on body weight	Decreasing volume of goiter	Decreasing proptosis	Decreasing cardio-vascular troubles	Decreasing digestive troubles	Decreasing fingers trembling
Number	615	578	46	319	96	638
%	85	80	30	92	95	98

The decreasing volume of goiter is more popular in patients affected by Basedow' disease than those with toxic nodular goiter (respectively 82% and 18%).

Ophthalmic symptoms are in particular very hard to find out and evaluate their seriousness. Subjective symptoms such as lacrimation, (feeling of) burning, photophobia, blurred vision etc... can be more or less relieved in 65% of the cases. However, the number of patient of decreasing proptosis can be determined in 30% of the patients with proptosis. No cases of increasing proptosis have been so far found after I-131 administration.

2.2. Nuclear medicine methods

Table V shows the results of thyroid uptake in hyperthyroid patients before and after treatment.

TABLE V. VALUE OF THYROID UPTAKE IN HYPERTHYROID PATIENTS AFTER TREATMENT: 2 HOURS AND 24 HOURS

Group of patients	After 2 hours (%)	After 24 hours (%)
Healthy people	14.5 ± 3.0	32.5 ± 7.0
Hyperthyroid patients before treatment	47.2 ± 15.9 (a)	70.9 ± 11.6 (a)
Hyperthyroid patients after successful treatment 4–12 months	15.9 ± 5.6 (b)	31.9 ± 6.4 (b)

Notes: (a) $p < 0.01$ as compared with healthy people.

(b) $p > 0.05$ as compared with healthy people.

Table VI shows the concentration of T3, T4 and TSH in different groups of patients ' plasma.

TABLE VI. CONCENTRATION OF T3, T4 AND TSH IN DIFFERENT GROUPS OF PATIENTS' PLASMA

Group of patients	T3 ($nmol.L^{-1}$)	T4 ($nmol.L^{-1}$)	TSH ($mU.L^{-1}$)
Healthy people	1.98 ± 0.54	109.17 ± 17.70	2.01 ± 0.91
Hyperthyroid patients before treatment	10.70 ± 6.72 (a)	282.12 ± 80.52 (a)	0.10 ± 0.04 (a)
Hyperthyroid patients after successful treatment 4–12 months	1.79 ± 0.95 (b)	109.88 ± 43.10 (b)	1.87 ± 0.85 (b)
Hyperthyroid patients after successful treatment 4–10 years	2.12 ± 0.48 (b)	110.21 ± 24.69 (b)	1.99 ± 0.89 (b)

Notes: (a) $p < 0.01$ as compared with healthy people.
(b) $p > 0.05$ as compared with healthy people.

We can deduct from data in these tables that if treatment is successful, the tests will show the results within normal frame.

Based on clinical features and results of thyroid hormone concentrations, we classificated the treated patients as follows:

- *Euthyroid status* is observed after 4 years from date of I-131 dose administration in 522 patients (accounting for 72.3%).
- *Persistent or recurrent hyperthyroidism*, with which the patient had to take more than one dose of I-131 to attain euthyroidism: 145 patients (20% of total patients).

- *The number of hypothyroid patients* has been increasing over observation time and appears as follows:
    - 4 to 12 months after date of radioiodine administration: 22 patients (accounting for 3%).
    - 4 years after date of I-131 administration: 56 patients (7.7%).
    - 6 years after date of firstly therapeutic dose administration: 101 patients (14%).

  So the cumulative hypothyroid rate during 6 years at our patients is:

$$\frac{101 x 100}{723 x 6} = 2.3\% \text{ per year}$$

3. CONCLUSIONS

3.1. This is an efficient treatment method. The percentage of patients attaining euthyroidism after 4–year following-up is 72.3%. Persistent and recurrent patients requiring high doses account for up to 20%. In the 1st period, this percentage is higher due to our hesitance for the determination of C in the formula to specify dose and, hence, the C-values are lower than that in successive periods. Therefore, better results are achieved as we have more experiences. In relation with the high figure of this percentage, another reason may be that the majority of our patients was previously treated by anti-thyroid drugs but not successful before being administered with I-131.

Therefore, our results of attaining euthyroid status for our patients are similar to those by other authors (6, 7, 8) but the percentage of persistent and recurrent patients in the report are higher.

3.2. We have not so far faced with noticeable complications from our 723 patients under following-up and also no genetic disorders are found in the patients' children after their treatment by I-131. Also, no thyroid storm occurs after I-131 administration. Therefore this is a safe method to be applied largely. That is the reason why from day to day the number of patients, which come directly to our Department without previous treatment by any other method, is growing. At first, the number is only about 11% and then reaches to 25% of the total patients. Our conclusion is that if no stable and clear results by internal medicine are found after one year of treatment, we 'd better resort to radioiodine treatment.

Anyway, there is still much worry on the long-term radiation effect; therefore, little application of I-131 has been made, especially to our children and women at child-bearing age.

3.3. The determination of dose in compliance with the above formula is rather convenient and simple. The specification of thyroid gland weight needn't to be too complicated but just by palpation and by ultrasonography and it is enough. The concentration of T3, T4 in the patients' plasma and even of FT3, FT4 will be important data to readjust suitable C value. However, we think that the C value can be higher to decrease the number of persistent and recurrent patients. This still needs a more attentive study.

A number of patients must use a very great total dose (>20 mCi or >740 MBq) to attain euthyroid status. Perhaps, the 1st radioiodine doses are not enough for being effective and preventing the absorption of the following dose; or these patients can be more radio-resistant than the others. No method is found to determine this factor.

3.4. Hypothyroidism is still considered as an unavoidable complication after I-131 treatment. The percentage of hypothyroid patients has been increasing over observation time but still lower than that reported in [6–8]. It is possible the dose is not strong enough because of patients

worry about being dull caused by hypothyroidism that makes us hesitant. Nowadays, thyroid hormone medicine are available and cheap. The treatment of hypothyroidism is not difficult and expensive. We should be; therefore, bolder in the use of I-131 to treat hyperthyroidism.

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5. TREATMENT OF BONE PAIN

32-PHOSPHORUS FOR BONE PAIN PALLIATION DUE TO BONE METASTASES, ITS SAFETY AND EFFICACY IN PATIENTS WITH ADVANCED CANCER*

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Abstract. Bone pain due to bony metastases can seriously affect a patient's quality of life. External irradiation, narcotic drugs and polyphosphates may cause important side effects or are expensive, therefore in many patients radionuclide treatment using a single dose of beta emitting bone seeking radiopharmaceuticals has become widely accepted. Except 32-Phosphorus (32-P) all of them are expensive and difficult to obtain in certain countries. The aim of the study was to evaluate safety and efficacy of 32-P for palliation of bone pain due to bony metastases by comparing it to 89-Strontium (89-Sr), the most commonly used radiopharmaceutical for bone pain palliation in the framework of a prospective IAEA co-ordinated multicenter study. A very strict protocol for unified patient inclusion and follow up was used. 93 cancer patients with osteoblastic bony metastases were included into the study, 48 were treated by 89-Sr (150 MBq) and 45 by 32-P (450 MBq). Pain score, analgesic consumption, quality of life, and indices of bone marrow depression were monitored 2 weeks pre- and up to 4 months post treatment. Favourable response to treatment was recorded in 75% of the patients treated with 89-Sr and in 60% of those treated with 32-P (p=0,122). There was no significant difference between the duration of favourable effect for both radiopharmaceuticals. Moderate decrease of white blood cell (WBC) and platelet counts, and haemoglobin (Hb) levels was detected more often in the 32-P treated group. Although 32-P appears to be more toxic, no toxic effects requiring specific treatment were seen in either group. Due to its comparable efficacy and safety, general availability and low cost its more widespread use should be encouraged to increase quality of life and reduce cost of medical care of patients with intractable bone pain due to cancer metastases.

1. INTRODUCTION

Bony metastases ultimately develop in over 80% of patients with metastatic breast and prostate cancers, and bone can be the sole site of metastatic spread. Bone metastases are frequently multiple and diffuse. A prominent symptom caused by bony metastases is pain, which can seriously affect a

* Work performed within the framework of the International Atomic Energy Agency Co-ordinated Research Project.

patient's quality of life [1]. Only a few patients with hormone resistant prostate cancer achieve significant clinical benefit from chemotherapy. For breast cancer, hormonal therapy and chemotherapy can palliate symptoms for a short duration, but complete responses are infrequent. Radiation therapy can provide significant palliation in up to 70% of the cases, but median time of relief to pain is often measured in weeks. Hemibody or total body external irradiation may cause important side effects such as bone marrow suppression, gastrointestinal symptoms, and radiation pneumonitis. Pain palliation using narcotic drugs also causes considerable side effects, while prolonged treatment with polyphosphates is expensive [2, 3].

Therefore in many patients with a large number of metastatic lesions radionuclide treatment using single dose of beta emitting bone-seeking radiopharmaceuticals has become widely accepted. 32-Phosphorus orthophosphate and polyphosphate, 89-Strontium, 90-Yttrium EDTA and several 131-Iodine, 186-Rhenium and 153-Samarium labelled diphosphonates have been evaluated [4, 5]. Except 32-Phosphorus all of them are expensive and difficult to obtain in certain countries.

32-Phosphorus decays by beta radiation of maximum energy of 1,71 MeV with mean range of beta particles in tissue of 3 mm and maximum range of 8 mm. It's physical half life is 14,3 days. Its biological half-life in the bone marrow is 7–9 days. Since it is incorporated into the nucleic acids of rapidly proliferating cells as well as into cortical bone concern was raised that bone marrow toxicity with possible severe consequences can outweigh benefit of bone pain palliation [6].

2. THE AIM

The aim of the study was to evaluate safety and efficacy of 32-P for palliation of bone pain due to bony metastases. For this purpose safety and efficacy of 32-P was compared with safety and efficacy of 89-Sr (7), the most commonly used and thoroughly evaluated radiopharmaceutical for bone pain palliation in the framework of a prospective IAEA co-ordinated multicentric study.

3. PATIENTS AND METHODS

Methods

To assess efficacy of both radiopharmaceuticals intensity of bone pain was recorded once daily on 1–10 subjective scale, and consumption of analgesics was determined as type of analgesic multiplied by daily frequency (analgesic index) for 14 days before treatment and for 4 months after treatment. General quality of life data were also recorded.

To assess safety of 32-P and 89-Sr total white blood cell (WBC) and differential count, platelet count, haemoglobin (Hb) concentration were measured 14 days before application of the radiopharmaceutical, on the day of treatment and every 14 days after treatment for 4 months to assess bone marrow suppression. Creatinine concentration was measured simultaneously to assess renal function. Presence of bleeding, infection, gastrointestinal problems etc. was recorded.

Patients

A very strict protocol for unified patient inclusion and follow up was used. Inclusion criteria were as follows. Confirmed multiple osteoblastic bony metastases, the extent of metastatic spread was semiquantified as 'Bone scan index' [8].

Pain should be caused by bone scan positive site, but spinal cord lesions and/or pathologic fractures were excluded. Patients had to have been on analgesic or narcotic therapy. Consumption of analgesics was determined using analgesic index.

No radiotherapy or chemotherapy was allowed during the last 6 weeks and no change in hormone therapy during the last 3 months before the injection of radiopharmaceuticals.

There had to have been no signs of bone marrow suppression (white blood cell count over 5000/mL and platelet count over 150 000/mL) and no significant renal failure (creatinine level below 200 umol/L).

Life expectancy had to have been more than 6 weeks and in case of female patients pregnancy had to be excluded. Written informed consent for the study was obtained from all patients.

Patients conforming to the above mentioned criteria were randomised to receive either 32-P (450 MBq 32-P as orthophosphate orally) or 89-Sr (150 MBq 89-Sr as chloride intravenously). The patients were unaware of the form of treatment they received. 93 patients who completed at least two months follow up were included into the study. 45 of them received 32-P and 48 89-Sr.

Profile of the patients before treatment is described in Table I. There were no statistically significant differences between the two groups as far as gender, age, metastatic spread, and degree of pain, as well as haematological values was concerned. Most patients had bony metastases due to prostate and breast cancer, but lung, colorectal, ovarian, bladder and even thyroid cancer patients were also represented. Most patients were treated by surgery, chemotherapy, radiotherapy, and hormonal therapy as indicated previous to radionuclide therapy.

TABLE I. PROFILE OF PATIENTS TREATED WITH 32-P AND 89-SR BEFORE TREATMENT (N OR MEAN VALUE)

	32-P	**89-Sr**	**p**
N	45	49	
Gender			0,147
Female	9 (20%)	16 (33%)	
Male	36 (80%)	33 (67%)	
Age	61,2	62,4	0,668
Bone Scan Index	39,9	45,7	0,234
Pain Score	6,5	6,7	0,633
Analgesic Index	6,1	6,9	0,349
Hb (g/L)	108,8	109,1	0,917
WBC	8030	7570	0,072
Platelets (10*3)	273	256	0,350
Primary cancer			0,261
Prostate	32	27	
Breast	9	16	
Other	4	5	

Statistical analysis

T-test and chi-square test for univariate comparison of treatment groups was used. Logistic regression was used when the outcome variable was considered to be dichotomous (e.g. response versus no response) and Cox's proportional hazards model was used to calculate the duration of effects.

4. RESULTS

Efficacy

The patients were classified as responders or not-responders at a meeting of all contributing investigators taking into account changes in individual pain score and analgesic index. Also duration of the favourable effect was determined in responders using the same criteria. 68% of all patients were considered to respond to treatment with radionuclides. 60% of patients responded to treatment with phosphorus and 75% to treatment with strontium. While difference between number of patients who responded favourably to 89-Sr and 32-P is not significantly different (p = 0,122), the probability of success in the 89-Sr treated group is 1,25 times greater than in the 32-P treated group (Table II).

TABLE II. EFFICACY: NUMBER OF PATIENTS TREATED WITH 32-P AND 89-SR WITH PAIN RELIEF (RESPONSE)

Treatment	Response		
	Yes	No	Total
32-P	27 (60,0%)	18 (40,0%)	45 (100%)
87-Sr	36 (75,0%)	12 (25,0%)	48 (100%)
	p=0,122		
Total	63 (67,8%)	30 (32,3%)	93 (100%)

For responders duration of the response was also considered important. We compared the length of response between both treatment groups using the methods of survival analysis. The time of interest, 'survival time', was time elapsed between the beginning of the response and end of the response. At any time point proportion of patients still having good effect of treatment with each radiopharmaceutical is calculated. No significant differences between both groups were observed (p = 0.737).

Safety

Other than changes in platelet and WBC counts and Hb concentration no adverse effects were observed in either group.

Greater proportion of decrease for all three haematological parameters was observed in the group of patients treated with 32-P than with 89-Sr and also pathological decreases of all three were more common in the 32-P treated group. Since this was a multicentric trial with different ranges of normal values for haematological parameters in their laboratories, adverse effects were evaluated as being present or absent.

Significant decrease of WBC was seen in 42% of patients treated with phosphorus and in only 19% of patients treated with strontium. This difference was statistically significant. Also platelet count decreased significantly in 56% of patients treated with phosphorus and in one third of patients treated with strontium. Also this difference was statistically significant. Haemoglobin levels dropped in 69% of patients treated with phosphorus and approximately in half of those treated with strontium. This difference was statistically not significant (Table III).

TABLE III. SAFETY: NUMBER OF PATIENTS TREATED WITH 32-P AND 89-SR WITH PATHOLOGICAL DECREASE OF WBC, PLATELETS, AND HB CONCENTRATION

	32-P	89-Sr	Total	p
N	45	48	93	
WBC	19 (42%)	9 (19%)	28 (30%)	0,014
Platelets	25 (56%)	16 (33%)	41 (44%)	0,031
Hb	31 (69%)	26 (54%)	57 (61%)	0,145

5. DISCUSSION

Both 89-Sr and 32-P are used mainly to suppress hyperproliferative cell lines rather than to eradicate them, therefore expected effect of such treatment is predominantly palliation of bone pain rather than treatment of bony metastases. Favourable response to treatment was recorded in 75% of the patients treated with 89-Sr and in 60% of those treated with 32-P, overall response being 67,8%. These values are in accordance with published figures for success of bone pain palliation using different radiopharmaceuticals in patients with different types of cancer (4,7).

“Survival time curves" were used to assess the duration of favourable effect. At any time point these curves give proportion of patients still having good effect of treatment for each radiopharmaceutical. There was no significant difference between the duration of favourable effect for both radiopharmaceuticals.

No statistically significant differences between the two groups of patients receiving different radiopharmaceuticals were found in efficacy indices.

Limiting side effects using radiopharmaceuticals for bone pain palliation is temporary myelosuppression, the severity of which can be influenced also by the extent of the tumour involving bone marrow, previous radiotherapy and chemotherapy as well as patient’s general condition. Decrease of WBC and platelet counts were recorded significantly more often in the patients treated with phosphorus than with strontium while no statistically significant differences was seen in frequency of Hb concentration decrease. Since 32-P is accumulated not only in the growing bone, as is 89-Sr, but also in the rapidly proliferating cells, such as bone marrow, more myelotoxic side effects could be expected in patients treated with 32-P than with 89-Sr.

Nevertheless decrease of WBCs, platelets, and haemoglobin levels was moderate and was clinically not considered important, since no toxic effects requiring specific treatment were seen in either group. Known risk of developing acute leukaemia several years after treatment with 32-P was not considered important in patients with widespread metastatic disease.

6. CONCLUSION

According to our results 32-P is slightly but not significantly less effective than 89-Sr for palliation of bone pain due to bony metastases. Although 32-P appears to be more toxic it is important to note, that no toxic effects requiring specific treatment were seen in either group. It seems that 32-P is as safe as 89-Sr using doses up to 450 MBq.

Due to its comparable efficacy and safety with other radiopharmaceuticals for bone pain palliation, general availability and low cost more widespread use of 32-Phosphorus should be encouraged to increase quality of life and reduce cost of medical care of patients with intractable bone pain due to cancer metastases.

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RADIOCHEMICAL STUDIES AND PHARMACOLOGICAL BEHAVIOUR OF ^{186}Re COMPLEXES OF PHOSPHONATE LIGANDS

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Abstract. This paper describes the synthesis and characterization of phosphonic acid derivatives and their radiochemical studies with ^{186}Re for the development of bone seeking radiopharmaceuticals. These ligands, when tagged with therapeutic radionuclides such as ^{186}Re, ^{188}Re could localize in the bone selectively. A series of α-aminomethyl phosphonic acid derivatives were synthesized by a Mannich type reaction. All the ligands were crystallized to solid derivatives and subsequently characterized using ^{1}H-NMR spectroscopy. The ligands were complexed with ^{186}Re having a specific activity of ~40 mCi/mg (~1.5 GBq). Complexes with RC purity 95% and above could be prepared by varying the reaction conditions. By carefully optimizing the reaction and storage conditions, complexes which were stable for over 3–8 days could be prepared. Biodistribution studies carried out in rats revealed varying uptake (18–28% skeletal uptake at 3 h post injection) for these ^{186}Re complexes.

1. INTRODUCTION

Primary tumours frequently metastasise to the bone and bone pain is the most prominent symptom associated with bone metastases. The pain becomes progressively severe as the disease advances. The use of therapeutic radionuclides which localize selectively at the metastatic sites is found to be an effective treatment for the palliation of pain. Phosphonic acid derivatives labelled with radionuclides emitting β^- particles are found to be effective palliative agents for the treatment of bone pain [1,2]. Ethylene diamine tetramethylene phosphonate (EDTMP) is one of the most widely used ligands as it forms stable complexes with different radionuclides with excellent biodistribution and high lesion affinity [3–5]. ^{153}Sm labelled EDTMP is by now an established therapeutic radiopharmaceutical and used in several centres across the world for pain palliation. Other phosphonic acid derivatives such as hydroxy ethylidene diphosphonate (HEDP) form stable complexes with $^{186/188}$Re and hence ^{186}Re-HEDP is also proposed to be used as a bone pain palliation agent [6–8]. Due to the multiplicity of the ligands and the radionuclides now currently available, it is of interest to see the variation in bone uptake with the complexes of different phosphonic acid derivatives to arrive at the best possible agent. Herein we report the work carried out on the synthesis of EDTMP and its homologous phosphonic acid derivatives, their radiochemical studies with ^{186}Re and biodistribution studies of the complexes in Wistar rats. The ligands synthesised are given in Fig.1.

2. MATERIALS AND METHODS

2.1. Materials

^{186}Re as ammonium perrhenate with >99% radiochemical purity, 35–40 mCi/mg (1.3–1.5 GBq/mg) of specific activity and radioactive concentration of 15 mCi/mL (0.55 GBq/mL) was prepared by the method reported by us earlier [9]. Whatman no. 3 chromatography paper was used for the paper electrophoresis studies. Stannous chloride was obtained from Sigma Chemical Company. Orthophosphorus acid, ethylene diamine, 1,3-diamine propane, 2,2-dimethyl-1,3-propane diamine,

1,4-diamino butane and formaldehyde were purchased from Aldrich Chemical Co., USA. Solvents used for synthesis and recrystallisation experiments were purified as per standard procedure.

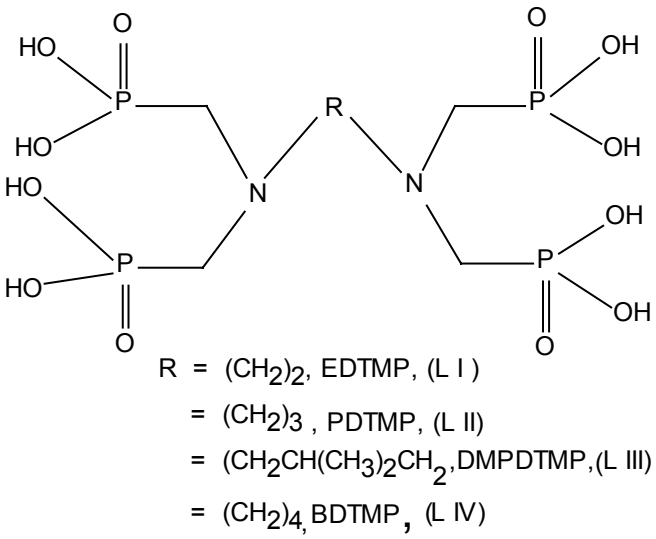

FIG. 1. Structure of ligands synthesized.

^{1}H-NMR spectra were obtained in a Varian VXR 300S spectrometer, using D_2O as the solvent and H_2O peak as the internal reference. A solid scintillation counter with NaI(Tl) crystal which is generally used for measuring ^{99m}Tc was used without any further adjustments for measuring radioactivity.

2.2. Syntheses of the ligands

Syntheses of the ligands were carried out by broadly following a reported procedure [10]. To a solution of orthophosphorus acid (4 mol) dissolved in Conc. HCl equimolar amount of the corresponding diamine was added slowly. The mixture was refluxed while formaldehyde (4 mol) was added dropwise to the refluxing mixture. At the end of three hours the reaction mixture was concentrated under vacuum. In the case of ligand I (EDTMP) precipitate was observed immediately on concentration while for all the other ligands the phosphonates precipitated on adding methanol or ethanol to the concentrated reaction mixture. The crude phosphonates were recrystallised from hot water or methanol: water (1:1) mixture to crystalline derivatives.

2.3. Preparation of ^{186}Re(V)-Phosphonates

Phosphonate ligands (30–50 mg) dissolved in 0.3 mL of bicarbonate buffer (0.5M, pH 9), 0.5 mL of 0.9% saline and 0.2 mL (100 μg, 0.54 mM ~ 148 MBq**)** of $^{186}ReO_4^-$ solution were mixed in a 10 mL vial. To this 0.02 mL of stannous chloride (100 mg/mL) dissolved in conc.HCl was added. The reaction mixture was purged with nitrogen and heated in a boiling water bath for 30 minutes and allowed to cool to room temperature. In some studies, the pH of the complex was adjusted to 7 with 1M NaOH solution.

2.3.1. Characterization of the Complexes

Paper chromatography

Paper chromatography was performed using Whatman 3 paper (1 × 10 cm strip). 5 μL portion of the test solutions were applied at 1.5 cm from the lower end of the strip. The strips were developed

in different solvents such as acetone and normal saline. The strips were dried, cut into eight equal segments and the radioactivity was measured.

Paper electrophoresis

5 μL samples were spotted on Whatman 3 chromatography paper 10–12 cm from the cathode and paper electrophoresis was carried out for 1h at 300 V in 0.02 M phosphate buffer at pH 7.5. The strips were cut into 1-cm segments and the radioactivity was measured.

2.4. Bio-distribution studies

Bio-distribution studies of ^{186}Re(V)-phosphonates were performed in male Wistar rats weighing 150–200 g. 80–100 μCi (3–3.7 MBq) of the complexes in ~0.3 mL volume was injected through tail vein and the rats were sacrificed at different time intervals by cervical dislocation. The tissues and organs were excised, rinsed with saline, weighed and counted over a NaI (Tl) scintillation detector with flat geometry (15 cm diameter). Distribution of the activity in different organs was calculated as percent injected dose/g as well as percent injected dose. Blood activity was calculated assuming blood volume as 7% of the total body weight. (All the bio-distribution studies were carried out in compliance with the national laws related to the conduct of animal experimentation).

3. RESULTS AND DISCUSSION

3.1. Syntheses

The synthetic scheme followed in this experiments provide almost quantitative yields of the phosphonate ligands. The characterization of the ligands were made with the help of ^{1}H NMR spectra recorded in D_2O. The peak multiplicities and integrations in the ^{1}H NMR spectra of the products were consistent with the expected features. The incorporation of the α-methylene phosphonate group was evident from the presence of a doublet at 3.2–3.4 ppm owing to the coupling with the adjacent phosphorus atom in the proton NMR spectrum of the product.

3.2. Quality control of ^{186}Re-phosphonates

Radiochemical purity of ^{186}Re complexes was characterised by paper chromatography studies. In paper chromatography using acetone as solvent, the complexes of all the ligands remained at the point of spotting. Under identical conditions $^{186}ReO_4^-$ moved towards the solvent front. Thus yield of the unreacted $^{186}ReO_4^-$ could be estimated. In paper chromatography using saline as solvent the complexes moved with the solvent front and hydrolyzed rhenium if present is expected to remain at the point of spotting. Thus presence of radiochemical impurities in the complexes of Re-phosphonates could be estimated by the above two paper chromatography techniques. In paper electrophoresis studies all the complexes moved towards the anode with a migration rate similar to that of $^{186}ReO_4^-$. The movement of the complex in electrophoresis indicated that the complexes formed are negatively charged.

3.3. Optimization studies for the preparation of ^{186}Re phosphonates

Several experiments were carried out to optimize the conditions for obtaining maximum complexation yield. Results of the studies on the effect of ligand concentration on complexation yield are given in Table 1. 0.5 mg of stannous chloride was used for these studies. It was observed that optimum amount of ligand required for the preparation of complexes of different ligands was in the range of 30–50 mg (~70–110μM).

The effect of the concentration of $SnCl_2$ on complexation yield was studied at the optimum ligand concentration and 100 μg (0.54 μM) of rhenium. It was observed that the amount of $SnCl_2$ needed was ~0.5 mg (2.7 μM) for ligand I and II, and 1 mg (5.6 μM) for ligand III and IV to get quantitative yields of Re complexes.

Effects of reaction temperature and time on complexation yields were also studied. Complexation reaction was carried out at pH 2 with optimum amounts of ligands, 2 mg of $SnCl_2$, at room temperature and at 100°C for different time intervals. The reaction was found to be complete (RC purity ~94%) immediately even at room temperature in the case of complex of ligand IV. In the case of other ligands, the reaction progressed slowly at room temperature with complexation yields >95% after 45min, 2 h and 1 h for complexes of ligand I, II and III, respectively. However, the reaction was found to be complete in 15–30 min in case of all the three ligands when the reaction mixture was heated in a boiling water bath.

The effect of pH on complexation yield was studied by adjusting the reaction mixture to different pH by using 1 M NaOH solution. Results of the complexation studies of ligands at different pH are depicted in Fig. 2. It was observed that the solubility of the ligands was very low at pH<2. At pH <2 most of the rhenium activity was found to be in the form of colloidal rhenium. At higher pH the complexation yield was low due to oxidation of Re to $^{186}ReO_4^-$. The optimum pH for complexation was found to be ~2. The complexation yield decreased drastically with increase in pH; in the case of complex of ligand II and IV, the complexation yield was <5% at pH 9. In the case of complex of ligand I and III the complexation yield decreased initially and then increased at pH 7 and then fell to lower values. The increase in complexation yield may be due to the easy availability of negatively charged phosphonate ions for complexation at higher pH. However, at higher pH the Re has a tendency to be found in the higher oxidation states, thereby reducing the complexation yield. The maximum complexation yield obtained at optimized conditions was ~95–97% in the case of complex of ligand I, II and III. The main radiochemical impurity was in the form of $^{186}ReO_4^-$ with <1% in the form of reduced hydrolyzed species. In the case of complex of ligand IV the maximum complexation yield obtained was 94% and the remaining activity was seen as colloidal Re.

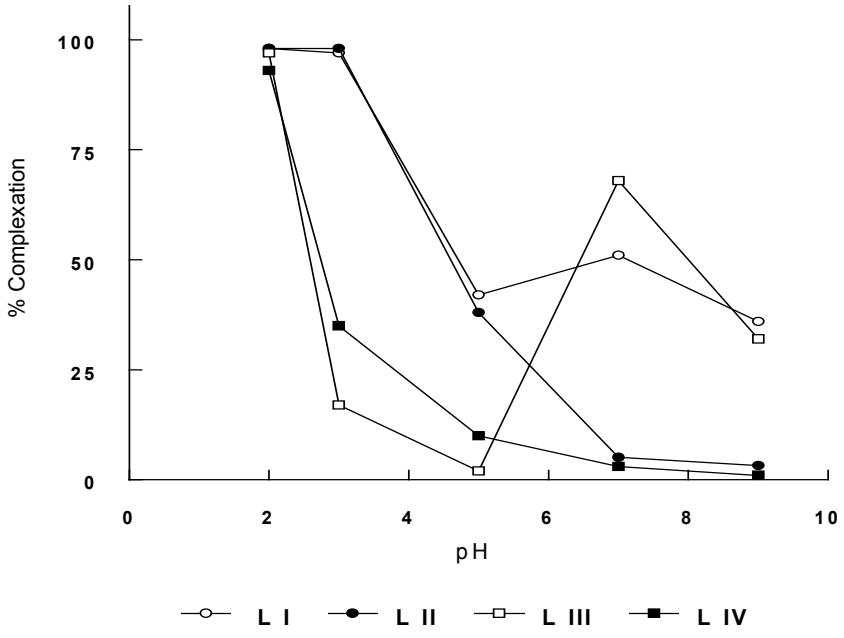

FIG. 2. Effect of pH on complexation yield

TABLE I. OPTIMUM AMOUNTS OF LIGAND AND STANNOUS CHLORIDE REQUIRED FOR THE PREPARATION OF ^{186}RE – PHOSPHONATES

Ligand	I	II	III	IV
Ligand (mg)	30	30	30	50
Stannous Chloride (mg)	0.5	0.5	1.0	1.0

** Reaction Volume was 1mL.*

3.4. Stability studies

Though high complexation yields with ^{186}Re could be obtained with all the ligands, the stability of the complexes varied depending on the ligand as well as the storage temperature. Though the complex of ligand IV could be prepared at room temperature the stability of the resultant complex was found to be significantly poor as compared to that of a complex prepared by heating the reaction mixture for 30 min in a boiling water bath. The complex prepared at room temperature showed decomposition within an hour. The rate of decomposition of the complex prepared at higher temperature varied depending on the time for which the complex was heated. Rate of decomposition decreased as the time of heating was increased. Optimum time of heating for getting maximum stability was ~30 min as shown in Fig. 3. The stability of the complexes could be improved to at least a day by preparing the complex at higher temperature. The experiment clearly suggests that, though near quantitative complexation could be achieved without heating, the heating step during complexation is essential to get a product with improved stability. These results are in agreement with the studies reported earlier [9, 11].

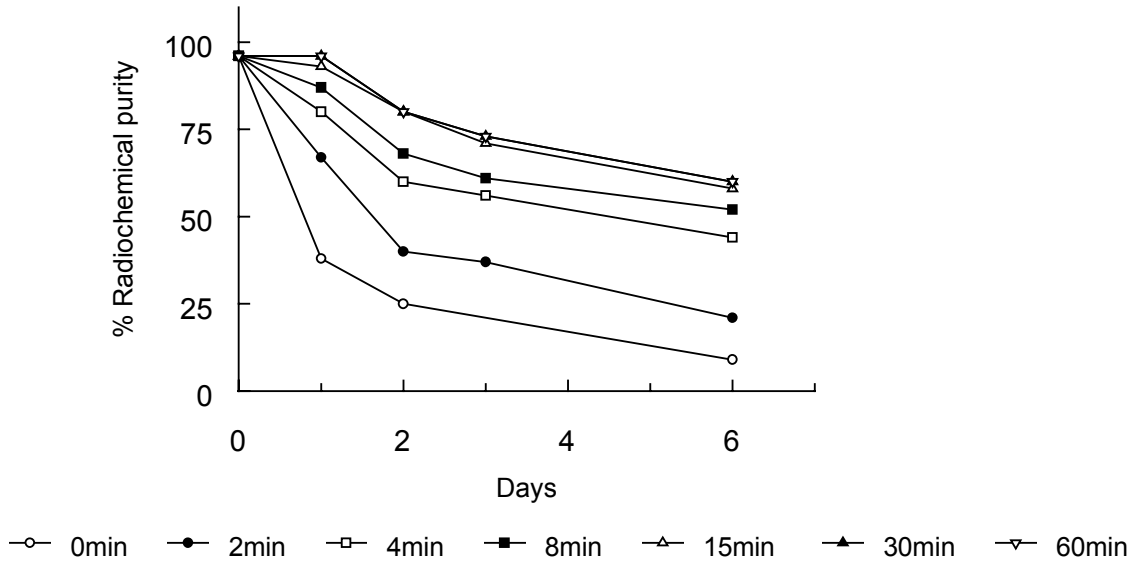

FIG. 3. Effect of heating time on the stability of ^{186}Re-phosphonate (ligand IV).

In order to use the complexes as injectable radiopharmaceuticals, it is desirable that the product is at physiological pH. Hence, stability studies were also carried out after adjusting the pH to 7. The stability of the complex of ligand I (^{186}Re-EDTMP) was studied up to 8 days. The complex was found to be stable at pH 2 for the extended period of storage whereas at pH 7 the complex was stable only for one day. Stability of the complexes of all other ligands was found to be poor at room temperature. The over all stability was found to be better when the complexes were stored at 4^0C. Even at 4^0C, the stability of the complexes adjusted to pH 7 was lower as compared to the one maintained at pH 2. Stability studies of the complexes stored at pH 2 and 7 at 4°C are summarized in Table II.

3.4. Biodistribution studies

Results of the bio-distribution studies are presented in Fig.4 and 5. Bio-distribution studies of ^{186}Re-phosphonates showed 18–28% skeletal uptake at 3 h p.i. for different ligands. Blood activity was in the range of 0.4–1.0% and renal excretion was 25–75%. Soft tissue uptake was more in the case of complex of ligand II and IV as compared to that of the complex of ligand I and III. Lowest soft tissue uptake was seen in the case of complex of ligand III. Retention in kidney was more with complex of ligand II whereas retention in liver was more in the case of complex of ligand IV. This may be due to the presence of colloidal Re (~5%) in the injected complex. Skeletal uptake observed after TABLE II.

TABLE II. STABILITY OF THE COMPLEXES AT 4°C WHEN STORED AT DIFFERENT PH

Description	Stability (days)	
	pH 2	pH 7
Ligand I	>8	6
Ligand II	8	2
Ligand III	4	2
Ligand IV	3	1

3 h. p.i. was retained even after 48 h. Skeletal uptake in case of complexes of ligand I (EDTMP) with ^{99m}Tc and ^{153}Sm was reported by other workers and these values are higher than what we have obtained with ^{186}Re [4]. The overall lower skeletal uptake of ^{186}Re complex of these phosphonic acid derivatives may be due to the *in vivo* oxidation of the complexes to ReO_4^- and its consequent excretion through the renal route. However, these complexes could be still useful as bone pain palliation agents which can be confirmed by carrying out skeletal uptake studies in animals having bone metastases. The hypoxic nature of tumour cells might increase the retention in tumour cells compared to normal cells [12].

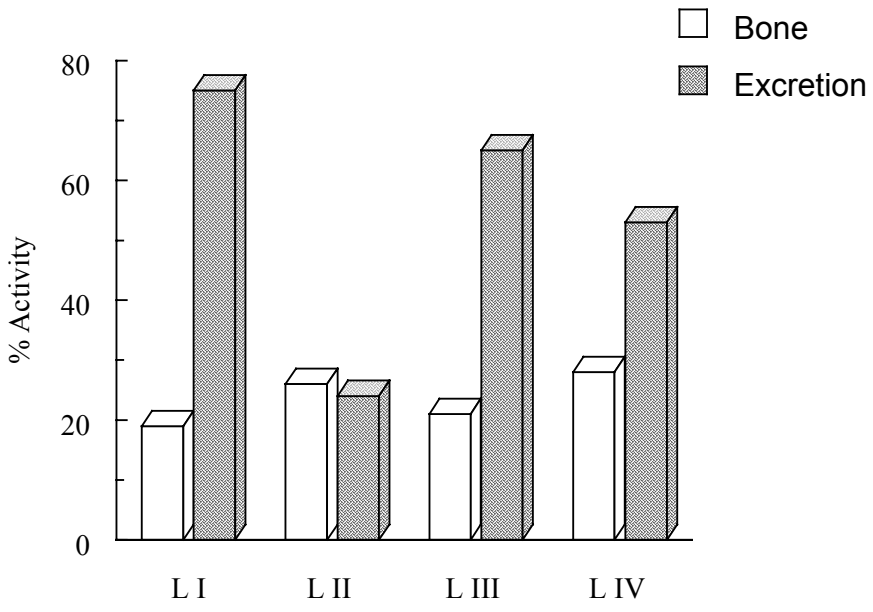

FIG. 4. % Uptake in bone and renal excretion after 3h p.i.

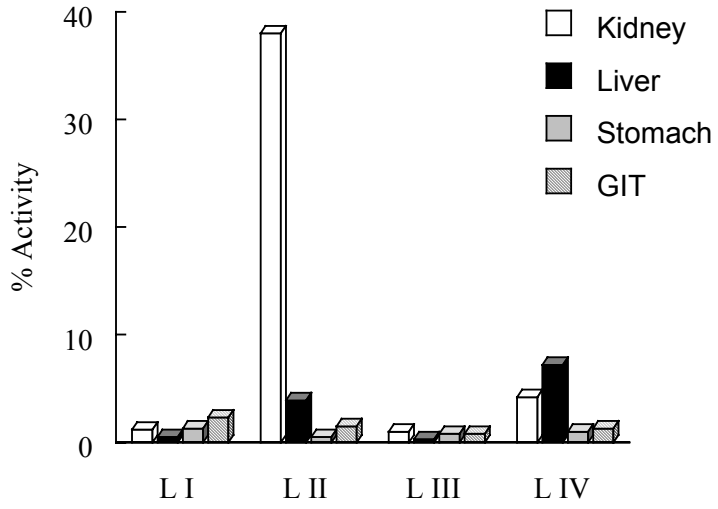

FIG. 5. % Uptake in non-targeted organ after 3h p.i.

4. CONCLUSION

The present studies indicate that rhenium complexes with phosphonic acid derivatives in high yields. The stability of the complexes under optimized condition ranged from 3–8 days. Skeletal uptake of the complexes was in the range of 18–28% at 3h p.i.. Reasonably good skeletal uptake with minimum retention in non-target organs was observed for the complexes of ligand III (^{186}Re-DMPDTMP). Retention of activity in kidney was observed in the case of complex of ligand II (^{186}Re-PDTMP) and in liver in the case of the complex of ligand IV (^{186}Re-BDTMP) along with skeletal uptake.

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PALLIATIVE EFFECT OF Re-186 HEDP IN DIFFERENT CANCER PATIENTS WITH BONE METASTASES

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Abstract. The clinical picture of bone metastases is manifested by pain and loss of mechanical stability. Standard treatment options for bone metastases include external beam radiotherapy and the use of analgesics. Due to a large number of lesions in many patients, the use of radionuclide therapy with beta emitters may be preferable. Re-186 hydroxyethydilene diphosphonate (Re-186 HEDP) is one of the radiopharmaceuticals suitable for palliative treatment of metastatic bone pain. The aim of this study was to investigate palliative and side effects of Re-186 HEDP in pts with different type of cancers. **Material&method:** Thirty one (17 male, 14 female) patients with cancer (10 prostate, 10 breast, 4 rectum, 5 lung, 2 nasopharynx) and bone metastases were included in the study. Therapy was started with a fixed dose of 1295 MBq of Re-186 HEDP. If necessary, the same dose was repeated at least 3 times after an interval of 10–12 weeks A total of 40 standard doses (1295 MBq Re HEDP, Mallinckrodt, Holland) were given; 6 pts received repeated doses (3 doses in 3 pts, 2 doses in 3 pts). The pts with bone marrow suppression were excluded from the study. The pain relief was assessed with ECOG and Karnofsky status index. All pts were evaluated with standard evaluation forms filled daily a maximum of 10 weeks. **Results:** The respond rate was found as 87.5% in pts with breast and prostate Ca, 75% in pts with rectum Ca, 50% in pts with nasopharynx Ca and 20% in pts with lung Ca. The overall response rate was 67.5%. The palliation period varied between 6 to 10 weeks. The mean palliation period was 8.1 ± 1.3 weeks. Maximal palliation effect was observed between the 3rd and the 7th weeks. Any serious side effects were not seen except mild haematologic toxicity. **Discussion & conclusion:** It is concluded that Re-186 HEDP is a highly effective agent in the palliaton of metastatic bone pain in pts with prostate, breast, rectum cancer, mildly effective in pts with nasopharynx cancer, but not effective in lung cancer. On the other hand, Re-186 seems to be a good alternative to Sr-89 because of its preferable physical charecteristics (as short half life and gamma energy emission), low side effect profile, early response and repeated repeatebility.

1. INTRODUCTION

Bone metastases are often the first presentation of distant disease in patients with cancer, especially prostate, breast and lung cancer [1]. The clinical picture of bone metastases is manifested by pain and loss of mechanical stability. The state is incurable and the only chance is palliative therapy which includes hormon application, chemotherapy and radiotherapy [3]. Standard treatment options for bone metastases are external beam radiotherapy and use of analgesic drugs [7, 8, 45]. Due to large number of lesions in many patients, radionuclide therapy with specifically localized internal beta emitters may be preferable [5, 6, 9].

Use of radioisotopes in palliative therapy [2] of bone metastases have started with P 32 (10B13) and continued with Sr 89 [14, 15, 16] and Re-186 [24–41], Sm 153 EDTMP (17B21), Sn 117m DTPA [22–23]. Favourable responses in patients with bone metastases of cancers were held with Sr 89. Unfortunately this radionuclide has a relatively long physical half life and does not emit gamma rays for post therapy quantitative imaging. Recently Re-186 HEDP has been proposed for pain palliation in Pts with metastatic bone lesions [24, 26]. Initial results showed that Re-186 HEDP is able to reduce pain caused by bone metastases. Because of its proper imaging qualities, 1.07 MeV beta radiation and physical half life, it has found wide use in palliative therapy of bone metastases.

The aim of this study was to evaluate the benefit of Re-186 HEDP in terms of pain relief as well as benefit from repeated doses and unwanted effects in patients with different types of cancer with bone metastases.

2. MATERIAL AND METHOD

10 prostate, 10 breast, 4 rectum, 5 lung, 2 nasopharynx carcinoma (17 males, 14 females, mean age:58 ± 5 year, range 38–84 year) patients were given Re-186 reaching a total of 40 standard doses (1295 MBq Re-186 HEDP, Mallinckrodt, Holland). Some of them received repeated doses of the same activity at 3 months intervals. Including criteria were:

1. At least four bone metastases demonstrated in the bone scan,
2. A Karnofsky performance status of maximum 60%,
3. At least 4.0 × 1000 leukocyte and 150 × 1000 platelet count,
4. Normal renal functions (30 mmol/L serum creatinine concentration or less),
5. At least 3 months life expectancy.

The patients with either bone marrow suppression or signs of nerve compression were excluded from the study. Tc 99m MDP bone scintigraphy was performed and bone scan indices were evaluated before the treatment. (34,35).

A standard dose of 1295 MBq Re-186 HEDP was given IV. to the patients with slow infusion. The patients were kept in nuclear medicine department for 6 hours after injection. The next day, anterior and posterior whole body scanning was performed. The daily symptomatic status of patients was recorded whereas blood analysis were performed weekly for 8 weeks after the therapy. A control Tc 99m MDP scintigraphy was performed approximately 30 days after the therapy. Especially for patients who did not have any pain relief despite therapy; a comparison of number and intensity of metastases was made by means of bone scintigraphy to determine the cause of pain increase.

3. RESULTS

3.1. Prostate cancer

10 patients in D3 phase who had multiple metastases and did not respond to hormonal and/or analgesic therapy received Re-186 HEDP. All had chemotherapy (and 5 of them had radiotherapy also) and had elevated PSA (mean 67 ± 13, 28–258) and PAP levels (mean 58 ± 45, 3–100), normal platelet and leukocyte counts, liver and kidney functions before the therapy. A total of 14 doses were applied (2 pts received 2 doses,1 received 3 doses the remaining 7 received 1 dose). 6 showed complete response, 2 had partial remission, 2 did not respond at all. The response was observed at the end of first week and continued up to 8–10 weeks. 4 patients showed flare up phenomenon. All had a decline in platelet and leukocyte counts starting with the end of first week and continued to decline for 4–5 weeks and reached to normal level within 5–6 weeks ($p < 0.05$). Biochemical blood analyses of kidney and liver functions remained normal whereas alkaline phosphatase levels declined within 4 weeks after therapy ($p < 0.05$). PSA levels increased 20% after therapy in 4 weeks ($p<0.001$) and PAP values showed about a 10% decrease ($p < 0.05$).

TABLE I. PROSTATE CA PATIENTS FINDING THE CHAGEMENT OF AFTER TREATMENT.

	Year	Diag (year)	BSI (%)	KT	RT	Resp (%)	Trom 1	trom2	Leuk1	leuk 2	ALP 1	ALP2
KV	69	5	50	+	+	%100	306000	211000	5100	5000	140	100
KV2						%100	190000	140000	8700	5000	180	100
CS1	71	6	80	+	+	%100	280000	200000	11100	8000	432	300
CS2						%100	250000	150000	9700	6500	750	400
CS3						%75	250000	90000	8500	5000	800	850
TB	65	3	80	+	+	%100	200000	120000	5900	4200	1109	1200
OK	70	4	80	+	+	%75	120000	90000	9000	7600	600	650
SC	69	1	70	+	+	%75	150000	129000	6900	5200	118	120
IA	75	6	50	+		%100	180000	126000	6100	3300	150	100
MD1	84	7	80	+		%100	158000	145000	7000	5200	85	80
MD2						%50	150000	38000	4400	3800	120	100
AD	60	2	50	+	+	%80	290000	200000	6500	4900	280	180
MC	64	3	60	+	+	%10	235000	180000	5800	4200	350	245
AÖ	64	2	60	+		%10	309000	273000	8100	9800	102	100
Mean	70±6	4±2	70			%87.5	212875± 74885	163250± 59830	7400± 1900	6030± 2100	347± 266	325± 295

3.2. Breast cancer

10 female patients (9 infiltrative ductal ca, 1 mucinous adenoCA) 38–52 years old (43 ± 5) received 12 standard doses of Re-186 HEDP. Two were considered to be inoperable and the others had modified radical mastectomy and lymph node resection. All of them received chemotherapy and hormone therapy, 8 of which also received radiotherapy. Four patients showed complete response. The other four showed decrease in pain level, but still needed low dose analgesics. The performance status were elevated. Only 2 patients did not experience any decrease in pain. The flare up phenomenon was observed in two patients who had complete response. No neurologic effects were observed. The thrombocyte counts were decreased slightly higher than leukocytes, but they returned to the original level in 6–7 weeks after treatment (20% fall in thrombocyte and 15% fall in leukocyte). Similar to prostate cancer, alkaline phosphatase showed a decrease within 4 weeks after treatment but returned to the same level at the end of 6–8 weeks (140 ± 48;105 ± 41IU/dl). No change was observed in CA 15–5 or CEA levels except two patients who showed a 10% decrease in Ca 15–5.

TABLE II. BREAST CA PATIENTS

	year	Diag (year)	RT	KT	BSI	Res(%)	trom 1	trom 2	leuk 1	Leuk 2	ALP1	ALP2
FD	42	5	+	+	50	%75	375000	351000	6440	4300	199	104
TC	51	3	+	+	30	%100	327000	127000	5900	3500	112	100
EM1	44	7	+	+	60	%100	328000	220000	8470	5400	166	98
EM2						%100	250000	120000	7000	5800	120	95
EM3						%90	240000	98000	6400	4200	180	120
SI	38	2	+	+	40	%75	274000	140000	5500	3500	67	129
SA	47	2	-	+	60	%100	389000	300000	7890	5500	129	80
ZS	38	4	+	+	70	%50	175000	126000	3600	3000	120	150
ZS	52	2	-	+	60	%20	450000	350000	6300	4100	100	78
MK	46	4	+	+	40	%75	350000	28000	4800	3600	350	220
SA	38	2	-	+	50	%100	256000	195000	6200	3900	37	105
FS1	59	12	+	+	55	% 75	158000	79000	5600	4780	222	200
FS2							120000	70000	8000	760	350	320
Mean	43±5	4±3			50	%87.5	303500±97000	214000±10100	6230±1390	4040±890	140±48	105±41

3.3. Nasopharyngeal cancer

Two patients (48 and 67 years old) received therapy. One did not show any response and died in the fifth week. The other patient who refused chemotherapy and volunteered for Re-186 HEDP therapy responded well. The response started at the end of the first week but this patient died in the sixth week. We could not evaluate the duration of palliation. Haematologic side effects were not observed to be at dangerous levels.

TABLE III. NASOPHARYNGEAL CA

	years	Diagnose	RT	KT	BSI	Response	Trom 1	Trom 2	leuk 1	leuk 2	ALP1	ALP2
MU	47	2	+	+	%40	-	60000	-	3700	-	300	-
KS	67	1	+	-	%20	% 85	381000	245000	11800	7100	1570	1280

3.4. Lung cancer

Five male patients (58 ± 6 years old) were treated with Re-186 HEDP. None of them were operated on and all of them received chemotherapy and radiotherapy. Only two patients experienced pain relief and decrease use of analgesics. Others did not seem to respond. No important side effects were observed.

TABLE IV. LUNG CA

	year	Diagnose	RT	KT	BSI	Response	Trom 1	Trom 2	Leuk 1	Leuk 2	ALP 1	ALP2
KU	85	1	+	+	10	0	356000	276000	12600	10900	238	100
HC	62	2	+	+	40	75	280000	190000	4600	3500	140	120
CM	59	1	+	+	20	0	360000	250000	3900	2500	280	190
MB	59	2	+	+	25	20	240000	200000	4200	3500	180	220
CD	64	1	+	+	30	0	363000	250000	11100	12000	180	200

3.5. Rectal cancer

Two females and 2 males with mean age of 49 ± 14 received Re-186 HEDP. Two patients showed complete response, one partial response and one no answer. The one with complete response, had pain again at the end of 8 weeks and received a second dose. The response of the second dose was complete and treatment was repeated 8 weeks after the second dose. The thrombocyte and leucocyte levels were decreased slightly after the first treatment and the degree of haematological effects increased after the second and third doses. The haematological toxicity was 30–40% decrease (grade 3) after the third dose. The duration of painless period decreased slowly after the third treatment.

TABLE V. RECTAL CA

	year	Diag (year)	RT	KT	Response	BSI (%)	Trom 1	Trom 2	Leuk 1	Leuk 2	ALP 1	ALP 2
FK	39	1	-	+	%5	50	70000	-	3200	-	250	-
ZA1	70	2	-	-	%100	20	400000	350000	6800	6000	465	300
ZA2					%90		250000	150000	7000	5400	480	400
MÖ	39	1	-	+	%75	60	200000	150000	11000	5200	1047	699
TA	51	1	+	+	%100	40	540000	480000	7060	4060	750	800
mean	49± 14	1.5			%75	30	380000± 170000	326000± 166000	8200± 2300	5000± 1000	754± 291	599± 166

4. DISCUSSION

Bone metastases are important indicators of distant spread in patients with cancer and they are clinically manifested by excessive pain. In the presence of bone metastases, therapy is palliative more than curative.

Since 1930s, radio-isotopes have been used with the purpose of palliation of the pain due to bone metastases [2, 5]. P32 was the first radio-isotope which has been used and Friedell and Straash [46] reported 90% palliation rate in bone metastases of breast cancer. Similar results have been reported in prostate cancer [10, 11, 12]. But this agent couldn't have been used widely because of bone marrow toxicity [13]. Later, Pecher et al. have used Sr89 and reported high palliative effect in breast and prostate cancer [47]. In 1979, Maxon used Re186 HEDP as an alternative to Sr89 and concluded it to have less side effects, the chance of repeatability and higher palliative rates [25, 26, 27, 35]. Nowadays Sm153 EDTMP, Sn117m DTPA are used widely in pain palliation because of advantages like less side effects, gamma rays and repeatibility in short periods. High palliation rates were reported with Sm153, also [20, 19].

Pain palliation rates were found to be 60–90% with Sr89 [14, 15, 16], 80–90% with Re186 HEDP [34, 44], and 70–80% with Sm153 (19, 20, 21) in cases with prostate cancer. In our study, palliative rate was found to be 87.5% in 10 patients with prostate cancer and this result was consistent with the literature. Pain palliation rates were found to be 70–80% with Sr89, 75–90% with Re186, and 60–70% with Sm153 [18, 19] in patients with breast cancer. In our study, Re186 HEDP was given to 10 patients with breast cancer and palliation rate was found to be 87,5%. There was complete pain relief in 4 patients and only in 2 patients no significant change in pain level could be observed. These findings are consistent with the literature. There isn't any distinct data concerning nasopharyngeal cancers. Presence of response to therapy has been reported in patients with multiple metastases. In our study, Re186 HEDP was given to 2 patients with nasopharynx cancer; response to therapy has been observed in one of them, but the other one died before any kind of evaluation. There is no evidence of palliation in patients with lung cancer. In a few cases, no effect on pain could be observed with Re186. Our study revealed the same results. Possible mechanisms were thought to be high cellular turnover rates and/or pleural and/or neural invasions in early stages. There are no result in the literature in patients with rectal cancers. Despite the limitation of the number, we found 75% palliation rate in this group. We concluded that further investigations with more patients were needed.

The durations of palliation were reported 3–6 months for Sr89 [16], 6–10 weeks for Re186 HEDP [28, 36], and 4–8 weeks for Sm153 [17, 21] in the literature. Despite the relatively long palliation period with Sr89 [14], it is not widely used because of retardation of therapeutic response and more side effects. The 8–10 weeks' long palliation period we observed in our study can be accepted as a satisfactory level when repeatability of the therapy was taken into account.

In earlier stages after radionuclide palliative therapy, increase in pain level (flare up) is observed. Later, major consequence is bone marrow toxicity. Flare up was observed in 10% of cases with Sr89 [15] and similar results have been reported with Re186 HEDP [29]. This observation has been thought to be due to cellular necrosis in early stages and/or secreted mediators during this process. Flare up phenomenon is reported to be seen more often in cases with multiple metastases and respond better to therapy. We observed flare up phenomenon in 6 of our 31 cases. BSI (bone scan index) rates and responsiveness to therapy were higher in these cases. Flare up phenomenon was thought to be a possible early indicator of responsiveness to therapy. No distinct data was observed with neither Sm153 nor Sn117m.

Bone marrow toxicity is the most important side effect of the therapy and it is more prominent with P32 [13], but can be seen with Sr89 also [15]. It is the major limitation in the use of P32. It is observed with Sr89 after the 4^{th} week in the rate of 20–30% [15]. This effect has been reported to be seen in a lower rate and for a shorter period with Re186 HEDP [33]. Sm153 [18] and Sn117m [22, 23]

are today's choices because of lower side effect profiles. In our study we observed bone marrow suppression after an average of 4 weeks in 15–20% of our cases. Repeated therapy courses caused increases in side effects but no none of the patients needed blood transfusions and no permanent bone marrow suppression was observed.

Temporary decreases in alkaline phosphatase levels were observed and these observations were correlated with other pharmaceutical studies [42]. Temporary decreases in PSA levels in patients with prostate cancer have been reported [43]. No change in other tumour markers has been observed. Changes in PSA levels have been observed with Re186 HEDP [40, 44]. In our study, changes in PSA levels were observed in patients with prostate cancer; these changes were thought to be due to PSA subtypes and necrosis in bone lesions was concluded to cause increases in blood PSA levels.

Neurological side effects have been reported after radionuclide therapy. Especially, in patients with parietal and temporal metastases. Re186 HEDP therapy has been found to cause symptoms of neurological compression [30, 31, 32].We did not observe any neurological symptoms in our patients. Site of the metastases and the possible relation with nerves were thought to be responsible from neurological side effects.

6. CONCLUSION

Palliation rate was found to be 87,5% in prostate and breast cancers and this finding is consistent with the literature. High palliation rates are reported in patients with rectal cancers (75%) and it is planned to control this result with more patients. The results of patients with nasopharyngeal cancer are hopeful (75% palliation rate), but further investigations are needed. A number of studies are being performed in this group of patients. There is not enough palliation in patients with lung cancer, and this finding is thought to be due to fast turnover rate of tumour cells and/or neural or pleural invasion in early stages.

The overall palliation rate has been calculated as 67,5% and this result is consistent with the literature.

It is concluded that Re-186 HEDP is a highly effective agent in the palliation of metastatic bone pain in patients with prostate, breast, rectum cancer, mildly effective in patients with nasopharyngeal cancer, but not effective in lung cancer. On the other hand, Re-186 seems to be a good alternative to Sr-89 because of its preferable physical characteristics (as short half life and gamma energy emission), low side effect profile, early response and repeatability.

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^{188}RHENIUM-HEDP IN THE TREATMENT OF PAIN IN BONE METASTASES

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Abstract. Systemic use of radiopharmaceuticals is a recognised alternative method for the treatment of pain in patients with multiple bone metastases. A new option, ^{188}Re-HEDP is proposed, using generator-obtained ^{188}Rhenium (β energy = 2.1 MeV, γ energy = 155 keV, half-life = 16.9 hours). After establishing parameters of biodistribution, dosimetry and image acquisition in mice, rats and rabbits, Phase I and II studies were conducted on 12 patients with multiple metastases from carcinomas, with pain surpassing other analgesic options. More than 50% pain relief was found in 91% of the patients, with total relief during a variable period in 41% of them allowing opiate and other analgesic drugs to be decreased or withdrawn, and showing a lower bone marrow contribution to total absorbed dose than that reported for other similar radiopharmaceuticals. Further study of this option is recommended in order to determine higher dose protocols without toxic bone marrow reaction possibilities.

1. INTRODUCTION

Considering death causes in developed countries, cancer is the second in frequency after cardiovascular diseases. Multiple metastases are the common evolution in a high percentage of cancer patients and pain is the main symptom involved. The therapeutic approach to this situation involves chemotherapy, hormonal therapy in cases in which the tumour reacts to hormonal stimulation, radiotherapy and analgesic drugs. In some cases tumours are resistant to hormonal therapy. Local field radiotherapy usually solves the situation in cases of few or single metastatic sites, but as a larger involvement develops, it turns inadequate as a pain relieving tool and the option of hemibody irradiation has significant toxicity. This situation has aroused interest in developing bone-seeking radiopharmaceuticals that can provide less toxic though effective pain relief. After wide initial experience with ^{32}Phosphorus and afterwards with ^{89}Strontium, various phosphonate radiopharmaceuticals were developed, such as ^{186}Rhenium-HEDP and the more used ^{153}Samarium-EDTMP. These compounds have demonstrated favourable biodistribution and dosimetry, and have been approved for clinical use. The objective of the present work is to assess the feasibility of the use of ^{188}Rhenium-HEDP as an option for pain palliation in clinical situations involving multiple metastatic disease. ^{188}Re was obtained from a ^{188}Tungsten/^{188}Rhenium (^{188}W-^{188}Re) radionuclide generator system developed at the Oak Ridge National Laboratory, TN, USA, thus representing an advantage for daily hospital work (1,2). In previously reported work, a lyophilised kit of HEDP for labelling with ^{188}Re was prepared and tested in mice, rats and rabbits showing rapid bone uptake and blood clearance with high renal excretion and good quality images were obtained using the 155 keV gamma photon (3).

2. MATERIALS AND METHODS

2.1. PATIENTS

Twelve patients were selected for treatment after they have provided written informed consent, all suffering from pain caused by multiple metastatic disease. The original cancer was from prostate (n = 6), breast (n = 5) and uterus (n = 1). The Ethical Committee of the University Hospital, School of Medicine, Montevideo, Uruguay approved the protocol used.

Admission criteria were: a) presence of painful bone metastases; b) failure of previous conventional analgesic therapy; c) bone scan showing multiple bone metastases; d) white blood cells and platelet count higher than 4.000/mm3 and 150.000/mm3 respectively; serum creatinine concentration of 1.5 mg/dl or less.

Exclusion criteria were: a) urinary obstructive pathology; b) renal failure; c) urinary incontinence; d) psychiatric disorders; e) spine compression; f) fracture on pathological bone.

2.2. PROTOCOL

The total dose was divided in a tracer dose and a complementary therapeutic dose. The tracer dose was administered as an intravenous bolus through a 3-way stopcock and flushed with saline solution. The first 5 patients were followed by serial blood sampling and urine collection during 24 hours. For the rest of the patients urine was collected at time intervals up to 6 hours after dose administration. Bladder catheterization was performed to all the patients in order to facilitate urine collection and minimise possible contamination, as well as to avoid possible urinary retention due to obstructive pathology and to diminish bladder wall irradiation. After 24–48 hours, the complementary therapeutic dose was delivered, following the same procedure. Two patients received a second therapeutic dose, 3–4 months after the first dose. One of the patients was accepted even though his platelet count was less than the admitted limit, because of humanitarian reasons.

Radiopharmaceutical

^{188}Re-HEDP (radiochemical purity >98%) has been prepared from lyophilised kits with ^{188}Rhenium from the alumina ^{188}W/^{188}Re generator provided by Oak Ridge National Laboratory.

Dose

Maximum administered activity limit for accumulated dose was established at 35 mCi (1.3 GBq); 0.45 ± 0.09 mCi/kg (16.7 ± 3.3 MBq/kg) considering safety as well as reasonable expectance of therapeutic benefit.

Image acquisition

Whole body scans were performed with a Sophycamera DSX (93 PMT) with a Medium Energy High Resolution collimator, with a 20% window centered at the 155 keV peak.

Sample processing and measurements

One mL of blood was measured for total activity, and centrifuged for plasma separation. Plasma was treated for protein binding by trichloroacetic acid (TCA) precipitation and radioactivity measurements of plasma, TCA supernatant and precipitate were performed. Total recovered volume of urine was measured and aliquots of 20 mL were assayed for radioactivity in a dose calibrator. Multiple regression analysis of blood and plasma profiles was done. Calculation of coefficient and microconstants were obtained by model fitting and elimination half-life (k_e) was calculated from urine

profiles by: $\ln(1-E/A_0) = -k_0 t$, where E is urine activity at time t and A_0 is the administered dose. Bone uptake as remnant dose at 24 hours was estimated as $A_0 — E_{max}$ where A_0 is the ^{188}Re-HEDP administered dose and E_{max} is total accumulated urine excretion.

Patient follow-up

After dose administration, patient control was performed by means of weekly interviews during 11 weeks approximately. This control consisted in: a) haematological follow-up by hemogram which included platelet, white and red cell count; b) clinical interview with physical examination of the patient and control of medication status. This evaluation was complemented by daily self-assessment of pain and drug intake, performed by means of protocol forms supplied to the patient or relative in charge, for daily record of pain using a 0–5 subjective pain scale and a drug intake register, in which the amount and kind of pain medication was recorded.

Dosimetry

Residence time in trabecular bone was considered equal to that of cortical bone, and calculated based in experimental data as follows:

$$\text{Residence time} = (0.5 \times C/A \times 1.443 \times t_{1/2}\ ^{188}\text{Re})h$$

where:

C — Bone uptake (mCi)
A — Administered dose (mCi)
$t_{1/2}$ ^{188}Re — 16.9 h

Dose absorbed to bone marrow was calculated using MIRDOSE3, introducing residence times.

3. RESULTS

For a total of 14 doses in 12 patients, only 2 doses resulted in no pain relief (14%). Five of the patients experimented periods of total relief of different duration (Table).

RESULTS: PAIN RELIEF
DOSES : 14

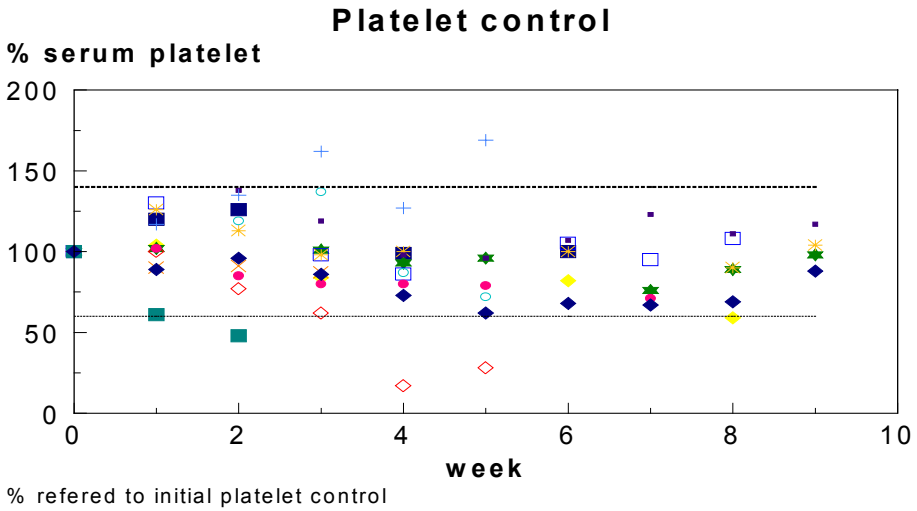
- ONSET
  - » 1 WEEK 3 PTS
  - » 2 WEEKS 6 PTS
  - » 3 WEEKS 2 PTS
  - » 4 WEEKS 1 PT
  - » NO RELIEF 2 PTS
- DURATION
  - » 6 WEEKS 2 PTS
  - » 5 WEEKS 2 PTS
  - » 3-4 WEEKS 5 PTS
  - » 2 WEEKS 2 PTS
  - » 1 WEEK 1 PT

Concerning drug intake, 4 patients were able to discontinue opiate therapy and 3 patients other analgesic drugs. Decrease in drug intake was observed in 7 cases for opiate treatment and in 5 cases of non opiate analgesics.

For the first 5 patients, followed up to 24 hours, bone uptake after therapeutic dose administration showed a mean ± standard deviation of 38 ± 17%. For the remaining 7 patients, having

received 9 administrations, estimated 24 hours uptake was: 41 ± 17% and 37 ± 16% for tracer and therapeutic doses respectively. Twenty-four hour bone uptake for all the 12 patients for therapeutic dose administration was estimated as 40 ± 16%.

Platelets showed mild variations in all but one patient who had a significant decrease in his count. This was the same patient that began the treatment with a lower than recommended platelet count. Nevertheless, no hemorragic symptoms were observed, and platelet count began increasing slowly with no additional treatment. Red cell count showed no variation, and white cells suffered a mild decrease in the same patient of the platelet decrease, and an increase in other patient, from the fifth week onwards.



Platelet Control Graph shows platelet count values of the 12 patients presented as percent of the initial value for each patient.

The 14 doses (tracer and therapeutic) of ^{188}Re-HEDP, administered to the 12 patients had a mean activity of 31 ± 6 mCi. Considering estimated bone uptake of 40 ± 16% and a residence time of 4.7 ± 1.8 hours, using MIRDOSE3, a bone marrow dose of 2.2 ± 0.8 rad/mCi and a total bone marrow dose of 65 ± 28 rads were calculated.

4. DISCUSSION

The protocol used to characterise the pharmacokinetic behaviour of ^{188}Re-HEDP was carried on in five patients in a limited study approved by the Ethical Committee. Prosecution of this protocol in a second population discontinued blood sampling for testing radiopharmaceutical clearance and was limited to urine determinations with bladder catheterization up to six hours. Considering that 70% of total eliminated activity was achieved at 6 hours, it was decided that it was not necessary to keep the patient under hospitalisation neither for urine collection nor for radioprotection reasons. After tracer and therapeutic doses, similar blood clearances were observed for the first 5 patients (4). This were also in agreement with the values reported for ^{99m}Tc-HEDP, except for long times where higher values were determined for ^{188}Re-HEDP. A three compartment model was the best fit for the five patients. Urine profiles show that even considering a 60% elimination of injected dose in 24 hours, a rapid excretion occurs in the first 6 hours post administration. No significant differences were found between estimated (n=14) and experimentally determined 24 hour elimination data (n = 5). Similar results were also obtained when comparing elimination data after tracer and therapeutic doses. Determination of bone uptake is valuable for dosimetry purposes to critical organ as well as for assessment of a potential correlation between this value and the therapeutic response as pain relieving agent.

Considering blood elements follow up, red cells suffered almost no variation after ^{188}Re-HEDP administration, while platelet and white cells variation referred to initial values was relatively mild. The only exceptions were:

a) One patient whose initial platelet count was lower than recommended, was in a terminal stage and dose was decided on humanity reasons, suffered a platelet and white cell number decrease followed by an increase, and died on the fifth week not from haematological alterations.

b) Another patient who showed an important white cell increase from the 5th week onwards that could be explained by intercurrent infection.

Dosimetry estimations showed that not only a higher bone uptake is indicative of higher absorbed doses to bone marrow, but also that residence time is an important parameter that can be evaluated through urine samples. Comparing estimated bone marrow absorbed dose values in rad/mCi for other therapeutic bone radiopharmaceuticals (^{32}P = 14, ^{89}Sr = 50, ^{186}Re-HEDP = 3, ^{153}Sm-EDTMP = 6), ^{188}Re-HEDP absorbed dose has been estimated in 2 rad/mCi.

Comparative series (5,6) using generator or irradiation obtained ^{188}Re, and reporting similar number and pathology patients, tested increasing doses of the radiopharmaceutical with haematological toxicity appearing at higher doses. Maxon et al. performed external dosimetry measures showing low potential radiation exposures to general population. Palmedo et al. found higher marrow toxicity at higher therapeutic doses, having reached up to 120 mCi in their series.

5. CONCLUSIONS

The use of short-lived, generator-produced ^{188}Rhenium as a bone seeking agent under the form of a phosphonate complex is a promising alternative for pain palliation in patients with multiple bone metastases. As an advantage, the use of long-lived generators makes Re-HEDP a very interesting choice in terms of cost-benefit and availability. For ^{188}Re-HEDP, very good quality radionuclide, low carrier amount and low contamination factor with low radionuclidic impurities ensures a safe performance and good therapeutic results. Further study is still required in order to increase the number of treated patients and to determine the doses and treatment timing that can achieve better results.

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^{153}Sm-EDTMP FOR PALLIATION OF PAIN FROM OSSEOUS METASTASES: PREPARATION AND BIODISTRIBUTION STUDIES*

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Abstract. The lung, breast and prostate cancers are the most frequent in our country. These cancers generally metastasize to the skeleton and a significant number of patients experience bone pain. The use of therapeutic radiopharmaceuticals which localize at metastatic sites has been found to be an effective new method for the treatment of pain and has many advantages over the use of analgesics and external radiation. Among these radiopharmaceuticals ^{153} Sm-EDTMP is the better agent thanks its short half-life, a high bone uptake and a rapid excretion from the body. In the present work, we report results of ^{153} Sm-EDTMP preparation, its uptake by shell of eggs as osseous model and its biodistribution in small animals.

1. INTRODUCTION

During the last years, a growing interest has been shown in the development of therapeutic radiopharmaceuticals for palliative treatment of metastatic bone pain [1].^{153}Sm-ethylenediaminetetramethylphosphonate (^{153}Sm-EDTMP) has become established as the better therapeutic agent in comparison with the pure beta emitters ^{89}Sr-chloride and ^{32}P-phosphate.

The unavailability of this radiopharmaceutical prevents actually its use by our nuclear medicine services. ^{153}Sm has excellent physical properties and can be produced in high yield and high specific activity by neutron irradiation of enriched ^{152} Sm targets. The gamma emission associated with its decay is useful for scintigraphic imaging.

The aim of the present work was to develop the in-house ^{153}Sm-EDTMP production by irradiating enriched ^{152} Sm targets in the Research Reactor NUR.

2. METHODS

2.1. ^{153} Sm-EDTMP PREPARATION

2.1.1. ^{153} Sm production

^{153} Sm was produced by irradiation of 2 mg samples Sm_2O_3 (98.7% enriched ^{152}Sm, provided by the IAEA), sealed in a quartz ampoule at a thermal neutron flux of 10^{13} n/cm^2.s in the Research Reactor NUR during 54 hours (discontinuous irradiation). The irradiation conditions were optimized. As target material, we used the samarium dioxyde and also the nitrate obtained by dissolution of the oxide in concentrated nitric acid followed by evaporation to dryness.

* A part of this work was carried out under an IAEA research contract N^o 7371/RB.

After irradiation,[153]Sm activity was recovered in a glove box directly from the quartz ampoule with syringe after dissolution of the target in hot dilute hydrochloric acid. The total volume was 2 mL.

Activity and radionuclidic purity were determined by gamma spectroscopy by counting an aliquot of obtained solution on HPGe detector. After two days cooling, the obtained specific activity was 44 mCi/mg.

Gamma spectrum of [153] Sm is shown in fig.1. The spectrum obtained with attenuation (lead shielding) contains the other characteristic peaks (463.3, 531.0, 608.9 keV) of samarium-153.A 4mm-thick lead canister was used for attenuation. In this case, the low energy photons (103.2 keV) are much more readily attenuated than the higher energy photons. 154Eu and 155Eu as potential gamma impurities [2] were not detected.

The radiochemical purity, determined by thin layer chromatography on ITLC-SG using distilled water as eluent, was higher than 98%. The solution of [153] Sm chloride was used without further purification.

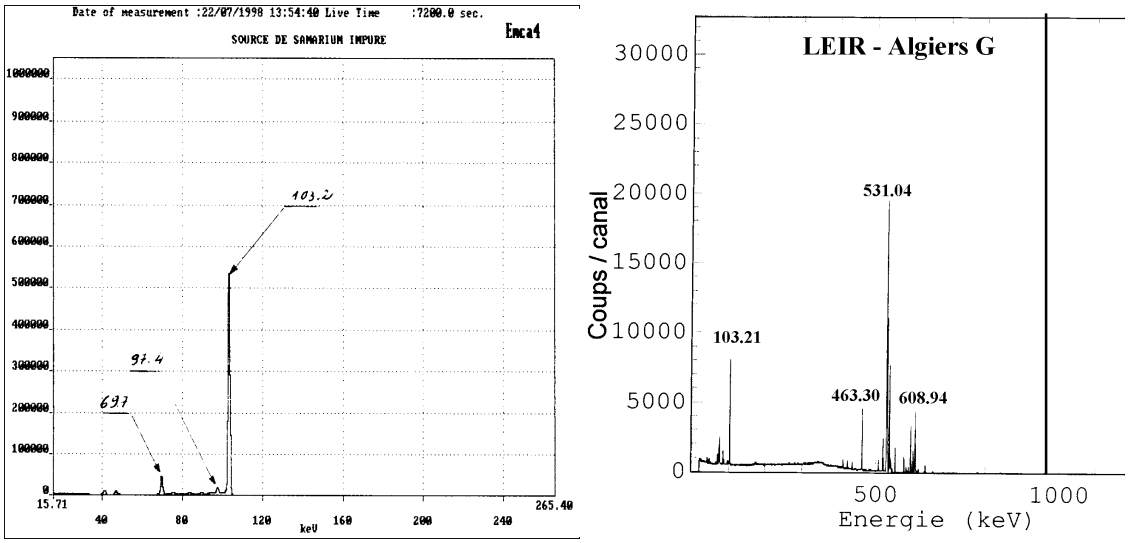

FIG. 1. Gamma spectrum of Samarium-153. Left, without attenuation.

2.1.2. [153] Sm complex preparation:

[153] Sm-EDMP was prepared by adding [153] Sm chloride to a solution of EDTMP. EDTMP was previously synthetized from phosphorous acid, ethylenediamine and formaldehyde using the Mannich-type reaction [2]. It was purified by successive recrystallizations and identified by IR spectroscopy. A stock solution of EDTMP was prepared by dissolution in distilled water and sodium hydroxide. The pH of the stock solution was adjusted to 11. The desired amount of ligand was placed in a vial and an appropriate amount of the [153]Sm-chloride solution was then added. The pH of the resulting solution must be 7–7.5 otherwise it was adjusted. The complex solution was kept at room temperature for 30 min before use. The complex yields and the radiochemical purity were determined using the same chromatographic system as mentioned above. The complex moved with the solvent front whereas free samarium remained at the start. It was noted on the radiochromatogramme, a small shoulder on the side of the main peak toward the origin. The small peak may represent another specie of the complex or simply some impurity present in the ligand. The best complex yield was obtained for EDTMP/Sm molar ratio 3–5/1 at a pH 7. Sterilization of the radiopharmaceutical was performed by autoclaving.

2.2. BIODISTRIBUTION STUDIES.

2.2.1. In-vitro uptake study

In-vitro uptake of ^{153}Sm-EDTMP by shell of eggs as osseous model was studied to confirm mechanism of its localization as it was previously done for ^{99m}Tc-diphosphonates [4]. The study was performed on hen eggs with shell and without shell. In the last case, the shell was removed from egg by incubating in dilute hydrochloric acid at room temperature during 96 h. The eggs were then immersed in a saline solution containing a known amount of the radiopharmaceutical and incubated during 2 h. Then there were washed and dried to prevent any contamination. The eggs were imaged by gamma camera (Fig. 2). The scintigraphic images revealed a high complex fixation in the shell. On the contrary, the eggs without shell were invisible and were not imaged. There is no complex fixation by the organic egg membrane. This confirm the high affinity of phosphonate complexes for calcium as phosphate in the mineral bone matrix or as carbonate in the case of shell of eggs, even though they are already coordinated to radionuclide (^{99m}Tc or ^{153}Sm). It was supposed that the ligand forms a bridge between the radionuclide and the calcium ions on the surface of hyroxyapatite [5]. The shell structure, constituted mainly of calcium carbonate crystals, may be a useful mineral model for the in-vitro study of phosphonate complexes uptake.

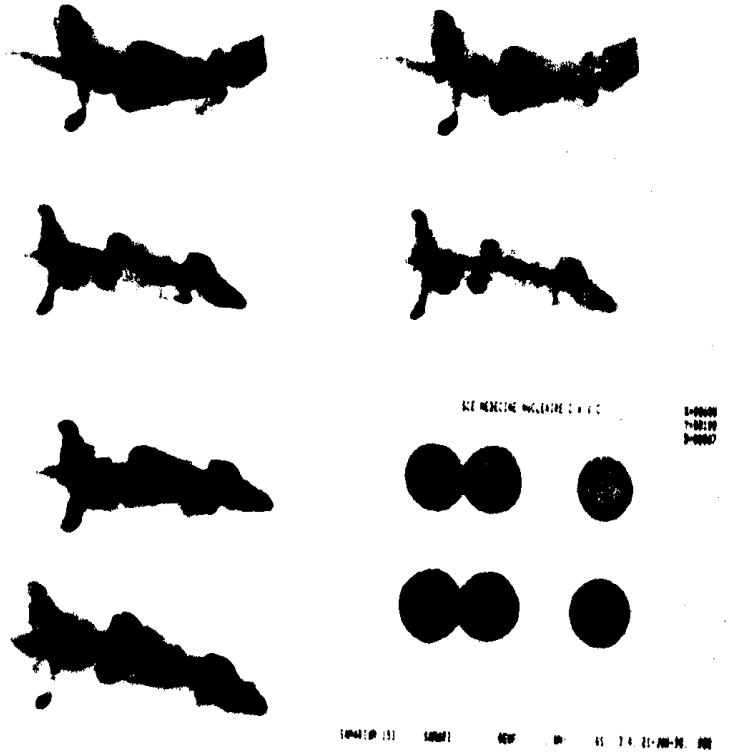

FIG. 2. Scintigraphic images of rats and hen eggs (right-down) obtained with ^{153}Sm-EDTMP.

2.2.2. In-vivo biodistribution

To evaluate biolocalization of 153 Sm-EDTMP and compare it to ^{99m} Tc-diphosphonates, scintigraphic studies were performed on rats. These studies were conducted with an Orbiter 75 camera (Siemens) equipped with a high resolution collimator and computer. Male rats (150–250g) were injected intravenously, into the jugular vein, with 0.2 mL of ^{153}Sm-EDTMP. Data acquisition commenced simultaneously. At 90 min post-injection data were acquired. The ^{153}Sm-chelate showed a lower blood concentration, higher bone uptake and higher bone-to-soft tissue ratios than the ^{99m}Tc-diphosphonates. Bone uptake and renal excretion take part in its rapid blood clearance. The

radioactivity in the skeleton increased as time progressed and remained unchanged 90 min post-injection. Scintigraphic images of rats, obtained at 90 min post-injection are shown in Fig. 2. Aside the skeleton, only the injection point (jugular vein) and the urinary system were visualized. No specific fixation in non-osseous tissues and organs was found.

3. CONCLUSION

The samarium-153, produced in Research Reactor NUR by irradiation of enriched target is suitable for the preparation^{153} Sm-EDTMP. The biolocalization characteristics of the prepared 153Sm-EDTMP complex in rat were satisfactory. It is planned to be further investigated by additional biological experimentation in animals before clinical evaluation.

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EFFICACY AND TOXICITY OF SAMARIUM-153-EDTMP LOCALLY PRODUCED IN THE TREATMENT OF PAINFUL SKELETAL METASTASES

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Abstract. Samarium-153 emits medium-energy beta particles an a gamma photon with a physical half-life of 46,3 hous. When chelated to ethylenediaminetetramethylenephosphonic acid (EDTMP), it is remarkably stable in vitro and in vivo. In this study we administered randomLy 0,5 and 1,0 mCi/Kg body weight (two groups), to 30 patients with painful metastatic bone cancer. Slight and spontaneously reversible myelotoxicity was observed. A bigger leukocyte and platelet suppression was obtained with 1,0 mCi/kg than 0,5 mCi/Kg dose. Pain palliation was obtained in 66% of the treated patients. Our preliminary results indicate that ^{153}Sm-EDTMP is a promising radiotherapeutic agent for palliative treatment of metastatic bone cancer pain where a reactor is available and at a very affordable cost.

1. INTRODUCTION

Several radionuclides have been used to treat metastatic bone cancer like ^{32}P, ^{89}Sr, ^{90}Y, ^{131}I, and ^{186}Re. In general, these radionuclides required chelation to ligands possessing the propensity to concentrate in malignant bone lesions.

Samarium-153 ethylenediaminetetramethylenephosphonic acid is a radiopharmaceutical developed at the University of Missouri [(1)] that appears to have bone cancer localization and pain palliation properties after intravenous administration. ^{153}Sm-EDTMP was obtained from enriched ^{152}Sm, irradiated at a 5 MW research reactor and labelled with EDTMP at a molar ratio of 15:1 and pH 7,1.[(2)]

In this report we evaluate the efficacy and toxicity of two different doses of ^{153}Sm-EDTMP in 30 patients with painful metastatic bone cancer.

2. METHOD

All patients has histologically documented cancer with painful bone metastasis. Skeletal metastases were documented by X ray, ^{99m}Tc-HDP bone scan and in some of them CT scan. All the patients must meet the inclusion criteria (see annex). Written consent was obtained from each patient.

Sterile 153Sm-EDTMP was prepared on the day before the treatment. In each preparation biodistribution, autoradiography, and radiochemical purity tests were done.

The total group was 30 patients (16M, 14F), mean age 64,2 (range 35–86). Patients were divided randomLy in two groups according to the dose they received: Group I 0,5 mCi/Kg body weight and Group II 1,0 mCi/Kg body weight. (see annex). They present different type of cancer (see annex). In three of them the metastases were localized in one or two bones but in the majority of them were multiples. Blood test and follow-up were done during 16 weeks

3. RESULTS

In one case it was not possible to evaluate the response to treatment because the patient dies during the first week. Other 8 patients died during the follow-up but the data was used for evaluation.

In 16 patients (57%) analgesic drugs were decreased substantially or not given at all. 8 patients continue with same drugs and in 4 (14%) cases was necessary to add more potent analgesic despite the use of Samarium.

Toxicity, defined as bone marrow suppression, was mild and transient. There was no difference between 0,5 or 1,0 mCi/Kg administered dose in erythrocyte suppression. But in leukocyte and platelet suppression we obtained almost the double with the bigger dose. No difference in recovery time was observe in any of the blood series.

Efficacy, defined as pain palliation, was obtained in 27 (94%) cases: slight in 8, moderate in 7 and complete in 12. In 2 patients the pain remains the same. The duration of palliation was variable. If we considered only the maximum time with minimum pain this time will be more than 8 weeks in 12 cases, between 4–8 weeks in 5 and between 2–4 weeks in 10 patients (see annex).

Flare phenomenon as a side effects was observed in 6 patients.

4. DISCUSSION

Patients with only bone metastases have a reasonably long survival but with bone pain. An effective and easy administered palliative treatment would be desirable. Radioactive bone-seeking drugs with the capability of delivering an effective radiation dose to all of the metastatic skeletal sites would be superior to external beam irradiation, since the latter has regional limitations. Unfortunately, due primarily to the undesirable myelotoxicity and in-vivo instability of agents, most of the radiopharmaceuticals used previously have not been widely accepted.

Samarium-153 emits two medium-energy beta particles and has a physical half-life of 46.3 hours with an average penetration range of 0.83 mm in water.[3] The high stability and biodistribution of 153Sm-EDTMP has been documented in a number of in vitro and in vivo experiments.[4–6]

Samarium 153-EDTMP has three very important features:

- it possesses a 103 keV gamma emission for scintigraphic imaging of its biological distribution
- the short physical life ($t_{½}$ = 1,8 days) reduces the need for long patient isolation and facilitates the disposal of urine and other body fluid.
- the high affinity for metastatic bone lesions that allows the simultaneous delivery of radiation to all targeted sites.

The flare phenomenon observed in 6 patients it is an independent feature, and it is not related to any type of response to palliation.

Myelotoxicity was observed only in leukocytes and platelets and is depending on the amount of the administered dose. The recovery started around the fourth week post-treatment.

In our group of patients we observed pain palliation in 19 (66%) patients and slight response in 8 (28%). No difference was observed depending on administered dose. However, because of the small number of patients and the diversity of their neoplasms in each dose group, an absolute dose response relationship could not be clearly established.

5. CONCLUSION

Samarium-153-EDTMP, a radiopharmaceutical with beta particle emission, exhibits a strong concentration at metastatic bone sites. Pain palliation is produced in the majority of the patients. A slight and spontaneously reversible myelotoxicity is observed. To better define its toxicity and efficacy, a large cohort of patients with diverse tumour types must be studied.

We believe that ^{153}Sm-EDTMP is a very good option to treat metastatic bone pain in those countries where a reactor is available and at a very affordable cost.

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ANNEX

PATIENTS INCLUSION CRITERIA

— Patients requires analgesics for control of pain
— Pain caused by positive bone scan lesions
— WBC count more than 3.500 cells/mm^3
— Platelet count more than 100.000 cells/mm^3
— Absolute granulocyte more than 1.500 cells/mm^3
— Serum creatinine less than 1,5 mg/dl
— Stable hormone regime for not less than 3 months
— No external radiation therapy within the last 6 weeks
— No symptom or signs of spinal cord compression
— Life expectancy greater than 4 weeks
— Can return to clinic for follow-up
— If female, not pregnant or nursing
— Not in a clinical trial of another drug
— Informed consent obtained from the patient

Assessment of patient overall condition

TYPE	MEANING
worse	increased
no change	unchanged
slight relief	noticeably improved
moderate relief	vastly improved
complete relief	no pain present

KARNOFSKY PERFORMANCE SCALE

POINT	DESCRIPTION
100	Normal,no complains
90	Minor signs or symptoms of disease
80	Some signs and symptoms of disease
70	Unable to carry on normal activity
60	Requires occasional assistance
50	Requires considerable assistance
40	Requires special care and assistance
30	Severely disable, hospitalization indicated
20	Very sick, hospitalization necessary
10	Moribund, fatal processes progressing rapidly

PAIN EVALUATION IN GENERAL

— intensity
— localization
— limitation of activity
— use of analgesic
— dose of analgesic
— relation with environment
— relation with family
— interference with sleep

TOTAL GROUP

Number of patients	30
Male	16
Female	14
Age (year)	64,2 (35–86)
Weight (Kg)	65,8 (43–100)
Administered dose (mCi)	48,2 (20–75)

GROUP

Dose	Number of patients
0,5 mCi/Kg	16
1,0 mCi/Kg	14

TYPE OF CANCER

TYPE	Number of patients
Prostate	14
Breast	10
Lung	1
Renal	1
AdenoCA	1
Unknown	1
Colon	1
Endometrium	1

METASTASES

Number of met.	Number of patients
Single	3
Multiple	27

DOSES

GROUP	AGE (year)		SEX		WEIGHT (Kg)	
mCi/Kg	avg	range	M	F	avg	range
0,5	64,9	42–83	10	6	70,5	43–100
1,0	63,4	35–86	6	8	60,7	51–75

RESPONSE TO TREATMENT

GROUP	NUMBER OF PATIENTS (%)				
mCi/Kg	worse	no change	slight	moderate	complete
0,5	---	1 (7%)	3 (20%)	3 (20%)	8 (53%)
1,0	---	1 (6%)	5 (36%)	4 (29%)	4 (29%)
Total	---	2 (6%)	8 (28%)	7 (24%)	12 (42%)

CAN NOT FOLLOW UP DUE TO

GROUP	NUMBER OF PATIENTS (%)
mCi/Kg	death
0,5	3 (10%)
1,0	6 (20%)
Total	9 (30%)

USE OF DRUGS AFTER THERAPY

GROUP	NUMBER OF PATIENTS (%)			
mCi/Kg	more potent	same drugs	decrease substantially	none
0,5	1 (7%)	3 (23%)	6 (47%)	3 (23%)
1,0	3 (20%)	5 (33%)	6 (40%)	1 (7%)
Total	4 (14%)	8 (29%)	12 (43%)	4 (14%)

DURATION OF PALLIATION

GROUP	NUMBER OF PATIENTS				
mCi/Kg	< 1 week	1–2 weeks	2–4 weeks	4–8 weeks	> 8 weeks
0,5	---	---	5	2	8
1,0	---	2	5	3	4
Total	---	2	10	5	12

ERYTHROCYTE SUPRESSION

GROUP	BLOOD COUNT		
mCi/Kg	avg. baseline	% max. drop	avg. time of max. drop
0,5	4.100.000	13%	3 weeks
1,0	3.800.000	14%	3 weeks

LEUKOCYTE SUPRESSION

GROUP	BLOOD COUNT		
mCi/Kg	avg. baseline	% max. drop	avg. time of max. drop
0,5	6.800	27%	4 weeks
1,0	7.400	51%	3 weeks

PLATELET SUPRESSION

GROUP	BLOOD COUNT		
mCi/Kg	avg. baseline	% max. drop	avg. time of max. drop
0,5	281.000	39%	4 weeks
1,0	290.000	63%	4 weeks

RANDOM COMPARISON STUDY OF THE CLINICAL RESPONSE TO ^{153}Sm-EDTMP 1.0 mCi/kg AND 1.5 mCi/kg

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Abstract. Sixty-seven patient with painful bone metastases were randomized to two groups. Group 1 (n = 34) received 1.0 mCi/kg of ^{153}Sm-EDTMP and group II (n = 33) received 1.5 mCi/kg. All of them met inclusion criteria and there was no significantly difference between the basic conditions of two groups. After receiving ^{153}Sm-EDTMP intravenously, all patients were kept in close follow-up weekly with blood counting, physician visiting and collecting patient's self-filling-in diary including pain score, Karnofsky performance scale and analgesic consumption. The follow-up duration was six weeks. The final overall condition assessed by physician were graded into no change (including worse), slight relief, significant relief and complete relief. Only significant relief and complete relief were considered as effectiveness for pain relief. Haematological toxicity grade was evaluated based on the nadir of WBC ad PLT counts. The results indicated that the higher dosage group had a higher effectiveness rate (75.76%) compared to the lower dosage group (67.65%), but without statistic significance ($x^2 = 0.5365$, $0.25 < P < 0.50$). The difference of haematological toxicity and other adverse reactions between the two groups were also insignificant. We concluded that 1.5 mCi/kg of ^{153}Sm-EDTMP could be used for those patients with better haematological function and 1.0 mCi/kg used for those patients with poorer haematological function.

1. OBJECTIVE

The usual dosage of ^{153}Sm-EDTMP used in the treatment of metastatic bone pain is 0.5 ~ 1.0 mCi/kg. But the efficacy of this dosage is not much satisfying. The aim of present study is to compare the clinical response to 1.0 mCi/kg and the higher dose (1.5 mCi/kg) in hopes of promoting efficacy without increasing toxicity significantly.

2. MATERIALS AND METHODS

Sixty-seven patients were randomized to two groups. Group I (n = 34) received 1.0 mCi/kg of ^{153}Sm-EDTMP and group II (n = 33) received 1.5mCi/kg.

The basic conditions of two groups presented in Table I and II.

TABLE I. THE BASIC CONDITIONS OF TWO GROUPS

Group	n	M	F	Age (yr)	Bone pain score	No. of bone foci	WBC (10^9/L)	PLT (10^9/L)	Follow-up duration (wk)
I	34	22	12	54.3 (29~84)	6.1 (4~16)	9.1 (1~10)	7.13 ± 1.7	224.3 ± 65.5	5.8 (5~6)
II	33	22	11	61.1 (24~77)	8.0 (6~12)	8.9 (2~10)	7.14 ± 1.8	202.3 ± 60.8	6.0 (4~7)

TABLE II. PRIMARY MALIGNANT LESIONS OF THE PATIENTS

Group	n	Lung	Breast	NPS	Prostate	Colorectal	Others
I	34	14	6	6	1	2	5
II	33	9	5	6	3	3	7

All patients with cancer and suffer from bone pain caused by positive bone scan lesions. The bone pain score was calculated by multiplying pain degree and pain frequency (Table 3). The basic bone pain scores of all patients evaluated before receiving ^{153}Sm-EDTMP were higher than 6 with the exception of two patients with score 4. The Karnofsky performance scales of all patients were less than 70.

TABLE III. BONE PAIN SCORE

Pain degree	Score	Pain frequency	Score
none	0	none	0
mild	1	<1/d	1
moderate	2	1~3/d	2
severe	3	>3/d	3
intolerable	4	continuous	4

All of them met other inclusion criteria (Table IV). ^{153}Sm-EDTMP was prepared by China Institute of Atomic Energy (CIAE). Radiochemical purity >98%, specific radioactivity > 2 mCi/mg.

TABLE IV. PATIENT INCLUSION CRITERIA

1. Patient requires analgesics for control of pain caused by positive bone scan metastases
2. lesions. Pain score should be more then 6. Karnofsky performance scale less than 70.
3. White blood cell (WBC) count is more than 3.5×10^9/L.
4. Absolute granulocyte count is more than 1.7×10^9/L.
5. Platelet (PLT) count is more than 80×10^9/L.
6. Liver and kidney function are normal or mild abnormal.
7. Patient, if on hormones, is on stable hormone regimen for not less than three months.
8. Patient had no external radiation therapy within the last 6 weeks.
9. Patient has no symptoms or signs of spinal cord compression.
10. Patient can return to clinic for follow-up.
11. Patient has life expectancy greater than 6 weeks.
12. Patient, if female, is not pregnant or nursing.
13. Patient is not in a clinical trial of another investigational drug.
14. Informed consent is obtained from the patient.

The study was approved by the Institute Ethical Committee.

Therapy procedure

1. After receiving ^{153}Sm-EDTMP intravenously, all patients were kept in close follow-up weekly with blood counting, physician visiting and collecting patient's self-filling-in diary including pain score, Karnofsky performance scale and analgesic consumption. In case of positive clinical response, the follow-up duration was six weeks at least. Otherwise, it was four weeks. Weekly and after study finished, physician assessed patient's overall condition based on the changes of pain score, Karnofsky performance scale and analgesic consumption. The final overall conditions were graded into no change and worse (0), slight relief (I); significant relief including moderate relief and marked relief (II) and complete relief (III) (Table V). Only significant relief and complete relief were considered as effectiveness for pain relief.

TABLE V. PHYSICIANS GLOBAL ASSESSMENT

Overall condition	Score	Comment	Efficacy grade
Pain was worse discomfort and analgesic consumption increased daily activity decrease	-1	Worse	Null (0)
No change	0	No change	Null (0)
Pain relieved slightly discomfort and/or analgesic consumption decreased a little	1	slight relief	Slight (I)
Pain was noticeably improved but still present and may cause some discomfort or decrease daily activities	2	Moderate relief	Significant (II)
Pain was vastly improved and, although present, was scarcely troublesome and did not interfere with daily activities	3	Marked relief	Significant (II)
No pain presented, normal daily activities were performed and no analgesic was needed	4	Complete relief	Complete (III)

2. Haematology toxicity grade was evaluated based on the nadir of counts as presentation in Table VI.

TABLE VI. HAEMATOLOGY TOXICITY GRADE

	Haematology toxicity grade				
	0	I	II	III	IV
WBC ($\times 10^9$/L)	$\geq$4	3~3.9	2~2.9	1~1.9	<1
PLT ($\times 10^9$/L)	$\geq$100	75~99	50~74	25~49	<25

3. Liver and kidney toxicity were evaluated based on the results of liver function test and renal function test respectively. Liver and renal function values increased but $\leq 1.25 \times N$, increased in the range of 1.26N ~ 2.5N, 2.6N ~ 5N, 5N ~ 10N and > 10N were considered as toxicity grade 0, I, II, III and IV, respectively, where N was the upper limit of normal value.

3. RESULTS

3.1. Pain relief (Table VII)

In group I, eighteen patients had pain released, analgesic reduced and general condition improved significantly, and 5 patients had pain released completely. Therefore the effectiveness rate of this group was 67.65% (23/34). The effectiveness started within 2 weeks post ^{153}Sm-EDTMP and lasted more than 3 weeks. In group II, The effectiveness rate was 75.76% (25/33) and it's starting time and lasting duration were the same as group I. As compared to group I, the higher dose group had a higher effectiveness rate, but without statistic significance ($x^2 = 0.5365$, $0.25 < P < 0.50$).

TABLE VII. ^{153}SM-EDTMP EFFICACY IN PAIN RELIEF

Group	n	Patient's overall condition post treatment				Effectiveness
		0	I	II	III	
I	34	5	6	18	5	23 (67.65%)
II	33	2	6	23	2	25 (75.76%)

3.2. Haematology toxicity (Table 8,9)

In either of the two groups, haematology toxicity was significant and almost the same in gravity. After receiving ^{153}Sm-EDTMP, WBC counts decreased 50.5% ± 14.7% and 49.0% ± 22.8% in group I and group II respectively. The nadir time of WBC counts was 3.2 ± 1.7 wks and 3.7 ± 1.6 wks. PLT counts decreased 60.9% ± 21.2% and 56.1% ± 20.6%, and the nadir time of PLT was 4.0 ± 1.0 wks and 3.9 ± 1.4 wks in group I and group II, respectively. In group II, The sum of WBC toxicity grade II, III and IV was 10, more than that of group I (8). On the contrary, the sum of PLT toxicity II, III and IV in group II was 15, less than that of group I (21). After the nadir time, WBC and PLT increased gradually, but they did not recover to the pre-treatment level at the end of the 6th week. None of life-threatening haematology toxicity was noticed. So, as a whole, the difference of haematology toxicity between the two groups was insignificant.

TABLE VIII. ^{153}SM-EDTMP HAEMATOLOGY TOXICITY

		WBC		PLT	
Group	n	decrease (%)	nadir time (wk)	decrease (%)	nadir time (wk)
I	34	50.5 ± 14.7	3.2 ± 1.7	60.9 ± 21.2	4.0 ± 1.0
II	33	49.0 ± 27.8	3.7 ± 1.6	56.1 ± 20.6	3.9 ± 1.4

TABLE IX. ^{153}SM-EDTMP HAEMATOLOGY TOXICITY GRADE

		WBC					PLT				
Group	n	0	I	II	III	IV	0	I	II	III	IV
I	34	18	8	4	4	0	8	5	16	4	1
II	33	10	13	7	3	0	9	9	11	3	1

3.3. Other adverse reactions (Table X)

Mild liver toxicity and mild kidney toxicity was found in 2 patients and 1 patient in group I and in 4 patients and 1 patients in group II, respectively. In group I, transient weakness and anorexia, vomiting, and cough were noted in 6, 8 and 2 patients, respectively. In group II, transient dizziness occurred in 1 patient, anorexia in 4 patients and vomiting in other 5 patients.

In group II, One death occurred 6 weeks after injection and was thought to be related to the underlying disease. One cerebral haemorrhage occurred and was not thought to be related to the haemotology toxicity of ^{153}Sm-EDTMP, because his platelet counts was more the 75×10^9/L.

TABLE X. OTHER ADVERSE REACTION

	No. of case	
Adverse reaction	Group I	Group II
Weekness and anorexia	6	4
Vomiting	8	5
Dizziness	0	1
Cough	2	0
Mill liver toxicity	2	4
Mild kidney toxicity	1	1

3.4. Flare phenomenon (Table XI)

Flare phenomenon was found in 12 patients of group I and 10 patients of group II. There was no significant relation between this phenomenon and pain relief grade (Table XI).

TABLE XI. FLARE PHENOMENON AND RELATION TO PAIN RELIEF GRADE

		Flare phenomenon				
		Pain relief grade				
Group	n	0	I	II	III	total
I	34	2	4	6	0	12
II	33	3	1	3	3	10

4. DISCUSSION

Dosage-response relationship in radiopharmaceutical therapy of painful bone metastases was an important and interesting subject to be studied for finding out the optimal administered activity which will yield the least toxicity with lightest therapeutic efficacy [1]. Collins C. et al. [2] treated patients with doses beginning at 0.5 mCi/kg, escalating in 0.5 mCi increments to 3.0 mCi/kg. They found that pain responses occurred at 1.0 mCi/kg and 2.5 mCi/kg level was insignificant (70% vs 80%) at 4 wk. Serafini AN et al. [3] reported again pain responses occurred at 0.5 mCi/kg and 1.0 mCi/kg level at 4 wk were 68% and 72% respectively. Our present result also showed there was no statistically significant between pain responses occurred at 1.0 mCi/kg and 1.5 mCi/kg ($p > 0.25$) at 4 wk. Although the good pain responses to dosage varying by a factor of five were in the 65%~80% (interestingly, the pain control occurred in also 65% ~ 80% range for 32P, 89Sr and 186Re and 117mTin treatment), there seems to be a trend of higher response with higher dosage (Table XII). But an attempt to show an 80% response in really different from a 65% response, with an alpha (p) value of < 0.05 and beta of 0.9, would require in excess of 700 patients for the study [4]. Needless to say, it is a daunting and extremely expensive undertaking.

TABLE XII. DOSAGE-PAIN RESPONSE RELATIONSHIP OF ^{153}SM-EDTMP AT 4 WK

Authers	0.5 mCi/kg	1.0 mCi/kg	1.5 mCi/kg	2.5 mCi/kg
Serafini AN et al.	27/40 (68%)	28/39 (72%)		
Pan ZY et al.		23/34 (68%)	25/33 (76%)	
Collins C et al.		14/20 (70%)		15/20 (80%)
Total	27/40 (68%)	65/93 (70%)	25/33 (76%)	15/20 (80%)

Because the follow-up time was only six weeks, we could not get the information about pain response lasting time and the survival of the treated patients. Several reports showed higher dosage with longer duration of palliation and longer survival. Collins C et al. reported increased dose level showed increased marrow suppression. In present study, higher dosage with more marrow suppression was not noticed yet.

Based on the facts that higher dosage has the potential of longer term efficacy and higher haemotologic toxicity, it might be suggested that higher dosage (for example 1.5 mCi/kg) could be used for those patients with better haematological function and lower dosage (1.0 mCi/kg) used for those with poorer haematological function.

ACKNOWLEDGEMENTS

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RADIOCHEMICAL AND BIOLOGICAL STUDIES, INCLUDING IN NON-HUMAN PRIMATES, TOWARDS INDIGENOUS DEVELOPMENT OF ^{153}Sm-EDTMP FOR METASTATIC BONE PAIN PALLIATION

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Abstract. The combination of ease of formulation and superior biological features of ^{153}Sm-EDTMP in terms of safety and efficacy for metastatic bone pain palliation, together with the prospect of better logistics of production, has prompted extensive efforts by many groups world over for its preparation and evaluation. Our efforts have been directed towards exploring the feasibility for formulation of ^{153}Sm-EDTMP suitable for human use by neutron activation in medium flux reactors of the freely available and inexpensive natural samarium oxide target. The emphasis in biological studies was placed on tests in larger animals (monkeys) as a prelude to clinical evaluation. Feasibility to achieve reasonably high specific activity of 300–700 mCi/mg Sm at EOB with natural samarium has been adequately demonstrated. The radioeuropium contamination, estimated by γ–spectrometry to be <0.5% at 3 d after EOB, need not be deemed restrictive in the context of therapeutic application envisaged. Satisfactory formulation of ^{153}Sm-EDTMP from natural samarium at high radioactive concentrations of 40–50 mCi ^{153}Sm/mL, acceptable biolocalization, as revealed by both biodistribution studies in rats (femur uptake of 2–3% injected dose at 1h p.i. and retention up to 120 h p.i.) and gamma camera images in monkeys and adequate stability have been feasible. Excellent quality bone images of monkeys were recorded showing rapid clearance from blood, visualization of skeleton, clearance from kidneys within 2 hours and retention in skeleton up to 116 hours p.i. No significant activity in other soft tissues was noted. Comparative evaluation of the product prepared from enriched samarium as well as using in-house synthesized EDTMP has, likewise, revealed identical biolocalization features. EDTMP dose tolerance test in mice showed a safety factor of about 100 for a product made from natural samarium at an adult human dose of 50 mCi ^{153}Sm. Feasibility for production, reasonable safety and satisfactory biolocalisation of the indigenous product has been adequately established so as to warrant clinical trials in patients.

1. INTRODUCTION

Treatment of intractable bone pain suffered by cancer patients having extensive metastases has been an enormous clinical problem. Other compounding factors to bone metastases have been described as pathologic fracture, immobility, loss of independence and tremendous emotional sequelae, including depression, fear and isolation [1]. The use of analgesics, often narcotics, has contributed to further reduction in the quality of life by the side effects (lethargy, constipation). The silver lining in this bleak scenario has been the proven efficacy of radiation therapy, both as teletherapy in the case of focal lesions and more commonly by internal administration of therapeutic bone seeking radionuclide formulations [1–3]. The latter offers many, significant advantages and can be cited as the most notable contribution of nuclear medicine in recent years. The goal of radiation therapy has not only been for pain palliation in patients, but also for their improved functional status in day to day life. A recent addition to this goal is the possibility that radionuclide therapy may be able to prevent, or at least delay, the onset of new painful metastatic disease [1].

Amongst the radiopharmaceutical products used, ^{32}P as orthophosphate has been the oldest candidate. In spite of concerns about its relatively higher myelo suppression, well conducted clinical evaluation in India and a multicentre study under a CRP of IAEA, have revealed its utility in select group of patients considered eligible on the basis of screening for platelets and leucocytes counts prior to treatment [4, 5]. The approval of FDA for the calcium analogue, $^{89}SrCl_2$, called Metastron™ in June 1993, a more efficacious and safer agent, expanded the applications the world over, in turn,

focussing attention on the considerable benefits accruing to suffering patients [6]. The magnitude of improvement in the quality of life of treated patients has been found to be phenomenal and all out efforts pursued to harness further such benefits. The phosphonate complexes of superior therapeutic radionuclides of ^{186}Re and ^{153}Sm were also shown to be more promising for the palliative treatment of metastatic bone pain [7,8]. Recent efforts have indicated the distinctly favourable features of ^{117m}Sn(IV)-DTPA [9] and the potential of ^{188}Re(V)-DMSA [10].

The combination of favourable logistics of production of ^{153}Sm, ease of formulation, better chemical definition and superior biological features earmark ^{153}Sm-EDTMP (ethylenediamine tetramethylene phosphonate) as more preferable for regular clinical use. The prospect of indigenous production of this product even by developing nations with medium flux reactors appeared promising [11] and detailed investigations to standardise procedures for preparation and testing were considered desirable. The fact that there could be a short supply or even non-availability of enriched samarium (^{152}Sm) targets was recognised and hence natural samarium targets were mostly used in the present study, along with a few lots of enriched targets for comparison. It was, however, aimed to develop protocols applicable for both. Studies on formulation and evaluation of a number of batches of ^{153}Sm-EDTMP, for ease of production, purity, stability, safety and efficacy of bone uptake and retention as well as excretory pattern, have been carried out. The emphasis in biological studies was placed on tests in larger animals (monkeys) as a prelude to clinical evaluation and the results are presented in this paper.

2. MATERIALS AND METHODS

^{153}Sm was prepared by neutron irradiation of 5–10 mg natural Sm_2O_3 (American Potash) at a neutron flux of 6–8 × 10^{13} n. cm^{-2}. s^{-1} in the Dhruva reactor for about 7 days and dissolved in dilute hydrochloric acid. Two batches were also prepared from enriched samarium (>98%; gift sample from IAEA) for comparative evaluation. Complexation of ^{153}Sm with EDTMP (gift sample from commercial source) was carried out by addition of appropriate quantity of an alkaline EDTMP solution to samarium solution, mixing, adjusting pH to 7–8 and heating over a water bath at 70°C for 5 minutes. Complexes with ligand to metal (L/M, L:M) mole ratio of 20–125:1 were prepared. Products were also formulated using in-house synthesized EDTMP for comparative evaluation [12].

The formulations were evaluated for radiochemical aspects such as radioactive concentration, radiochemical purity and stability by paper chromatography using normal saline and ammonia-ethanol-water:0.2:2:4 as solvents. Aliquots of the product were incubated with human serum and samples drawn for RC purity analysis at different time intervals, in order to study the stability in serum.

Radionuclidic purity was estimated by high resolution γ spectrometry using HPGe detector coupled to a 4K MCA (ORTEC 92 × spectrum master and a PC loaded with ORTEC Maestro-II software). ^{152}Eu source was used for energy and efficiency calibration. Since the main gamma ray energy of ^{153}Sm and ^{155}Eu is much below the lowest gamma energy of ^{152}Eu, care was taken to validate the extrapolation of the efficiency calibration curve below 120 keV range, by assaying the activity of ^{156}Eu by the gamma emissions of both 811.8 and 89 keV and finding comparable values [Table-I(a)].

The biodistribution studies in male Wistar rats were carried out following the procedure described earlier [11]. The uptake expressed as% injected dose (% i.d.) in femurs (or femur/g) at 1 hour post injection (p.i.) was considered a good index of product quality. Long term retention upto 120 hours p.i. was also studied.

For blood clearance studies, rabbits (2.5–3 kg) were injected with an appropriate volume of the formulation into the ear vein through a butterfly needle arrangement. One mL blood was drawn at pre-determined time intervals and the activity in blood counted at a suitable geometry in a NaI(Tl) counter, in comparison to a standard, diluted to appropriate volume to approximate the total volume of rabbit blood. The activity in blood was expressed as% i.d.

EDTMP dose tolerance studies were performed on male Swiss mice (sets of 5 each) by slow administration (30 sec/mouse) of 0.1 to 0.2 mL EDTMP solution through the tail vein. The animals were kept under observation for about 15 days.

Dynamic and static images in monkeys were acquired using a gamma camera (Siemens-Orbiter/Diacam) at the Christian Medical College Hospital, Vellore, following administration of 0.4–1 mCi ^{153}Sm/kg into the leg vein of anaesthetised monkeys (3–8 kg).

3. RESULTS

On irradiation of natural samarium targets, ^{153}Sm of specific activity (at EOB) in the range of 300–700 mCi/mg Sm was obtained, while with enriched samarium targets, the specific activity was in the range of 600–1000 mCi/mg Sm (Table-I). Formulations ranging in radioactive concentration from 8–50 mCi/mL, Sm content 0.02–0.2 mg/mL and EDTMP content 1–46 mg/mL were prepared in this series of studies.

Radiochemical Purity (RCP) and Stability

While radiochemical purity tests form a part of the routine QC of ^{153}Sm-EDTMP, the emphasis in this set of experiments was on studying the stability of formulations at relatively high radioactive concentration of 40–50 mCi/mL and high ligand to metal (L/M) mole ratio of > 50:1. The latter was essential to minimize liver uptake as will be discussed later. Fig. 1(a) shows the stability of two formulations at 50 mCi/mL and 12.5 mCi/mL over a period of 8–10 days. The stability at ambient temperature was intentionally studied, though the product is normally envisaged to be stored refrigerated/frozen. It can be seen that there is no significant decrease in RCP for formulation at 12.5 mCi/mL throughout the period of 8 days. The formulation at 50 mCi/mL, however, shows a decreasing trend in RCP beyond 3–4 days. As expected, the same formulation when stored at 0°C did not exhibit any significant drop in RCP even beyond 4 days. It would be therefore possible to transport the product at ambient temperature and then store the product refrigerated.

TABLE I(A). RADIONUCLIDIC (RN) PURITY OF ^{153}SM: POSSIBLE SOURCE OF RN IMPURITIES

Radionuclide and t½	Eγ (keV)	γ abundance (%)	Probable route of production
^{154}Eu 8.5y	123.1	40.5	^{153}Sm $\xrightarrow[46.3\ h]{(\beta,\gamma)}$ ^{153}Eu (n,γ) ^{154}Eu σ = 390 b
^{155}Eu 4.68y	86.5 105.3	32.7 21.8	^{154}Sm (n,γ) ^{155}Sm (22.7%) $\xrightarrow[22.1\ min]{(\beta,\gamma)}$ ^{155}Eu σ = 5.5 b
^{156}Eu 15.2d	811.8 89	10.3 8.96	^{155}Eu (n,γ) ^{156}Eu σ = 4040 b

NOTE: Gamma ray intensity data taken from Gamma Ray Catalog, U. Reus, et al. GSI Report 79–2.

The stability of the product was also followed as a function of L/M mole ratio at radioactive concentration of ~50 mCi/mL. Formulation at L/M mole ratio of >100 showed consistently high RCP of >95% even when stored at room temperature [Fig. 1(b)].

The stability of the product in serum was also found to be satisfactory, over 98% and the chromatogram profile (Whatman 1 paper & normal saline solvent) of the product in saline and serum was comparable.

TABLE I(B). RADIONUCLIDIC (RN) PURITY DATA OF ^{153}SM

Lot No.	Sp. Act. at 2 d after EOB (mCi/mg)	μCi *Eu/mCi ^{153}Sm at 2 d after EOB		
		^{154}Eu	^{155}Eu	^{156}Eu
$1	193	0.005	0.13	0.68
2	357	0.011	0.15	1.36
3	342	0.013	0.15	1.47
4	365	0.018	0.17	2.26
$5	174	0.003	0.08	0.51
6	273	0.009	0.13	1.12
7	239	0.007	0.13	0.97
#8	497	0.014	0.0017	---
#9	319	0.004	0.006	---

Lots 1 to 7 prepared from natural samarium (26.7% ^{152}Sm).
 # Lots 8 & 9 prepared from enriched ^{152}Sm (>98%), 7 day & 3 day irradiation, respectively.
$ Lots 1 & 5: ~4 day irradiation.

Radionuclidic (RN) purity

Table-I reveals the extent of radio-europium contamination observed while using natural and enriched samarium targets. Due to some technical and operational reasons, currently planned short term irradiations of 48–60 hours duration are not regularly feasible in our reactor. The lots 1 & 5 in Table-I(b) simulate typical irradiation conditions intended for regular production purposes. Even for ^{153}Sm prepared from natural samarium targets after 7 days of irradiation, depending upon the reactor operation conditions, a maximum of ~0.02 μCi ^{154}Eu, 0.2 μCi ^{155}Eu and 2.5 μCi ^{156}Eu were detected per mCi ^{153}Sm, at reference time of 2 days from EOB. Samples containing enriched samarium showed contamination of ^{154}Eu of the same order, ^{155}Eu decreased by nearly two orders of magnitude and practically no ^{156}Eu. These values are broadly accountable from the possible route of formation of the impurity nuclides [Table-I(a)]. Improvement of RN purity (but at the cost of reduced yield) could also be achieved by radiochemical purification [13], but is not deemed essential in the context of therapeutic application envisaged and the known similarity in biological behaviour of Sm and Eu chelates of phosphonates.

Biodistribution studies

Biodistribution studies in rats showed femur uptake of 2–3% injected dose or 4.5–5.5% i.d./g femur at 1 h p.i. The uptake in other major organs of liver and kidneys was <1% i.d. The long term biodistribution studies revealed satisfactory retention in femurs upto 120 hours p.i., 2.5(0.04), 2.48(0.32), 2.96(0.11) and 2.67(0.29)% i.d. at 1, 24, 48 and 120 hour p.i., respectively, and no significant activity retention in any other tissues/organs.

Femur uptake per gram calculated and used for comparison of products was found to be a superior index.

These findings were valid for products of about 15 mCi/mL radioactive concentration and prepared from ^{153}Sm of specific activity <100mCi/mg Sm at L:M:: 20–25:1. However, a product formulated once from ^{153}Sm of specific activity ~700 mCi/mg Sm, radioactive concentration of 12mCi/mL and L/M mole excess of 20: 1 (EDTMP content: 1 mg/mL), showed significant liver activity (~7%), in addition to good femur uptake of 2.17 (±0.30)% at 1 h p.i. This was also validated by imaging studies in the monkey. Our observations are consistent with reports in literature [14], which state that the EDTMP concentration should be not less than 10 mg/mL to minimize the liver uptake of ^{153}Sm-EDTMP.

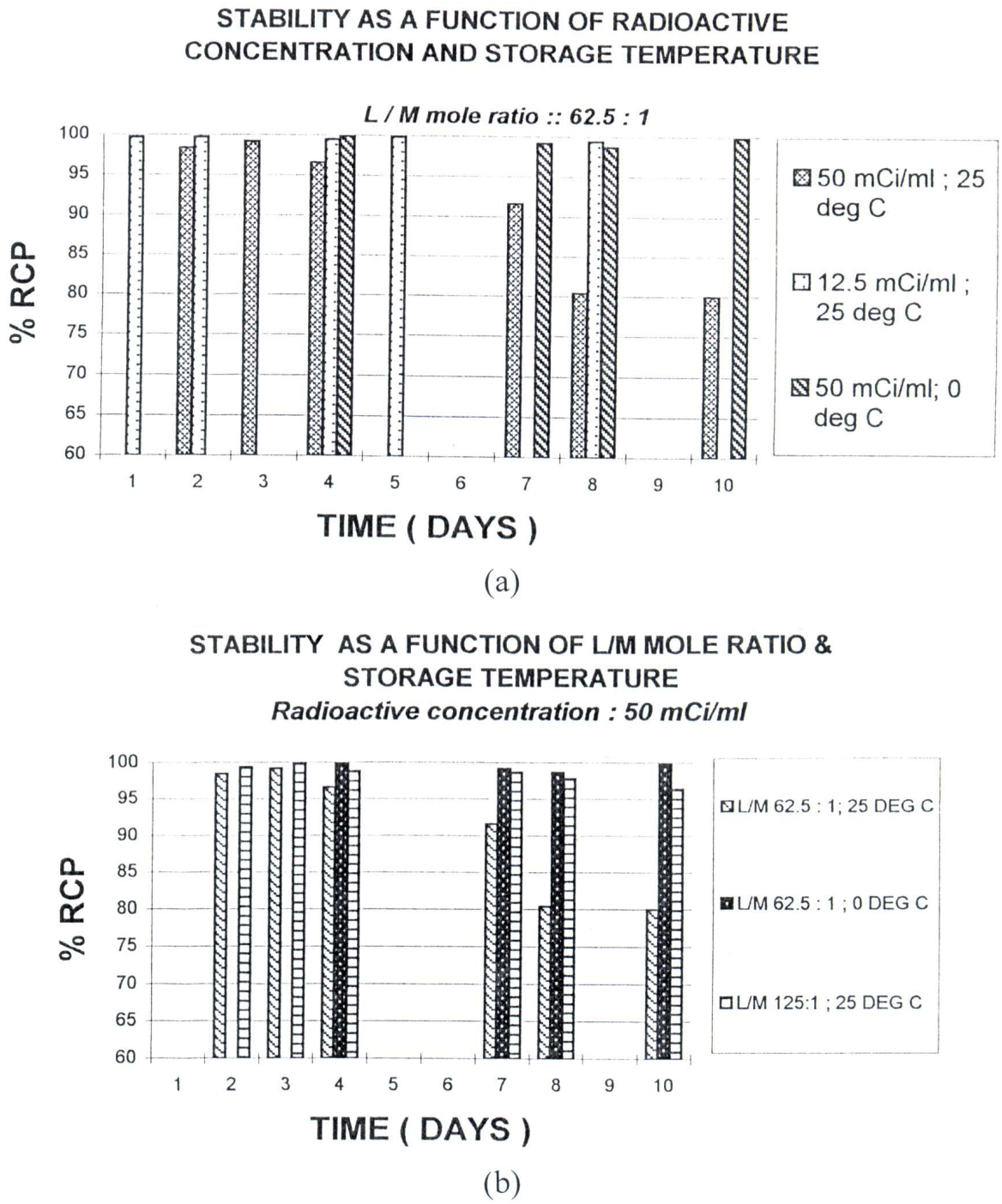


FIG. 1. Stability of ^{153}Sm-EDTMP formulations as a function of (a) Radioactive concentration and storage temperature (b) L: M mole ratio and storage temperature.

Consequently, all products prepared at high radioactive concentration (40–50 mCi/mL) and high specific activity were formulated at a L/M mole excess of 50 to 100. These formulations showed satisfactory biodistribution with no significant activity seen in the liver (Table-II). Table-II also gives comparative values of biodistribution at 1 h p.i. of products prepared from both natural and enriched samarium targets. It can be seen that the results are comparable.

TABLE II. BIODISTRIBUTION STUDIES IN RATS OF ^{153}SM-EDTMP FORMULATIONS USING NATURAL SAMARIUM AND ENRICHED SAMARIUM

Organ/Tissue	Formulation with natural samarium (Sp. Act. 391 mCi/mg Sm)		Formulation with enriched Samarium (Sp. Act. 482 mCi/mg Sm)	
	% i.d. at 1 h p.i. Mean (SD), n = 3		% i.d. at 1 h p.i. Mean (SD), n = 3	
	Lot 1	Lot 2	Lot 3	Lot 4
Blood/g	0.03 (0.01)	0.04 (0.01)	0.07 (0.04)	0.12 (0.05)
Stomach	0.32 (0.19)	0.18 (0.23)	2.06 (1.75)	1.54 (1.99)
Muscle/g	0.24 (0.20)	0.33 (0.12)	0.27 (0.08)	0.11 (0.07)
Liver	0.24 (0.03)	0.18 (0.03)	0.40 (0.05)	0.42 (0.04)
Kidney	0.31 (0.03)	0.27 (0.01)	0.58 (0.21)	0.59 (0.11)
Femurs	2.55 (0.09)	2.59 (0.11)	2.87 (0.34)	2.78 (0.87)
Femur/g	5.32 (0.2)	6.35 (0.62)	5.46 (0.54)	5.15 (1.41)
S. Intestine	0.50 (0.22)	0.41 (0.11)	1.30 (0.80)	0.68 (0.12)
L. Intestine	0.16 (0.07)	0.32 (0.29)	1.80 (2.56)	0.36 (0.05)

Lot 1: 50 mCi/mL; [EDTMP]/[Sm]: 62.5: 1
Lot 2: 47 mCi/mL; [EDTMP]/[Sm]: 125: 1
Lot 3: 50 mCi/mL; [EDTMP]/[Sm]: 50: 1.
Lot 4: 44 mCi/mL; [EDTMP]/[Sm]: 100: 1.

Blood clearance studies in rabbits and monkey

Nearly 50% of injected activity was cleared within 1 minute from the vascular pool, the blood level further dropping to ~20%, ~10% and ~5% at 10min, 30min and 1 hour, respectively. ~1.5% i.d. was almost persistent in blood well beyond 2 hours. In view of the very rapid blood clearance noted, attempts were made to acquire dynamic images in monkeys injected under the gamma camera at 1 sec/frame mode for 1–2 minutes. The time activity curves generated over the entire heart to represent blood pool activity (after correcting for background activity in an area of similar pixel size) showed very high clearance rate, similar to observations in rabbits. Quantification of blood pool activity in relation to injected activity was, however, not done in the monkey studies.

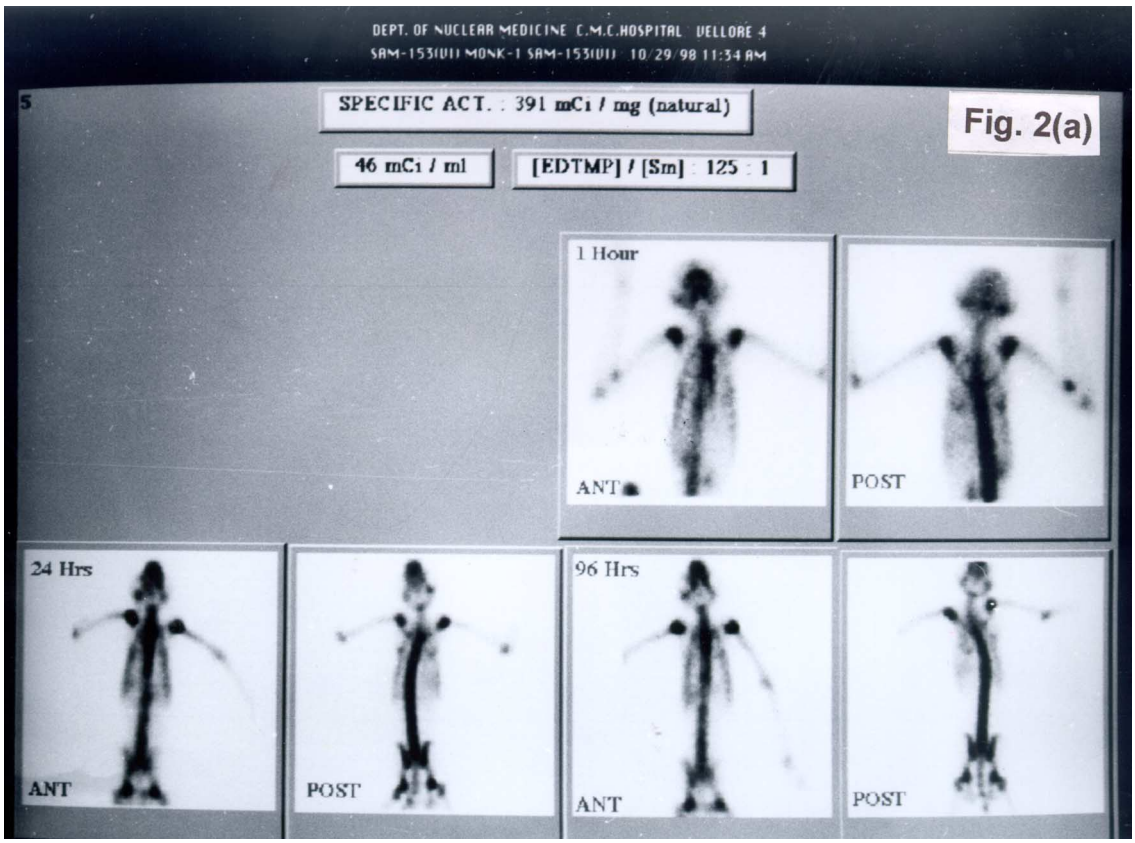
EDTMP dose tolerance studies

Up to 4 mg of EDTMP injected per mouse (Body weight: ~25 grams) was found to be well tolerated. None of the mice died during the period of observation or showed any significant change in body weight during the period of observation of 15 days. However, transient symptoms of behavioural changes like irritability, excitability and asphyxia were observed immediately p.i., which subsided quickly thereafter.

Imaging studies in monkeys

Images acquired as early as 20–30 min showed rapid clearance from blood, visualization of skeleton, renal activity up to 2 hours and clearance thereafter, progressive increase in skeletal activity and retention up to 116 hours p.i. (maximum duration of study). No significant activity in any other soft tissues was noted. Excellent quality bone images of monkeys were recorded.

Likewise, the quality of images from formulation using an authentic sample of EDTMP (gift from a collaborator) and that synthesized in-house [12] revealed no significant differences, as can be seen from Fig. 3(a) and 3(b).



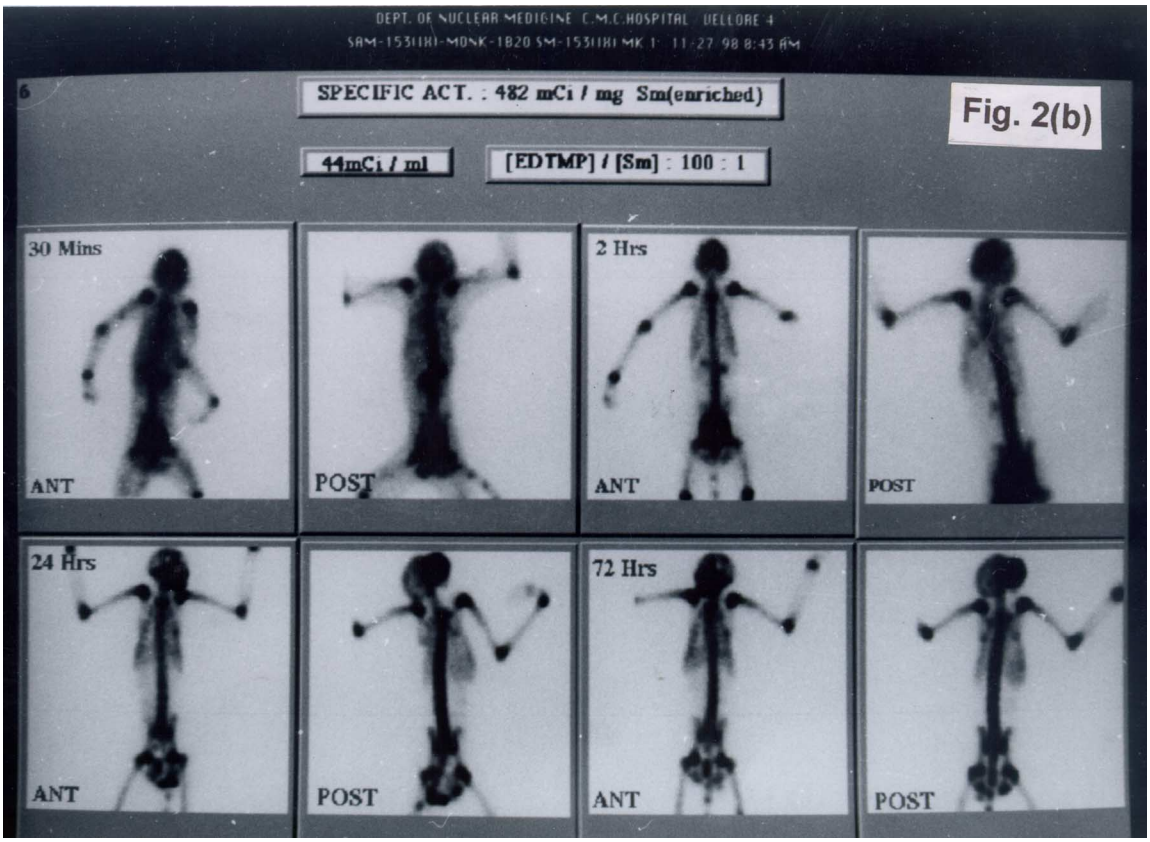
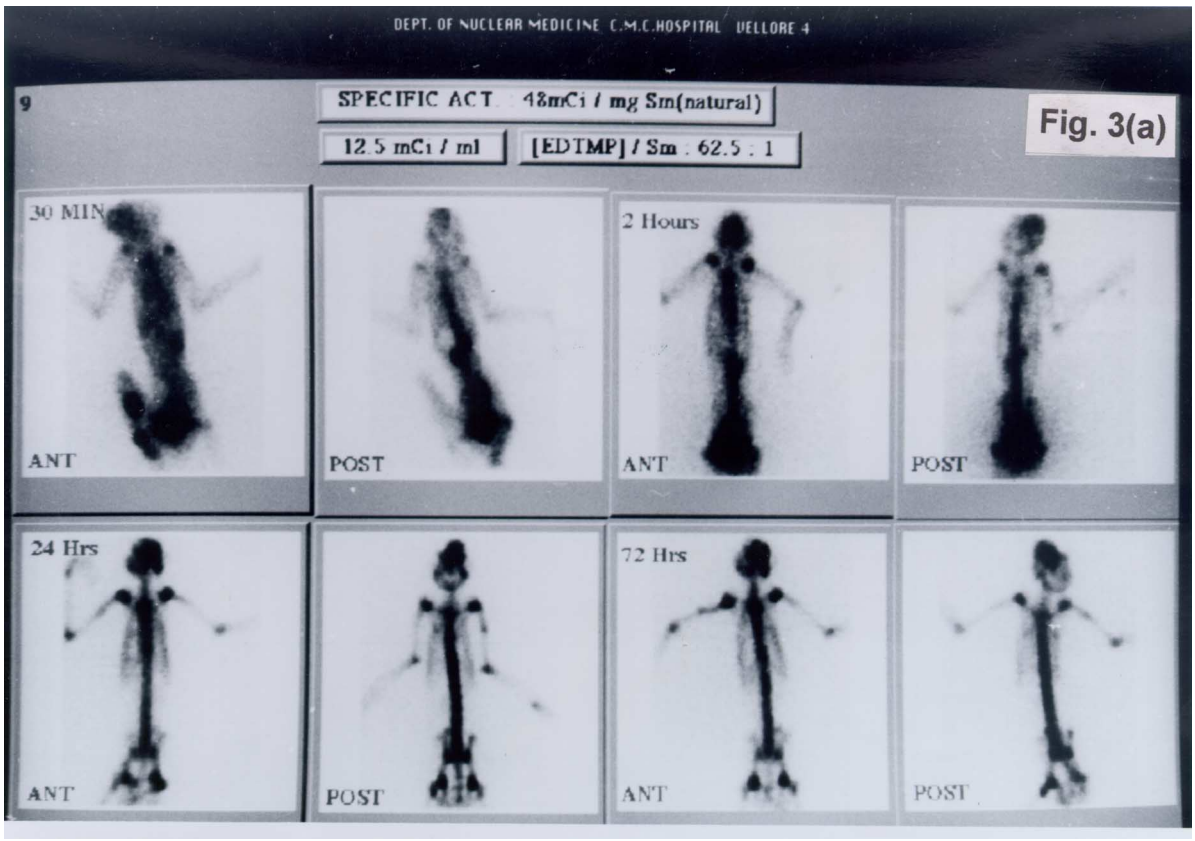


FIG. 2. Monkey images of 153Sm-EDTMP formulations
(a) using natural samarium targets: 46 mCi/mL, 391 mCi/mg Sm, L: M:: 125: 1
(b) using enriched samarium targets: 44 mCi/mL, 482 mCi/mg Sm, L: M:: 100: 1.



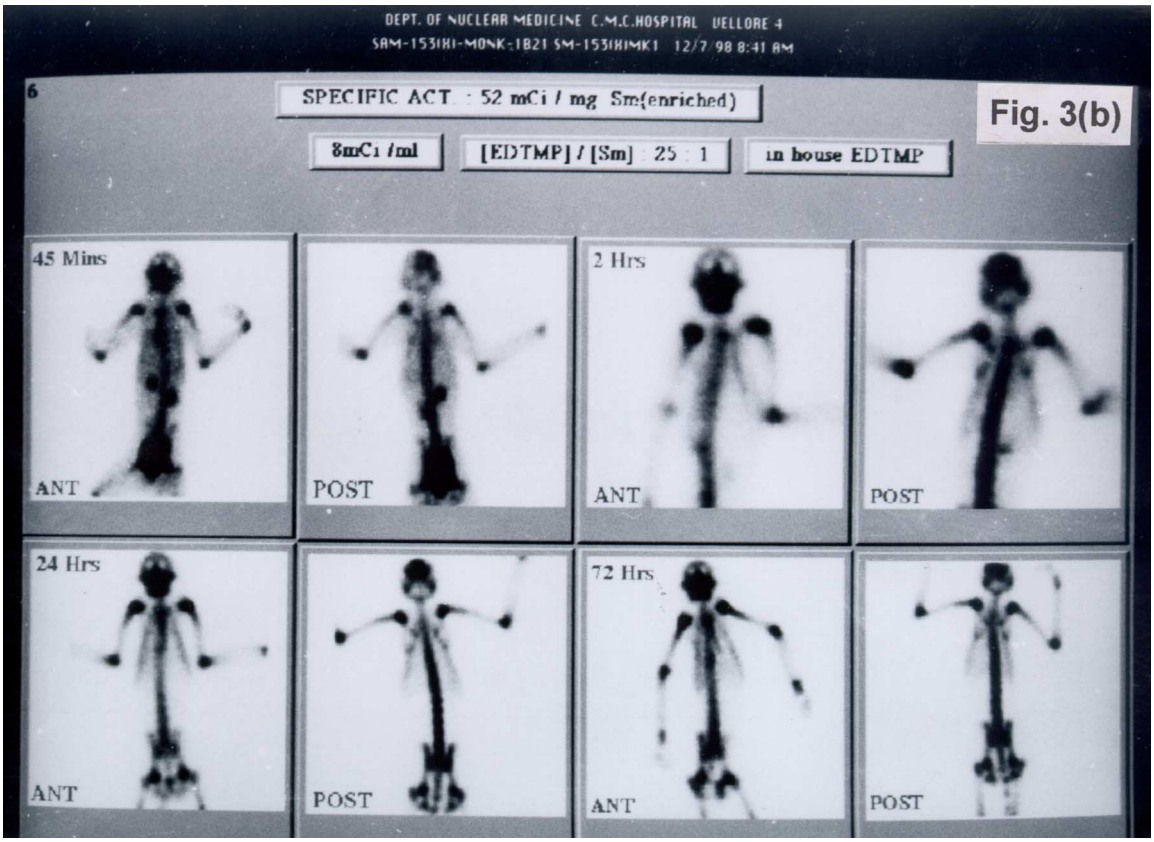


Fig. 3: Monkey images of 153Sm-EDTMP formulations
(a) using authentic sample of EDTMP: 13 mCi/mL, 48 mCi/mg Sm, L: M:: 62.5: 1
(b) using in-house synthesised EDTMP: 8 mCi/mL, 52 mCi/mg Sm, L: M:: 25: 1.

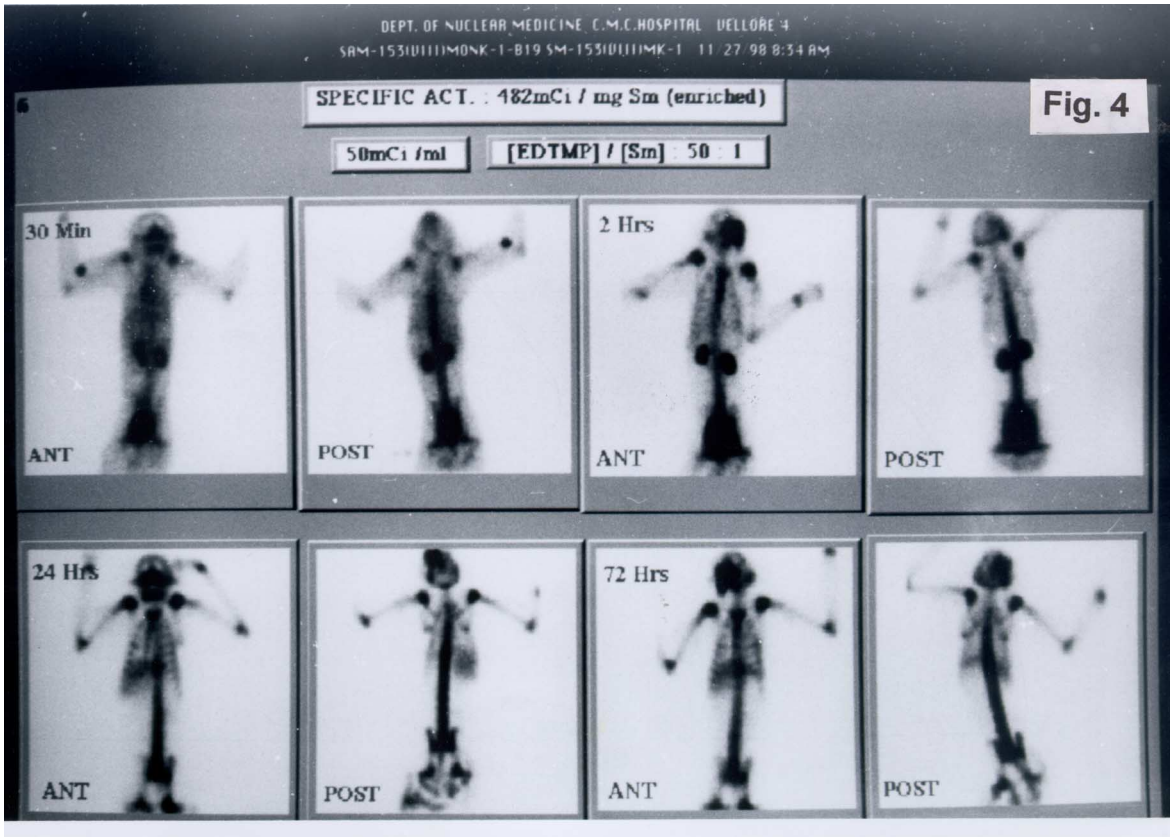


FIG. 4. Monkey images of ^{153}Sm-EDTMP formulation with high specific activity ^{153}Sm (obtained from enriched samarium target) and at L: M:: 50: 1. (50 mCi/mL, 482 mCi/mg Sm).

The results of comparative evaluation of product of similar specific activity, radioactive concentration and L/M mole ratio, but formulated using natural and enriched samarium targets, are shown in Fig. 2(a) and 2(b). No significant difference in image quality could be observed, which is consistent with the biodistribution results shown in Table-II.

Consistent with reports in literature [14] that formulation using enriched samarium require a high L/M mole ratio of > 80: 1 to minimize liver uptake, our studies with the product formulated with ^{153}Sm of specific activity 500mCi/mg Sm at L:M:50: 1 revealed liver visualization (Fig. 4). No significant liver activity was noticeable, however, in the product formulated at L:M:100: 1 with the same lot of ^{153}Sm [Fig.3(b)].

It was also observed that there was no liver activity in the product prepared from the same batch of ^{153}Sm, but after decay for about a week, even at a lower L:M mole ratio of 25:1 [Fig. 4 & 2(b)]. In other words, higher L:M mole ratio is essential for formulation of Sm-EDTMP from ^{153}Sm of high specific activity of the order of >350mCi/mg Sm.

4. DISCUSSION

It has been feasible to achieve reasonably high specific activity, 300–700 mCi (at EOB)/mg Sm, with natural samarium targets. Our data have also shown that the extent of radio-europium contamination with natural samarium targets is <0.5%. This need not be deemed restrictive in the context of therapeutic application envisaged and the known similarity in biological behaviour of Sm and Eu chelates of phosphonates.

Our studies reveal that a product of acceptable quality and adequate stability at high radioactive concentration of 40–50 mCi/mL could be satisfactorily formulated. Optimized formulations of ^{153}Sm-EDTMP prepared from both natural and enriched samarium exhibit identical biolocalization features as proven both by biodistribution in rats and imaging studies in monkeys. The same formulation protocol standardised is applicable for both. The EDTMP synthesised in-house [12] following a reported procedure, was found to yield a satisfactory product and could be used for regular indigenous manufacture.

For adult human dose envisaged at 50 mCi ^{153}Sm (~1 mCi ^{153}Sm/kg), a safety factor of about 100 could be shown for the product prepared using natural samarium (^{153}Sm of minimum specific activity of 300 mCi/mg Sm at EOB) and formulated at [EDTMP]: [Sm]:50–70:1. Practicable shelf life of 3 days from the EOB would be, however, advisable from a conservative viewpoint of stability, safety and radionuclidic contamination. The former two restrictions would also apply for a product made from enriched samarium, since high specific activity ^{153}Sm would require to be complexed with greater excess of EDTMP at L/M of 100: 1 in order to minimize the uptake in liver.

Our results have thus established the feasibility for production, reasonable safety and satisfactory biolocalization of indigenous product so as to warrant clinical trials in patients.

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6. RADIATION SYNOVECTOMY

^{166}Ho LABELLED HYDROXY APATITE PARTICLES FOR RADIOSYNOVECTOMY

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Abstract. The preparation of ^{166}Ho labelled hydroxyapatite (HA) particles for radiosynovectomy applications is described in this paper. ^{166}Ho was prepared by irradiating Ho_2O_3 in the Dhruva reactor at a flux of $1.8x10^{13}$ neutrons/cm^2/s. The irradiated target was dissolved in 0.1 N HCl solution. Gamma spectrometry of the processed ^{166}Ho did not show gamma peaks for any other radionuclide other than ^{166}Ho, thereby confirming the radionuclidic purity of the radioisotope. The irradiation resulted in the production of ~17 GBq of ^{166}Ho activity at the end of six hours post EOB and the corresponding specific activity was ~3.6 TBq/g of Ho. HA particles were synthesised by a reported method. Labelling studies were carried out with and without citric acid as a transchelating agent. Radiochemical purity of the ^{166}Ho-HA particles was ascertained by paper chromatography and by paper electrophoresis techniques. Labelling yield up to 100% could be achieved at pH 7, with 40 mg of HA particles and 8.6 μg of ^{166}Ho. Use of citric acid was not found to be essential for getting consistently high yields. ^{166}Ho-HA particles prepared were found to be stable for 72 h. The high labelling yield and good stability of the ^{166}Ho labelled particles indicate that these particles could be used for radiosynovectomy applications after obtaining satisfactory biodistribution results.

1. INTRODUCTION

Development of therapeutic radiopharmaceuticals for the effective management of synovial inflammation and related arthritis problems is one of the areas of current interest [1–5]. Earlier reported methods involving the use of inorganic colloids of radionuclides such as ^{32}P, ^{198}Au resulted in excessive leakage from the injected joints thereby resulting in high radiation risk to the patient [6]. Hence, the current research is directed towards the development of isotopically labelled particulates which have exceptionally high *in vivo* stability. A number of radioisotopes are now used or proposed to be used for radiosynovectomy [7–10]. A radioisotope to be used in radiosynovectomy should have β^- radiation with energies sufficient to penetrate and ablate the inflamed synovium without causing any serious damage to the underlying bone and cartilage of the joint. A small fraction of low energetic γ radiation will be useful to image the distribution of the radiopharmaceutical inside the body by gamma scintigraphy. The ideal half life of the radioisotope is ~1–2 days. The chemical nature of the radiopharmaceuticals should be such that if the radioisotope is released *in vivo*, it should get cleared from the system as quickly as possible. The isotope as such should not have much affinity to any organs or bones.

The use of particulates (10–20 μM size) labelled with beta particle emitting isotopes such as ^{166}Ho, ^{153}Sm ,^{186}Re, ^{165}Dy and ^{90}Y are currently used for radiosynovectomy. Hydroxyapatite (HA) [$Ca_{10}(PO_4)_6(OH)_2$], is one of the preferred particulates for this application as it is a major chemical constituent of skeletal bone matrix and gets converted into Ca and PO_4 ions in the body and gets completely eliminated over a period of six weeks. Preparation of ^{153}Sm and ^{166}Ho labelled hydroxyapatite particles and their biodistribution studies are already reported [4,7,9]. ^{166}Ho is one of the preferred radionuclides for radiosynovectomy due to its excellent characteristics such as 26.9 h half life and $E_{\beta\text{-max}}$ of 1.8 MeV corresponding to a soft tissue penetration of 8.5 mm. ^{166}Ho decay is also associated with the emission of gamma photons of 80 KeV (6%). ^{166}Ho can be made in high specific activities as the target ^{165}Ho is available in 100% natural abundance and have a high (66 barns) thermal neutron capture cross section.

The present paper describes the synthesis of HA particles and radiochemical studies of the HA particles with ^{166}Ho.

2. MATERIALS AND METHODS

All the chemicals used in the experiments were of AR Grade. Calcium nitrate, Ammonium hydrogen phosphate and Citric acid were purchased from E. Merck Chemicals Co. Ho_2O_3 target of Specpure grade was obtained from American Potash Chemical Corporation. Hydroxyapatite was synthesised in our laboratory but matched with an authentic sample provided during an IAEA course held at Beijing, China. Computer controlled X-ray diffractometer PW 1820 with PW 1710 microprocessor and automatic powder diffractometer was used for this purpose. Ni filtered CuK_α X-radiation was used for the generation of the pattern.

^{166}Ho activity measurements were made in a calibrated ion chamber when the activity handled were more than a few MBq. Aliquots from this solution were taken for labelling studies. A solid scintillation counter with NaI(Tl) well type crystal was used for measuring the ^{166}Ho activity in the experimental samples. The window of the counter was set between 60–100 KeV. Radionuclidic purity of ^{166}Ho was estimated by using HPGe-4K MCA detector system connected to a computer having Nucleus Inc. PCA-ADC card and software. The detector was precalibrated with respect to ^{152}Eu source and the energy vs. efficiency relation was worked out. ^{166}Ho sources of ~37–74 KBq strength aliquoted in 1 mL solution in thin walled stoppered glass vials were counted for 30 minutes. Photopeak at 81 keV was observed and the corresponding counts were collected.

2.1. Production of ^{166}Ho

^{166}Ho was produced by irradiating $^{165}Ho_2O_3$ target in the Dhruva reactor. 5 mg of Ho_2O_3 was weighed and sealed in a quartz ampoule and irradiated at a neutron flux of 1.8×10^{13} neutrons/cm^2/s for one week and cooled for 6 hours. Irradiated Ho_2O_3 was dissolved in 5 mL of 0.1 N HCl by gentle warming. The resultant solution was evaporated to near dryness and reconstituted with 10 mL of 0.1 N HCl solution. The activity was assayed by measuring the ion current in an ion chamber. The R.N. purity of the isotope formed was estimated by gamma spectrometry. 15–20 GBq of ^{166}Ho activity was recovered after radiochemical processing. The specific activity of ^{166}Ho was ~3–4 TBq/g.

2.2. Synthesis of hydroxyapatite (HA) particles

Hydroxyapatite, $Ca_{10}(OH)_2(PO_4)_6$, was synthesised by following a reported procedure [11]. $Ca(NO_3)_2.4\ H_2O$, 79 g (0.33 M) was dissolved in 300 mL of double distilled water and the pH of the solution was adjusted to 12 by dropwise addition of conc. NH_3 solution. $(NH_4)_2HPO_4$, 26.4 g (0.2 M) was separately dissolved in 500 mL of double distilled water and the pH of this solution was also adjusted to 12. The two solutions were mixed vigorously at which time precipitation was observed. The precipitated HA cake was heated for 10 min. at 70°C and cooled to room temperature. The precipitate was filtered and washed with 200 mL of hot double distilled water which was followed by a further wash with 100 mL of ethanol. The precipitate was dried initially at 150°C for one hour and then at 240°C for another hour. After cooling, the HA cake was broken to small lumps and ground to finer particles and sieved. Particles below 125 μm size was collected and subjected to further attrition in a high speed grinder in order to get HA particles of 5–20 μm size. Particle size analysis was carried out using LA-500 Horiba Laser Diffraction particle analyser. HA particles obtained from CIAE, Beijing were also used in some of the studies.

2.3. Radiochemical Studies

2.3.1. Preparation of ^{166}Ho-Citrate

15 mg of citric acid monohydrate was dissolved in 1.0 mL of 0.1 N HCl to which 20 μL of ^{166}Ho activity (30–37 MBq) was added and vortexed for 30 s. The reaction was allowed to proceed to completion by incubating for 30 min at room temperature.

2.3.2. Labelling of hydroxyapatite particles

200 μL of the ^{166}Ho-citrate was mixed with 40 mg of HA in a test tube and to which 800 μL of double distilled water was added. The contents in the test tube was vortexed for 1 min and kept mixing for 1 h in a shaker at room temperature. The contents were centrifuged at 2000 RPM for 5 min. The activity associated with the supernatant and precipitate was measured. The percent activity associated with the HA particles was calculated from this data. The washing step was done as follows. The liquid in the ^{166}Ho-HA particles was drained off and 4 mL of 0.9% saline solution was added to this. The contents were vortexed, centrifuged at 2000 RPM for 5 min. The activity in the supernatant and the particles were measured. The radiolabelling yield and the extent of activity leached from the particles upon wash were calculated from the above counting data.

Experiments were also conducted by directly using $^{166}HoCl_3$ in order to study the need of citric acid as a transchelating agent. In both the cases, experiments were carried out with different amounts of Ho by adding appropriate quantities of inactive Ho carrier to the activity.

2.4. Quality control techniques

2.4.1. Paper chromatography

Radiochemical purity of the complex was estimated by paper chromatography technique using Whatman 3MM chromatography paper and 0.9% saline as solvent. 5 μL of the ^{166}Ho-citrate or 5 μL of the hydroxyapatite suspension was applied at 1.5 cm from the lower end of the chromatography paper. The chromatography strips were developed in 0.9% saline solution. After run, the strips were cut into 1-cm segments and the radioactivity was measured.

2.4.2. Paper electrophoresis

5 μL samples were spotted on Whatman 3MM chromatography paper, 10–12 cm from the cathode and paper electrophoresis was carried out for 1 h at 300 V in 0.02 M phosphate buffer at pH 7.5. After run, the strips were cut into 1-cm segments and the radioactivity was measured.

3. RESULTS AND DISCUSSION

3.1 Synthesis of hydroxyapatite particles

The synthesis of the hydroxyapatite particles is fairly straight forward and was achieved in high yields by following the reported procedure. X-ray powder diffractometry pattern of the synthesised and authentic samples matched well. The generated pattern of the synthesised sample was also compared with the ASTM standard data card for HA and the results also matched well.

3.2. $^{166}HoCl_3$

The RN purity of the ^{166}Ho was estimated by gamma ray spectrometry. No other gamma peak other than the 81 KeV photopeak of ^{166}Ho was observed. The results indicated that the isotopic preparation is not contaminated with any other gamma emitting radionuclide. As the target taken was of Specpure quality no other activation products are expected. The specific activity of ^{166}Ho formed was in the range of 3–4 TBq/g, which was adequate for the preparation of radiolabelled particles for therapy.

Results of the paper chromatography and paper electrophoresis of $^{166}HoCl_3$ is given in Fig 1 and 2, respectively. In paper chromatography, ^{166}Ho as $HoCl_3$ was found to remain at the point of spotting, whereas in paper electrophoresis, $^{166}HoCl_3$ solution was found to move a little towards the cathode.

The entire activity was seen as a single sharp peak. The chromatography results indicate that ^{166}Ho is present as a single radiochemical species most likely $HoCl_3$.

3.3. ^{166}Ho-citrate complex

In paper chromatography, ^{166}Ho-citrate moved towards the solvent front (Fig. 1), whereas in paper electrophoresis it was spread in the paper both towards the anode and cathode. As the migration of Ho-citrate was different as that of the starting radiochemical species, paper chromatography could be used for the estimation of the complex yield.

Experiments were carried out with different amounts of citric acid (5–60 mg/mL) and $^{166}HoCl_3$ solution at pH 4–5 in order to see the Ho-citrate complex formation. It was observed that the paper electrophoresis and paper chromatography patterns are identical for all the concentrations of citric acid (5–60 mg/mL) studied.

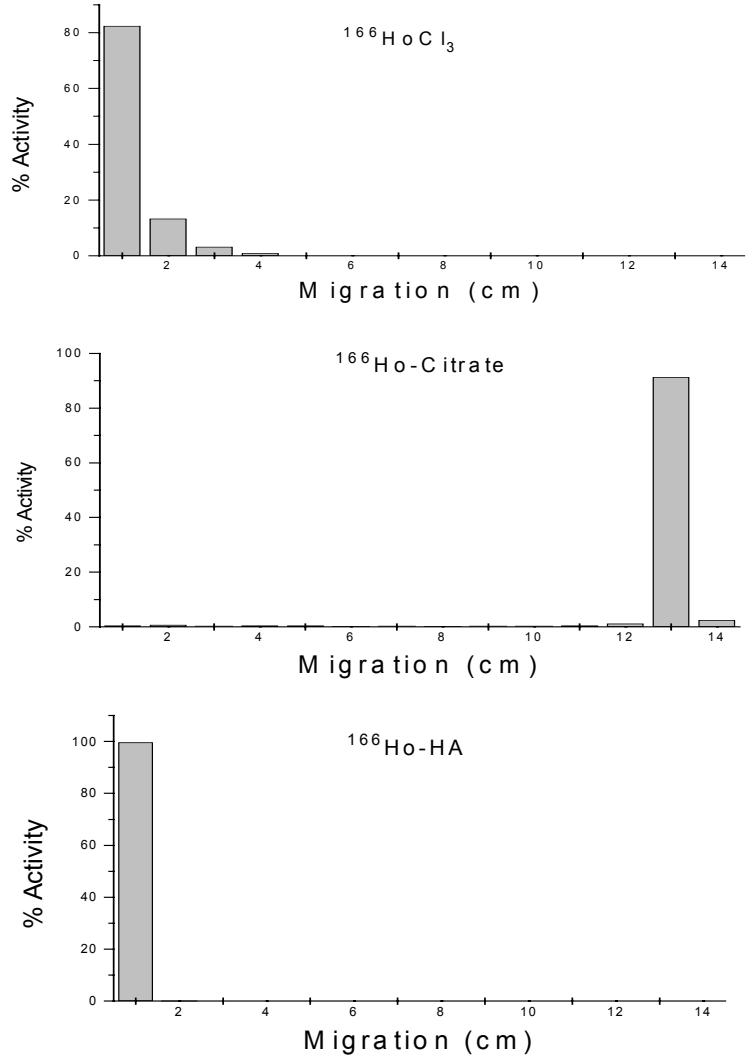

FIG. 1. Paper chromatography pattern of $^{166}HoCl_3$, ^{166}Ho-Citrate and ^{166}Ho-HA particles.

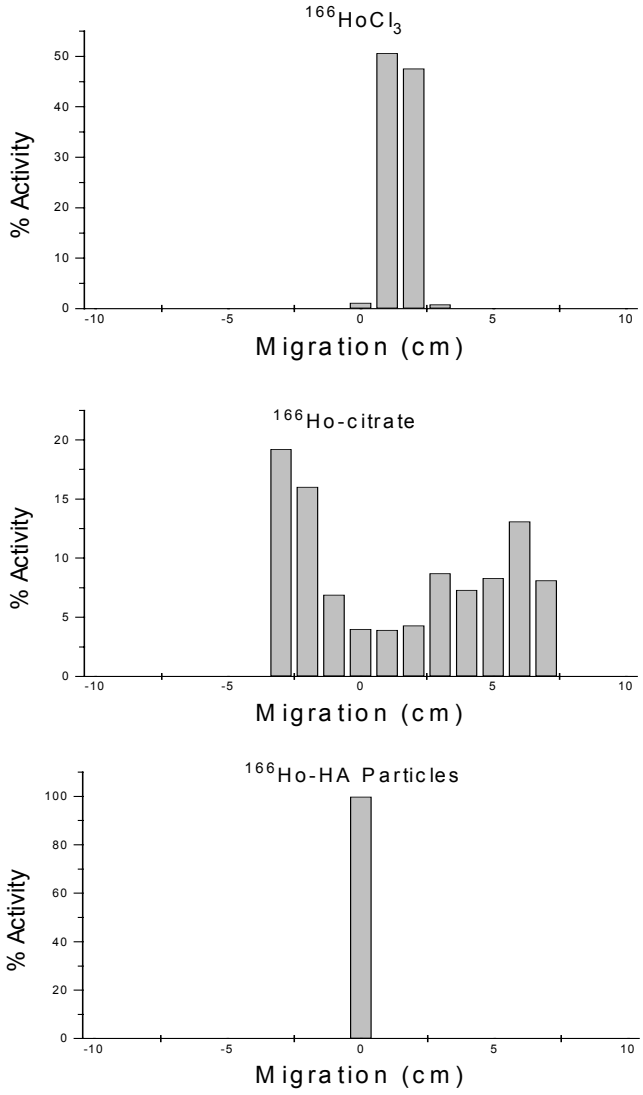

FIG. 2. Paper electrophoresis pattern of $^{166}HoCl_3$, ^{166}Ho-Citrate and ^{166}Ho-HA particles.

3.4. ^{166}Ho-HA particles

Labelling studies with different amount of HA particles were carried out. ^{166}Ho solution prepared with 15 mg of citric acid was used for these studies. The results are given in Table I. It was observed that the labelling yield increased with increasing amounts of HA particles. However, the labelling yield was only around 96% even at 100 mg of the particles. Hence, further optimisation studies were carried out by varying the amount of citrate used in these studies.

TABLE I. LABELLING YIELDS WITH DIFFERENT AMOUNTS OF HA. 15 mg/mL CITRIC ACID WAS USED AND THE REACTION WAS CARRIED OUT AT pH 4–5.

	Hydroxy apatite (mg/mL)				
	20	40	60	80	100
Batch 1	43.7	74.0	85.8	93.2	96.1
Batch 2	50.1	76.9	88.1	92.8	97.8

The effect of citrate concentration on the labelling yield is given in Table II. 40 mg of HA particles was used for these studies. It was observed that as the citrate concentration increases, the labelling yield decreases significantly. The labelling yield in the absence of ^{166}Ho-citrate is near 100%, indicating that it is not essential to have citrate for getting labelling with HA particles. In stead of acting as a transchelating agent, the citrate ions might be competing with HA particles for complexation with ^{166}Ho.

TABLE II. COMPLEXATION YIELDS WITH DIFFERENT CONCENTRATIONS OF CITRIC ACID. 40 mg OF HA PARTICLES WAS USED FOR THE STUDIES

	Citric acid Concentration (mg/mL)					
	0	5	10	15	30	60
Batch 1	99.9	99.1	91.7	99.4	68	39.1
Batch 2	99.8	98.9	90.6	96.9	69.6	43.2

The effect of pH on labelling yield was also studied. Two sets of experiments were carried out. 40 mg of HA and 15 mg of citrate was used for the first set of studies. In the second set of studies no citrate was used. It was observed that the labelling yields did not vary much with change in pH till pH 7. A marginal increase in complexation yield was seen at pH 10 when large quantities of carrier Ho was added. Results of a detailed study on the effect of ^{166}Ho concentration with and without citric acid and the effect of pH on the complexation yield are summarised in Table III and IV. Though the labelling yield was found to be uniformly good at all the pH studied, pH 7 was selected for further studies as it is nearer to the physiological pH.

From the results summarised in Table III and IV, it can be seen that labelling of HA with ^{166}Ho as $HoCl_3$ proceeds with optimum yield and there is no need for the use of citric acid. As Ho forms basic oxide with a stable (+3) oxidation state, it may not require a transchelation agent for getting labelled with HA. Labelling of HA with a radionuclide through a complex formed between a transchelating agent and radionuclide species may be required only when the radionuclide species cannot form a stable complex with HA. Hence, the intermediate step involving the preparation of

TABLE III. LABELLING YIELD AT DIFFERENT PH OF REACTION MIXTURE AND DIFFERENT AMOUNT OF ^{166}Ho. 15 mg OF CITRIC ACID AND 40 mg OF HA ARE USED IN THE STUDIES.

		Labelling Yield (%)			
Amount of ^{166}Ho (μg)	pH	2	4	7	10
	% Yield	99.6	99.7	99.8	99.5
0.86	% After wash	nd	nd	nd	nd
	% After 24 h	99.9	99.9	99.8	99.7
	% Yield	98.6	97.7	99.9	91.9
1.72	% After wash	99.7	99.7	99.8	99.7
	% After 24 h	99.9	99.9	100	99.8
	% Yield	80.2	69.7	71.7	89.4
8.6	% After wash	97.2	94.2	96.5	96.6
	% After 24 h	99.8	99.8	100	99.9
	% Yield	58.3	43.3	48.3	55.2
111	% After wash	85.1	87.9	88.9	97.7
	% After 24 h	98.6	98.8	99.2	99.7
	% Yield	47.1	47.6	49.1	59.6
221	% After wash	87.4	88.2	90.8	91.6
	% After 24 h	86.6	83.2	81.6	99.3

nd: not done

TABLE IV. LABELLING YIELD AT DIFFERENT pH OF REACTION MIXTURE AND DIFFERENT AMOUNT OF ^{166}Ho. 40 mg OF HA IS USED IN THE STUDIES. NO CITRIC ACID IS USED.

	Labelling Yield (%)				
Amount of ^{166}Ho (μg)	pH	2	4	7	10
0.86	% Yield	99.7	99.6	99.7	99.8
	% After wash	nd	nd	nd	nd
	% After 24 h	99.8	99.7	99.7	99.8
1.72	% Yield	99.4	99.8	99.8	99.8
	% After wash	90.0	99.7	99.8	99.8
	% After 24 h	99.9	99.9	99.9	99.8
8.6	% Yield	98.2	97.4	96.7	98.4
	% After wash	98.9	98.4	98.5	98.8
	% After 24 h	99.8	99.9	100	99.3
111	% Yield	79.3	86.9	91.3	98.0
	% After wash	95.8	97.5	98.9	99.5
	% After 24 h	99.3	99.6	99.5	100
221	% Yield	79.7	82.9	88.0	89.3
	% After wash	98.1	99.1	99.6	99.8
	% After 24 h	98.0	94.7	98.5	99.5

nd: not done

^{166}Ho-citrate complex could be eliminated here. The HA labelled with $HoCl_3$ also has shown excellent stability even after 96 h after its preparation. Since HA itself acts as a buffer around pH 7.0, $^{166}HoCl_3$ solution after adjusting its pH to 7.0 may directly be added to HA to prepare the labelled particles.

From the results of complexation studies with different amounts of ^{166}Ho, it was observed that the amount of ^{166}Ho used has a bearing on the complexation yield. At very low concentration of ^{166}Ho (0.86 μg, ~ 3 MBq) the labelling yield was found to be almost 100%, whereas at higher concentrations the labelling yield slightly reduced. However, the reduction in yield was less pronounced when citric acid was absent. The activity associated after first wash and 24 hour leaching studies showed that almost the entire activity is associated with the particles, irrespective of the amount of Ho used as well as the presence or absence of citric acid. Experiments were also conducted by varying the amount of Ho added by diluting the ^{166}Ho with carrier Ho (0.86, 1.72, 8.6, 111 and 221 μg). 111 μg of no carrier added Ho correspond to 10–15 mCi, a therapeutic dose. HA amount was kept constant at 40 mg. It can be seen from the results that ^{166}Ho labelled HA particles could be prepared at most of the Ho concentration studied.

In addition to the labelling yield, the stability of the labelled particles is an important consideration. In order to study the stability, leaching studies with saline solution and stability of the complex under storage was also studied. The results of these studies are also given in Table III. From the results it can be seen that the there is hardly any leaching of the activity from labelled HA particles at lower concentrations of Ho. Even at higher concentrations of Ho, the leaching is insignificant.

4. CONCLUSION

^{166}Ho labelled HA particles with near 100% activity tagged to the particles can be prepared for radiosynovectomy applications. Though other workers have used ^{166}Ho-citrate as transchelating agent, our studies suggest that use of citric acid is not essential for getting quantitative yield or for getting better stability. The ^{166}Ho-HA particles prepared are stable for 96 h, by which time no leaching of

activity from the particles was observed. The particles prepared can be used for biodistribution studies in experimental animal models.

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RADIATION SYNOVECTOMY WITH SAMARIUM-153 PARTICULATE HYDROXYAPATITE: A PRELIMINARY REPORT

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Abstract. The suitability of Samarium-153 particulate hydroxyapatite (Sm-153 PHYP) as a radiation synovectomy agent was evaluated in 14 patients (12 knee joints and 4 ankle joints) with chronic synovitis. Sm-153 PHYP was injected intra-articularly and flushed through with a mixture of xylocaine and triamcinolone acetonide. Whole-body images were acquired immediately and 72 hours after injection in order to demonstrate the distribution of radiopharmaceutical after intra-articular injection. Mean extra-articular activity accumulation was calculated. In six patients (37.5%) activity was noted in the lung immediately after injection (mean 0.2% of injected activity). In eight patients (50%) and seven patients (43.8%), 0.19% and 0.09% of the injected activity accumulated immediately in the liver and the regional lymph nodes, respectively. Good distribution of radioactivity in the joint space was seen. We believe that Sm-153 PHYP is useful for radiation synovectomy as an out-patient procedure because the procedure is very easy and is associated with low extra-articular leakage.

1. INTRODUCTION

Radiation synovectomy using various radiopharmaceuticals has been used to alleviate the pain and swelling of rheumatoid arthritis for more than 40 years [1]. After an injection of a beta-emitting radiopharmaceutical into the joint space, some of the injected radioactivity is absorbed by phagocytic lining cells along the synovial surface. As radionuclide decays, regenerating synovium will be irradiated.

Sm-153 PHYP that is recently used in this field, can be locally prepared by the Isotope Production Division, Office of Atomic Energy for Peace, Thailand [2]. Sm-153 decays by emission of gamma radiation (29.8%) with beta radiations of 810 keV (20%), 710 keV (50%) and 640 keV (30%). The penetration in soft tissue is 2.5 mm. It has a physical half-life of 1.95 days. With 103 keV gamma photon from the decay of Sm-153, extra-articular and intra-articular distribution of activity accumulation in patients can be assessed by gamma camera. We evaluated the biodistribution of Sm-153 PHYP from whole-body images in patients treated for chronic arthritis.

2. MATERIALS AND METHODS

2.1 Patients Selection

Fourteen patients with active and persistent arthritis who were refractory to intra-articular steroid injection were enrolled. There were 13 females and one male. Their age ranged from 33–81 years (mean 57 years). Pregnant or breast feeding females, patient younger than 18 years old and patients

with extensive cartilage and bone destruction (Stage 3, 4 of Steinbrocker's classification) were excluded.

2.2 Methods

Intra-articular injection of 555 MBq of Sm-153 PHYP was given by the rheumatologist as an outpatient therapy. To be sure that the needle was in the correct intra-articular position, synovial tapping from the large joint through a 21-gauge needle was tried first. Most of the effusion was removed as much as possible before the radiopharmaceutical was injected into the joint and flushed through with a mixture of 2% xylocaine and 10 mg of triamcinolone acetonide. The total minimum volume of injection was 5 mL for knee joint and 2 mL for ankle joint. To make the volume of radiopharmaceutical solution appropriate for the particular joint, xylocaine and triamcinolone acetonide were also helpful to minimize the transient local reaction and effusion after injection. The activity in the injection apparatus was measured both before and after injection. Immediately after injection, the joint was passively flexed to augment intra-articular distribution. The patients remained nonweight-bearing for 4 hours after injection. Then they were allowed to leave the department 4 hours postinjection and advised to rest but allowed to resume their normal activities the following day.

For extra-articular activity analysis, anterior and posterior whole-body imagings were acquired immediately and at 72 hours following injection using a single-headed gamma camera (Toshiba GCA-901A) with a low-energy, high resolution collimator with a 20% window centered at 103 keV for Sm-153. For intra-articular distribution analysis, anterior and lateral static images of the injected joint were performed following the whole-body imaging. SPECT images were acquired in five cases.

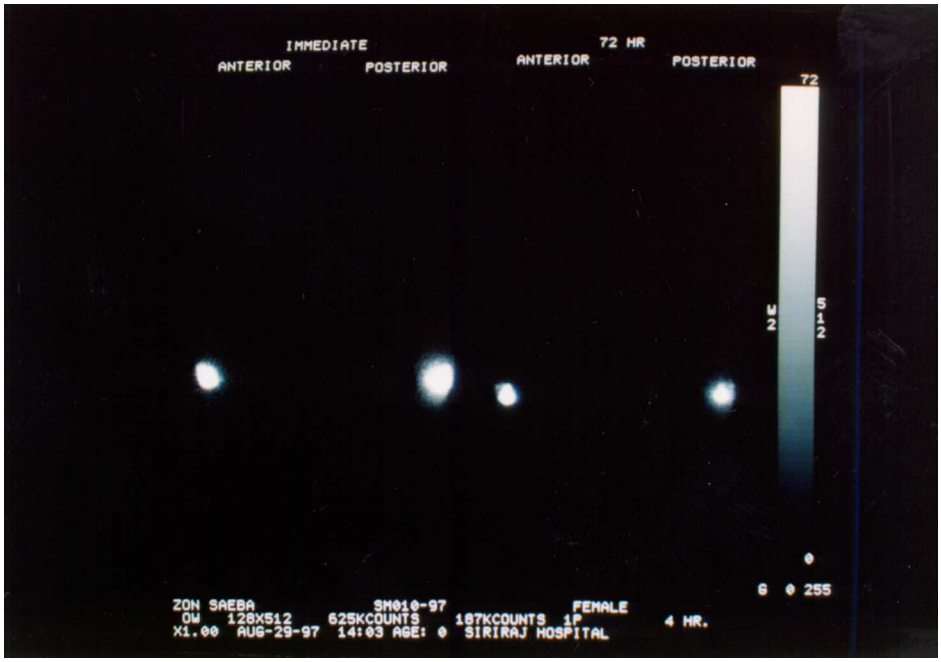

FIG. 1. Anterior and posterior whole-body imagings immediately (left) and at 72 hours (right) after Sm-153 PHYP synovectomy of the right knee showed no extra-articular activity.

3. RESULTS

Sixteen intra-articular injections were performed (12 knee joints and 4 ankle joints) Two patients received two injections. Mean injected activity was 595.3 MBq (range 258–736.3 MBq). Immediately after injection, no extra-articular activity was evident in four patients (25%) whereas 72–hour images showed no extra-articular accumulation in two patients (12.5%) (Fig. 1). Mean extra-articular activity accumulation was calculated from whole-body imaging data. Lung activity was

noted in six patients (37.5%) both immediately (mean 0.2% of injected activity) and at 72 hours after injection (mean 0.36% of injected activity) as shown in Table I. Accumulation of activity in regional lymph nodes occurred in 7 patients (43.8%) immediately after injection (mean 0.09% of injected activity) and appeared in nine patients (56.3%) at 72 hours after injection (mean 0.2% of injected activity). Whole body images immediate and 72 hours after injection showed mean activity of 0.2% and 0.8% of injected activity in the liver in 8 patients (50%) and 13 patients (81.3%), respectively.

More than 90% of the injected activity in the joint was seen even at 72 hours after intra-articular injection. Distribution of the activity in most patients was noted in the joint space with maximal activity in the suprapatellar bursa.

TABLE I. ACTIVITY LOCALIZATION IN DIFFERENT ORGANS POST RADIATION SYNOVECTOMY

	Mean activity in different organs (% intra-articular injection)		
Time following injection	Lung	Liver	Lymph nodes
Immediate	0.2 (n = 6)	0.19 (n = 8)	0.09 (n = 7)
72-hour	0.36 (n = 6)	0.8 (n = 13)	0.2 (n = 9)

4. DISCUSSION

A major problem associated with an intra-articular injection of radiocolloids is extensive leakage of radionuclides. It was suggested that leakage would be reduced by either a period of bed-rest or rigid splinting [3]. Radiation synovectomy with radioactive particles is believed to partly overcome this problem. In this study we found extra-articular activity in many patients but the amount of the leakage was very low. Thus it will be possible to perform synovectomy with Sm-153 PHYP as an outpatient therapy and it will be convenient for patients as well as physician.

From the distribution of leakage activity, we believed that Sm-153 PHYP passed into the blood because of the injury of synovial vessels during injection and the leakage activity may be due to the particle of PHYP. If the activity had been resulted from free Sm-153, it should have been demonstrated in the kidneys or bone [4]. There was no acute symptomatic complication from these small amounts of extra-articular leakage in our studied group.

Local preparation of Sm-153 PHYP that has been supported by the International Atomic Energy Agency leads to an appropriate utilization of national resources and low expense. We believe that Sm-153 PHYP may be useful for radiation synovectomy as an out-patient procedure because the procedure is very easy and associated with low-extra-articular leakage.

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7. MONOCLONAL ANTIBODIES

PREVENTIVE STUDY OF GASTRIC CANCER PERITONEAL MICROMETASTASIS IN NUDE MICE WITH ^{188}Re-LABELLED MONOCLONAL ANTIBODY 3H11

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Abstract. In advanced gastric cancer, especially when the serosa is invaded, the implantation of cancer cells in the peritoneum is common, and it affects patients' survival time severely. Based on successfully ^{labelled} monoclonal antibody 3H11 with ^{188}Re, we investigated the effect of RIT (radioimmunotherapy) with ^{188}Re-3H11 on preventing the establishment of gastric cancer cell peritoneal micrometastasis in nude mice. After 1×10^6 BGC - 823, gastric cancer cells were injected into the peritoneal cavity of each mouse, 45 BABL/C nude mice were divided into 9 groups. Each group received the various doses of ^{188}Re-3H11 or ^{188}Re-IgG or saline I.P.16 hours postoperation. The injected volume of each mouse was 1.0 mL. The results showed that the survial time depended on injected doses from 0 to 37MBq. The survival time was 170 $\pm$ 25.3 days after 37MBq ^{188}Re-3H11 were treated . It was over 5 times that of the saline group and about 3 times that of the 74MBq ^{188}Re-IgG group ($p<0.05$). The mice hemograms were reduced to lowest after injection 14 days, but they recovered after 28 days. Conclusion: Through properly injected doses, early postoperative ^{188}Re-3H11 I.P. is effective and safe in the prevention of intra-peritoneally injected gastric cancer cells from surviving, growing and disseminating in nude mice.

1. INTRODUCTION

In advanced gastric cancer, especially when the serosa is invaded, the implantation of cancer cells in peritoneal is common and it affects patients' survival time severely. So it is an important to prevent the plantation of cancer cells in peritoneal in order to increased 5 years survival ratios in patients with gastric cancer.

Dr Lu reported that 131I-3H11 I.P. postoperatively was effective and safe in the prevention of intra-peritoneally injected gastric cancer cells from surviving, growing and disseminating in nude mice and effectively reduce the incidence of liver metastases and the number of metastatic nodules in nude mice , with a prolongation of survival.

^{188}Re (β;Emax, 2.12MeV; γ;155keV, abundance of 15%) is a very attractive isotope for radioimmunotherapy, since it is obtained from a ^{188}W/^{188}Re generator in a carrier-free form on a daily basis. So it is a better therapeutic isotope than ^{131}I and ^{90}Y in aspect of RIT [1].

On the basis of successfully ^{labelled} monoclonal antibody 3H11 with ^{99m}Tc and ^{188}Re[2,3], we investigated the effect of RIT with ^{188}Re-3H11 on preventing the establishment of gastric cancer cells peritoneal micrometastasis in nude mice.

2. MATERIALS AND METHODS

2.1 Materials

^{188}W/^{188}Re Generator (Kexin Company, Shanghai China), SnCl2, A.R. (Sigma , American), UV (Sweden), 2-ME, A.R (American), ICON SPECT (Siemens, Germany)

2.2 Preparation of ^{188}Re-radio^{labelled} -3H11

Intact anti-gastric cancer monoclonal antibody 3H11 was reduced with 2-mercaptoethanol for 15min at room temperature and purified from excess thiol on a Sephadex G50 Column eluted with 0.05mol/L ABS (previously described). The reduced antibody 3H11 (1.0mg)was mixed with 1.0×10^{-3}g $SnCl_2$ and 15×10^{-3}g glucoheptonate and 7.0×10^{-3}g tartrate and 1.1×10^9Bq $Na^{188}ReO_4$ .The final

pH of mixed solution was about 50–5.5. It reacted 1.5–2.0h at room temperature. And then the product was purified by Sephadex G50 Column again [1,3].

3. ANIMAL TESTS

3.1. Biodistribution

The animal models of gastric cancer were established in nude mice by injecting 5×10^6 of 823 cells (0.1ml) subcutaneously into the axilla. When the tumour were approximately 0.3–0.4cm in diameter, the animals were given injection of the radio$^{\text{labelled}}$ Mabs 7.4 MBq by tail-vein. Five mice in each group were sacrificed by cervical dislocation at 24 h, 48 h and 72 h postinjection, respectively. Samples of tumour, blood and normal tissues were weighed, and then counted in an automatic γ well counter as well as a sample of the injection. The percentage of injected dose/g of radioactivity (i.d.%/g) in tissue were calculated. Tumor/normal tissue (T/NT) location ratios were determined from the cpm in tumourous and normal tissues.

3.2. RIT in nude mice

After 1×10^6 BGC -823 gastric cancer cells were injected into the peritoneal cavity of each mouse., 45 BABL/C nude mice were divided into 9 groups. Each group received the various doses of ^{188}Re-3H11 or ^{188}Re-IgG or saline I.P., 16 hours postoperation. The injected volume of each mouse was 1.0 mL. The injected doses of each group were saline, 7.4 and 37 MBq ^{188}Re-IgG, 7.4 MBq, 18.5 MBq, 37 MBq, 55.5 MBq and 74 MBq ^{188}Re-3H11 respectively. They were observed through hemogram, tumour formation and survival time.

4. RESULTS

The chemical purity of 3H11 was more than 95%,Kd was $5.68 \times 10^9 M^-$. The radiolabelling yield was more than 90%. The immunoreactivity of ^{188}Re-3H11 was over 70%.

TABLE I. BIODISTRIBUTION OF 188RE-3H11 IN NUDE MICE BEARING 823 GASTRIC CANCER XENOGRAFTS. DATA ARE MEAN ±S.D. OF FIVE ANIMALS AT EACH TIME POINT.

Time	24h		48h		72h	
Organ	id%/g	T/NT	id%/g	T/NT	id%/g	T/NT
Blood	6.29 ± 0.41	1.15 ± 0.03	3.28 ± 0.54	2.45 ± 0.55	2.06 ± 0.17	2.71 ± 0.53
Heart	1.37 ± 0.21	5.52 ± 0.98	0.95 ± 0.15	8.59 ± 0.47	0.70 ± 0.05	7.52 ± 0.28
Liver	2.20 ± 0.61	3.50 ± 0.81	1.39 ± 0.05	5.55 ± 0.95	0.81 ± 0.08	6.93 ± 0.62
Spleen	2.85 ± 0.78	2.67 ± 0.57	1.79 ± 0.50	4.40 ± 0.44	0.75 ± 0.09	7.06 ± 0.69
Kidney	4.03 ± 0.14	1.80 ± 0.11	3.55 ± 0.46	2.17 ± 0.20	2.18 ± 0.45	2.48 ± 0.41
Lung	1.95 ± 0.19	3.73 ± 0.15	1.55 ± 0.21	4.97 ± 0.32	0.95 ± 0.19	5.53 ± 0.95
Stomach	0.89 ± 0.06	8.21 ± 0.67	0.77 ± 0.13	10.4 ± 3.17	0.52 ± 0.06	10.2 ± 0.87
Intestine	0.65 ± 0.06	11.3 ± 1.06	0.58 ± 0.08	13.6 ± 3.90	0.42 ± 0.09	12.6 ± 1.27
Muscle	0.63 ± 0.10	11.8 ± 0.63	0.59 ± 0.07	16.8 ± 4.58	0.24 ± 0.05	21.8 ± 4.63
Bone	0.82 ± 0.07	8.91 ± 1.08	0.98 ± 0.21	8.22 ± 2.81	0.92 ± 0.17	5.71 ± 1.28
Tumour	7.60 ± 0.24		7.73 ± 1.36		5.25 ± 0.41	

The biodistribution results (Table 1) in nude mice demonstrated that ^{188}Re-3H11 was fast cleared from the blood and given rise to good T/NT ratios at 24 to 72h postinjection. The tumour uptake appeared to reach a peak at 24h postinjection and fell slowly thereafter. The id%/g of tumour was 7.60 ± 0.24 and 5.25 ± 0.41 at 24h and 72h postinjection respectively. The T/NT ratios were increased from 24h to 72h postinjection.

The mice hemogram were reduced to lowest 14 days after injection when the doses were 37 MBq and 55.5 MBq, respectively. But they recovered after 28 days. The mice hemograms were not significantly decreased when injected doses were less than 18.5MBq.

Two weeks after tumour implantation without therapeutically injected drugs, over a hundred cancerous nodules were found in serosa of each mouse in control group of three nude mice.

The relation between the survival time and therapeutic doses was shown in Figure 1. It showed that the survial time depended on injected doses during 0 to 37 MBq. The survival times of saline group, 7.4 MBq and 18.5 MBq, 37 MBq ^{188}Re-3H11group was 33.5 $\pm$ 3.3 days, 37.5 $\pm$ 4.2 days, 45.2 $\pm$ 6.8 days and 170 $\pm$ 25.3 days, respectively. But when injected doses was 55.5 MBq and 74 MBq, the survival time was significantly reduced. The animals died of internal hemorrhage 18 days after injection, and of intestinal liquefacient ulcer 5days after injection, respectively.

The survival time of injected 7.4 MBq ^{188}Re-3H11 group was more than 5 times than saline group and about 3 times than of 7.4M Bq ^{188}Re-IgG group ($p < 0.05$).

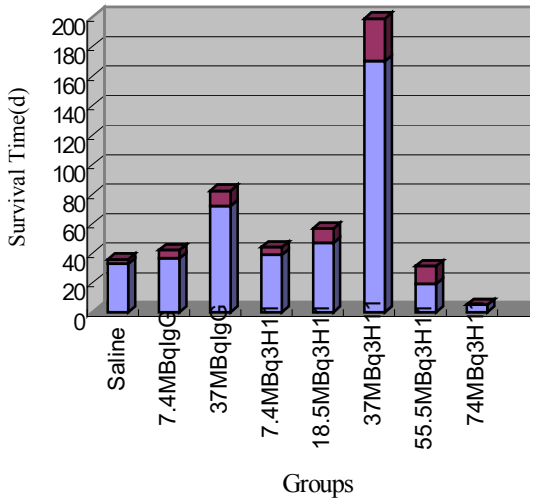

FIG. 1. The relation between therapeutic doses & survival time

5. DISCUSSION AND CONCLUSION

Currently, in most of clinical RIT in solid tumour, the percentage of injected pharmaceutical dose in tumour is less than 0.0001/g. The effective rate is less than 20%. There are two main reasons for the poor effect. In one hand, most of patients anticipated in the clinical test are in advanced stage. In this stage, the tumour's volume is large, its interstitial pressure is high and the absorbed dose of pharmaceutics to tumour is low, so the therapeutic effect is limited. In the other hand, the administration route of pharmaceutics in present is always by i.v.. The pharmaceutical dose to the tumour site is not enough to kill the tumour spheroid thoroughly.

So we think: (1) In the future, the real prospect of RIT is to kill the micrometastasis of tumour postoperation in order to prevent its recurrence; (2) The administration route should be changed to reduce the barriers to the tumour in order that the MoAb could be touched with tumour directly, and more MoAbs are absorbed by tumour and less are present in other organs and tissues. Based on the research work and considering the problem of RIT in the clinic, we think the real prospect of RIT for gastrointestinal conditions is to kill the micrometastasis of tumour postoperation.

The radioactivity was well concentrated in tumour from 24 h to 72 h after injection of ^{188}Re labelled 3H11. It is possible to make a study of RIT with ^{188}Re-3H11 on preventing the establishment of gastric cancer cell peritoneal micrometastasis in nude mice.

The survival time is related to injected doses. When the injection doses ranged from 0 to 37 MBq, the survival time was prolonged with raised injection doses. However, when the doses were over 55.5 MBq of ^{188}Re-3H11, the survival time was rapidly reduced. Thus it is very important to determine the proper dose range in order to effectively prevent micrometastasis form and prolong the survival time in nude mice.

Conclusion: Through properly injected doses, early postoperative ^{188}Re-3H11 I.P. is effective and safe in the prevention of intra-peritoneally injected gastric cancer cells from surviving, growing and disseminating in nude mice.

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LABELLING OF MoAb WITH $^{153}SmH_1ETA$: PRELIMINARY RESULTS

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Abstract. A method to label MoAb with Sm-153 using 1,5,9,13-tetraazacyclohexadecane N,N',N'',N''' tetraacetic acid (H_4ETA) as a bifunctional chelator was developed. H_4ETA and SmH_1ETA were synthesized in our laboratory and characterized by IR spectroscopy, TGA (thermogravimetric analysis), SEM (Scattering Electronic Microscopy), EDAX (Elemental Dispersion Analysis by X-rays) and EPR (Electron Paramagnetic Resonance) at 6 K. The $^{153}SmH_1ETA$MoAb was prepared by a simple incubation of the MoAb ior cea1, and the $^{153}SmH_1ETA$ complex at neutral pH and at room temperature for 24 h. The specific activity of the ^{labelled} antibody was 111 MBq/mg (3 mCi/mg). Sm-153(III) is commercially available with specific activities up to 318.2 GBq/mg. Therefore, under the conditions described above $^{153}SmH_1ETA$ ^{labelled} MoAb could be obtained with specific activity up to 1.14 GBq/mg (30.7 mCi/mg).

1. INTRODUCTION

The chelators used successfully to radio^{labelled} biologically important molecules such as antibodies and peptides with ^{99m}Tc are amine oximes (HMPAO), diaminothiols (DADT, BAT), hydrazinonicotinamide (SHNH, hynic), thiosemicarbazones as well as amidothiols like mercaptoacetyltriglicine (MAG_3)[1]. However when the biomolecules are required for therapeutic purposes, the traditional chelators are not the most appropriate. For example, despite the similarities in the chemical properties of rhenium and technetium, the labelling procedure employing Re has to be carried out by a multistep procedure and under acidic conditions which could affect the biomolecule integrity [2].

Whereas Samarium-153 has several favorable features as a radiotherapeutic agent. It possesses a 103 KeV gamma emission for scintigraphic imaging of its biological distribution allowing the *in vivo* absorbed dose calculation. The short physical half-life ($t_{1/2}$ = 1.8 days) of ^{153}Sm reduces the need for long patient isolation and facilitates the disposal of urine and other body fluid. ^{153}Sm gamma emission makes it to be a radionuclide of easy handling from the point of view of radiation protection. Due to its cross section (σ = 260), a reasonable high specific activity of samarium-153 can be produced even with fairly low flux nuclear reactors, which allow practical applications in nuclear medicine.

The aim of this work was to synthesize H_4ETA as a bifunctional chelator in order to examine the feasibility of labelling monoclonal antibodies (MoAb) with Samarium-153 under safety reaction conditions such as room temperature and neutral pH. For this purpose the synthesis and characterization of SmH_1ETA complex in macroscopic quantities were performed.

2. MATERIALS AND METHODS

2.1 Synthesis of H_4ETA.

H_4ETA was synthesized in our laboratory by reaction between chloroacetic acid and ano-N_4 ligand in aqueous solution at 0°C overnight followed by a precipitation at pH 2.0 and dried under vacuum (m.p. 242–244°C) [3]. The product was characterized by IR, RMN and thermogravimetric analyses.

2.2 Synthesis and characterization of SmH_1ETA.

The SmH_1ETA complex was prepared according to the reported in the literature [4]. A schematic representation of the route synthesis for the SmH_1ETA complex is as follows:

$SmCl_3 \bullet \times H_2O/H_2O + H_4ETA/NaOH/pH = 10 \rightarrow$ Solution/pH = 6–6.7 $\rightarrow$ Sol. Mixture

Sol. Mixture reaction/80–85^{0}C, 17 h $\rightarrow$ concentration/vacuum at 45^{0}C $\rightarrow$ SmH_1ETA

2.3 Preparation of $^{153}SmCl_3$ solution.

Samarium-153 chloride was obtained by neutron irradiation of 10 mg of enriched Sm_2O_3 (^{152}Sm, 99.4 %, from ISOTEC Inc.) in a Triga Mark III reactor at a flux in the central thimble of 3×10^{13} n$\cdot$ cm$^{-2\cdot}$ s^{-1} for 20 h. [5]. After irradiation 100 µL of 12 N chloride acid was added to the irradiation vial and stirred for 1 min followed by the addition of 900 µL of injectable water and also stirred for 2 min. The average radioactive concentration was 37 GBq/mL.

2.4 Preparation of $^{153}SmH_1ETA$ complex.

Sterile and apyrogenic V vials were prepared to contain 1.0 mg (2.17×10^{-3} mmoL) of HETA in 1.0 mL of 0.5 M bicarbonate buffer (pH 8.3) plus 20 µL of 2.5 N NaOH then 10 µL of $SmCl_3$ solution (4.9×10^{-4} mmoL Sm, 370 MBq) was added and the mixture, with a final pH 9.0, was incubated at 78°C for 3 h. Radiochemical purity was evaluated by TLC utilizing aluminum cellulose sheets (MercK) as the stationary phase with methanol: water: ammonium hydroxide (20 :40 :2) as the mobile phase. Sm^{+3} remained at the origin ($R_f = 0$) and $^{153}SmH_1ETA$ traveled with the solvent front with a R_f value of 0.9–1.0.

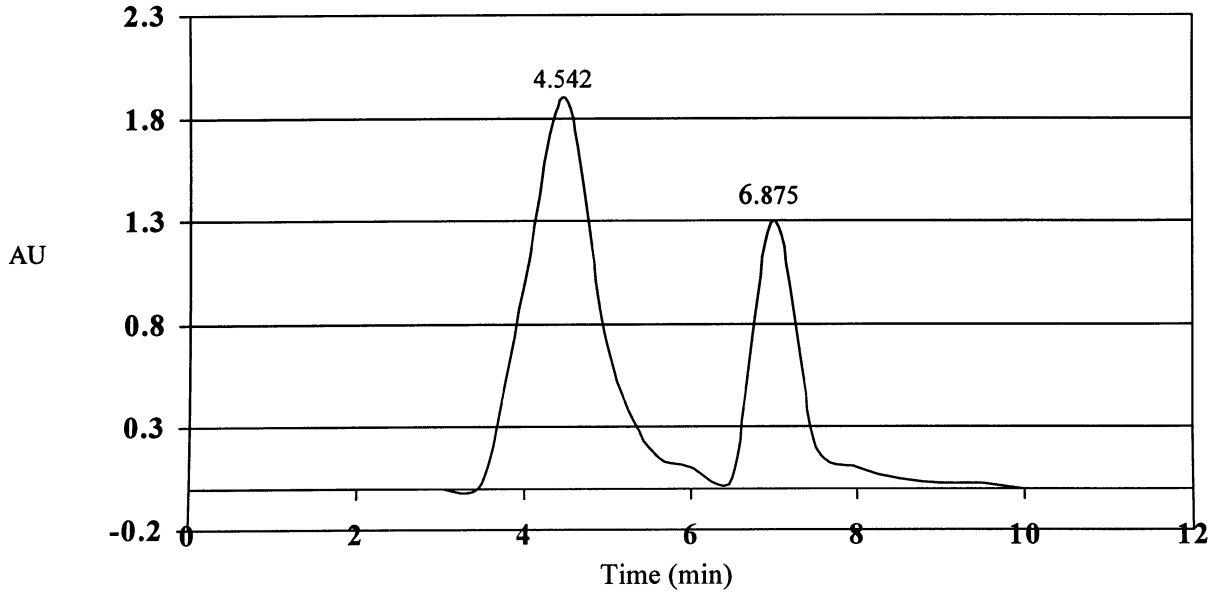


FIG. 1. HPLC separation (UV detector) of MoAb (Tr=4.5 min) and ^{153}Sm-HETA (Tr = 6.9 min).

2.5 Preparation of $^{153}SmH_1ETAMoAb$.

Murine monoclonal antibody (MoAb) IgG1 ior cea1 against carcinoembryonic antigen (CEA) was supplied by the Center of Molecular Investigations (CIMAB, Havana, Cuba) into vials containing 5.0 mL of a sterile and apyrogenic neutral phosphate buffer saline (PBS) solution with an antibody concentration of 1.0 mg/mL. To 1.0 mL of MoAb solution was added 1.0 mL of $^{153}SmH_1ETA$ solution and the mixture was incubated at room temperature (18–20°C) and neutral pH until 24 h.

2.6 Radiochemical quality control

Quality control of the ^{labelled} antibody was evaluated by size exclusion HPLC analysis employing a ProteinPak 125 SW gel filtration column (Waters), with photodiode array detector. 0.1 M phosphate pH 7.4 at a flow rate 1.5 mL/min was used as mobile phase. Under these conditions Sm^{+3} was retained into the column and for MoAb and $^{153}SmH_1ETA$ the retention time was 4.5 min and 6.9 min respectively (FIG. 1). The radiochromatographic profile was determined by collecting samples (Waters fraction collector) of uniform volume (0.5 mL) for counting in a external NaI (Tl) detector (NML, Laboratories, Inc.).

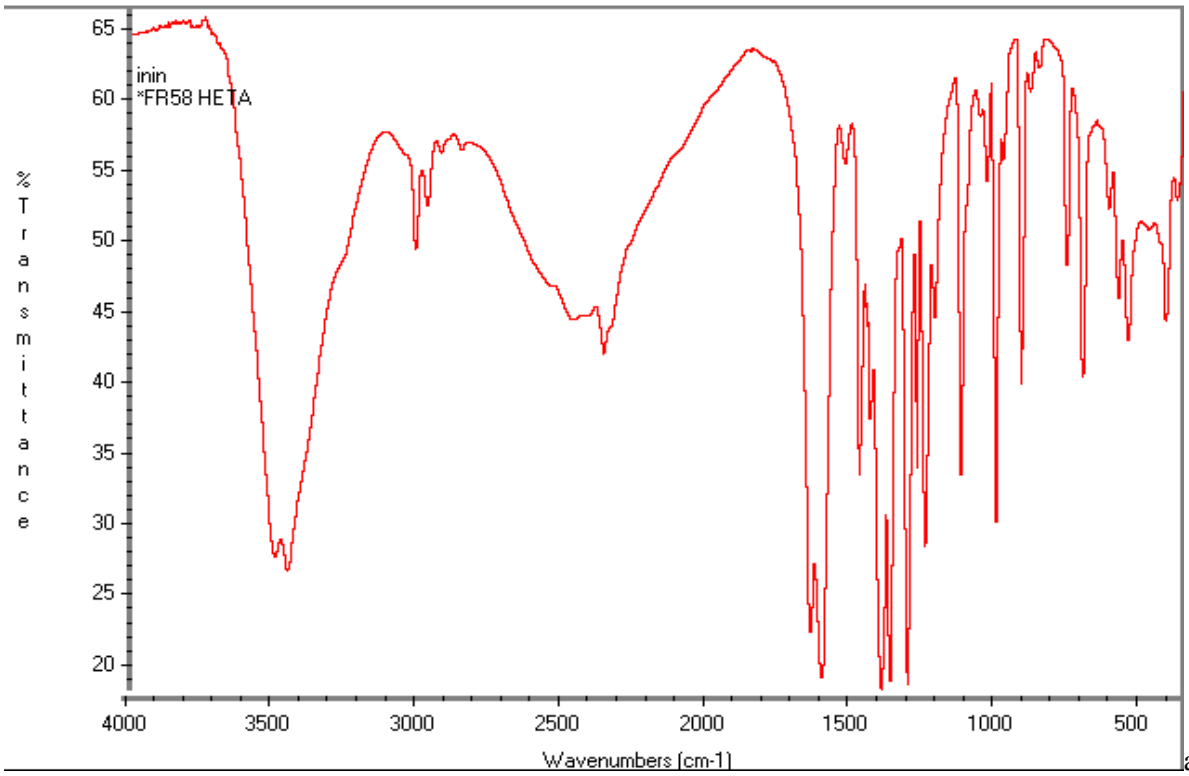
3. RESULTS AND DISCUSSION

The H_4ETA and the SmH_1ETA complex were obtained as reported elsewhere [3] and [4], respectively. Their preliminary characterization were carried out by IR spectroscopy, TGA (thermogravimetric analysis), SEM(Scattering Electronic Microscopy), EDAX (Elemental Dispersion Analysis by X-rays) and EPR(Electron Paramagnetic Resonance) at 6 K. Their IR spectra are shown in FIG. 2a,b ; as it is seen the formation of the complex modified the spectrum of the ligand. The main vibration frequencies assigned to O-H, CH_2-C=O, $O=CO^{-1}$ in the free ligand (3456, 2969 and 1647 cm^{-1} respectively) were shifted to lower energies and those corresponding to C-N-C (1126, 1036 cm^{-1}) and -CH_2-CH_2-(916, 704 cm^{-1}) to higher energies. As it was observed in other similar LnHETA complexes [4] the CH_2-N- band at 1477 cm^{-1} disappeared. This indicated the geometry change of the ligand after Sm(III) was coordinated. It is worthwhile to mention that a band between 2480–2290 cm^{-1} (corresponding to NH^+ group) did not disappear completely after coordination which suggested the presence of HOOC group, this is revealed by the formation of a zwitterion (NH^+-OOC) in the KBr matrix. No free ligand and nor Sm-Cl vibration frequencies corresponding to $SmCl_3$ were observed in the complex.

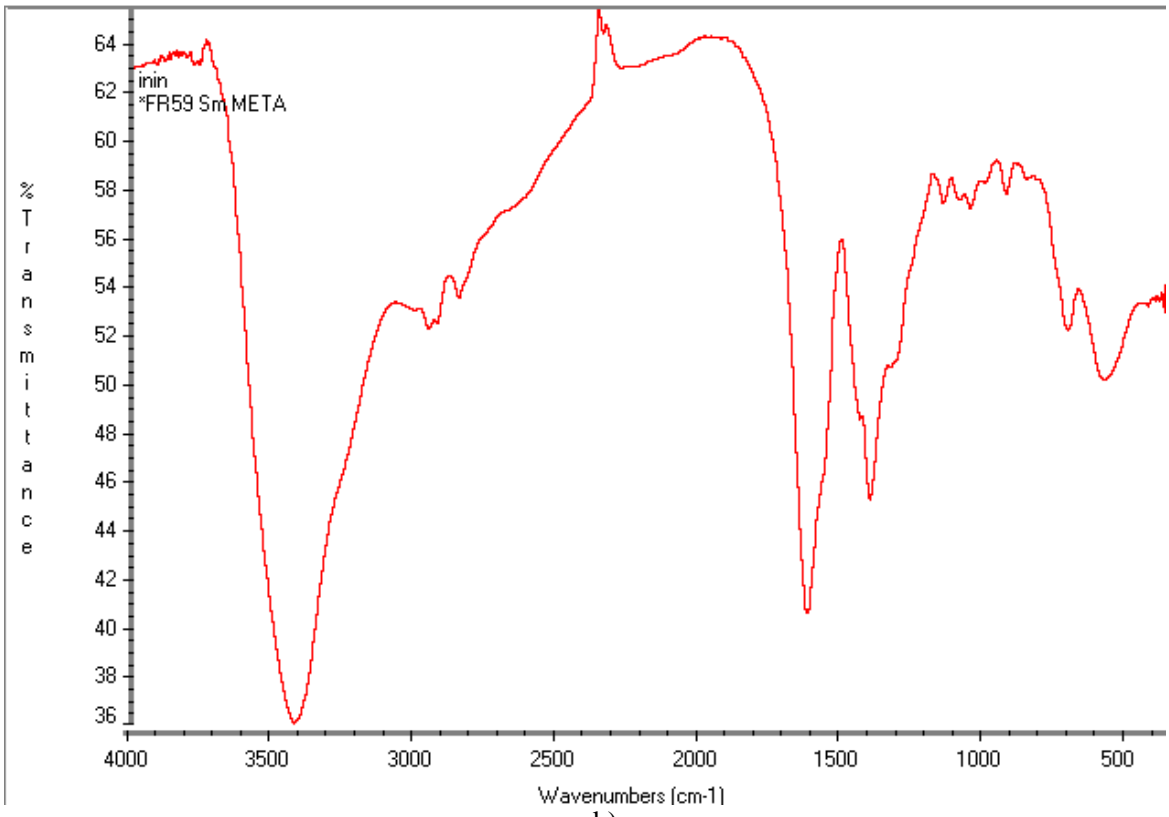
Semiquantitative microelemental analysis by EDAX, the minimum formula reported for similar compounds [4] and the TGA let us to propose that the complex was stabilized as $SmH_1ETA \bullet 3NaCl \bullet 3H_2O$. The feature of the EPR spectrum of this complex (FIG. 3) also suggested that samarium(III) was coordinated to the ligand. Besides, the SEM picture (FIG. 4) showed a homogeneous topology of the sample, which evidences an acceptable purity of one unique complex.

So far we can propose that the SmH_1ETA complex can be conjugated to the MoAb antibody through its no ionized HOOC- group, and thus allowed the labelling of the associated species as $^{153}SmH_1ETAMoAb$.

During the radiolabelling procedure, radiochromatographic profile showed that 10 min after incubation only 15.6 $\pm$ 3.2 % of the radioactivity was associated with the MoAb (FIG. 5A) and after 24 h it increased to 95 $\pm$ 2.1 % (FIG. 5B). Under these conditions approximately 0.628 mol and 3.5 mol of $^{153}SmH_1ETA$ were coupled to each mol of MoAb after 10 min and 24 h respectively. The formation of $^{153}SmH_1ETA$ ^{labelled} MoAb by a simple incubation of the antibody with the samarium complex, even when $^{153}SmH_1ETA$ was prepared as a stable complex, could be explained on the basis above described.



a)



b)

FIG. 2. IR spectra in KBr matrix of a) H_4ETA and b) $SmH_1ETA•3NaCl•3H_2O$.

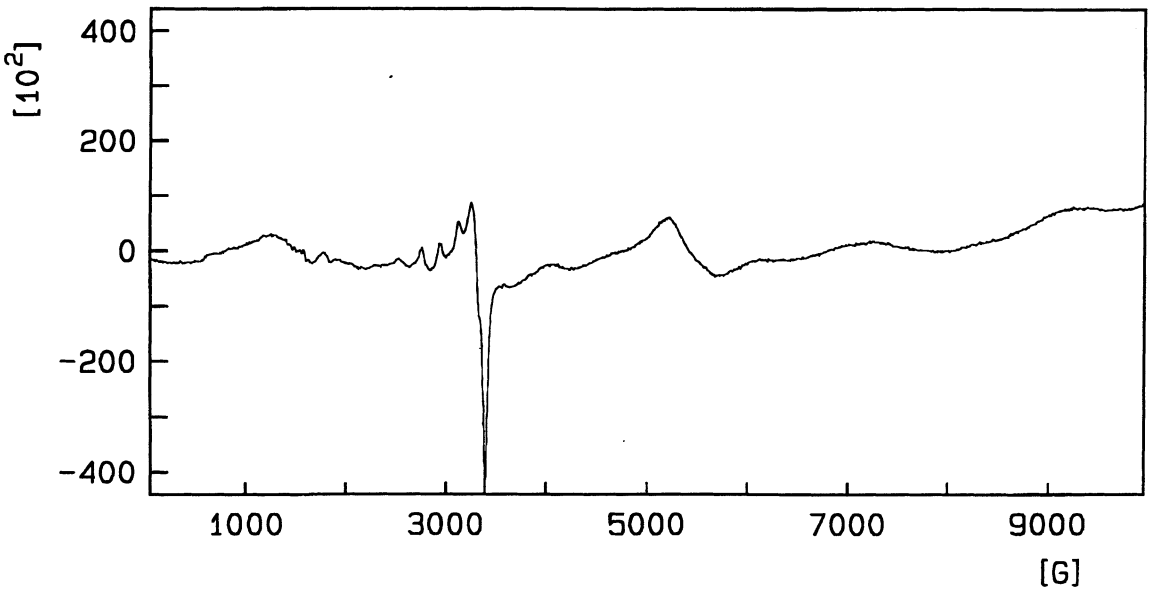


FIG. 3. EPR spectrum at 6 K of $SmH_1ETA•3NaCl•3H_2O$.

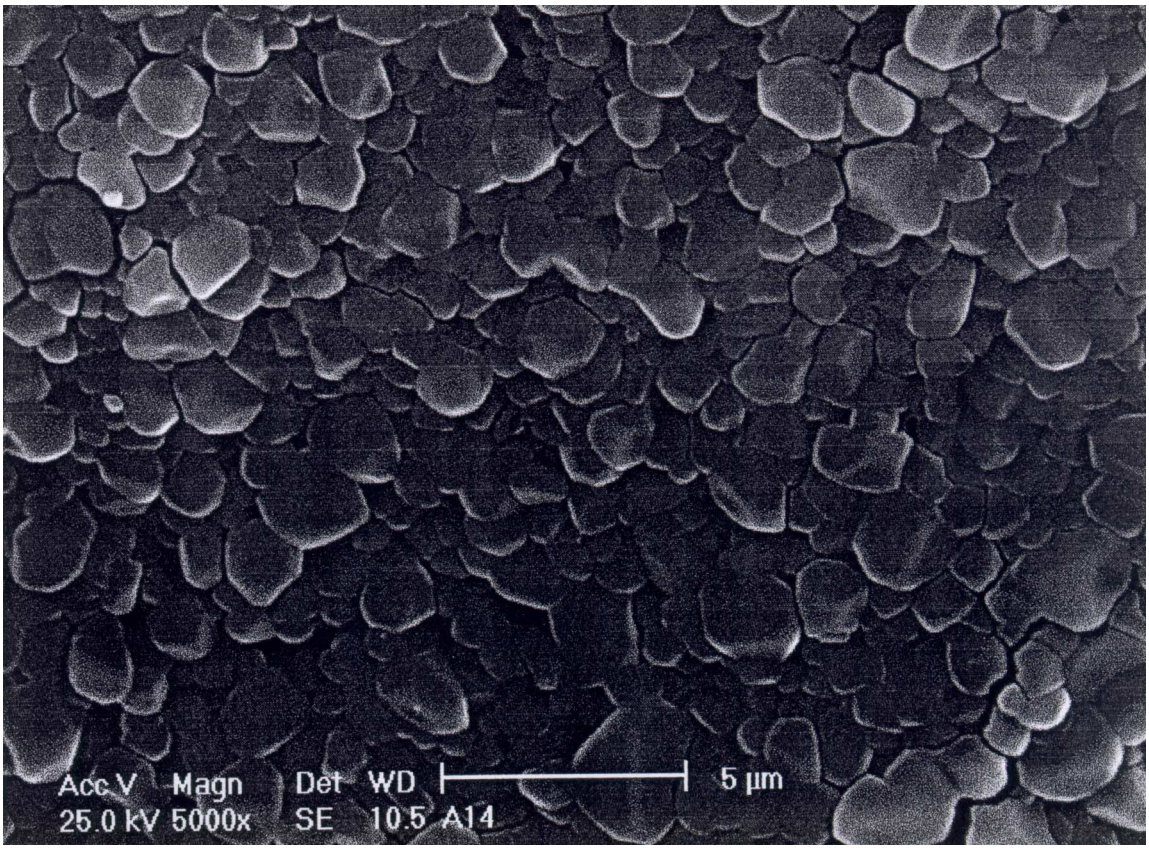


FIG. 4. SEM picture of $SmH_1ETA•3NaCl•3H_2O$.

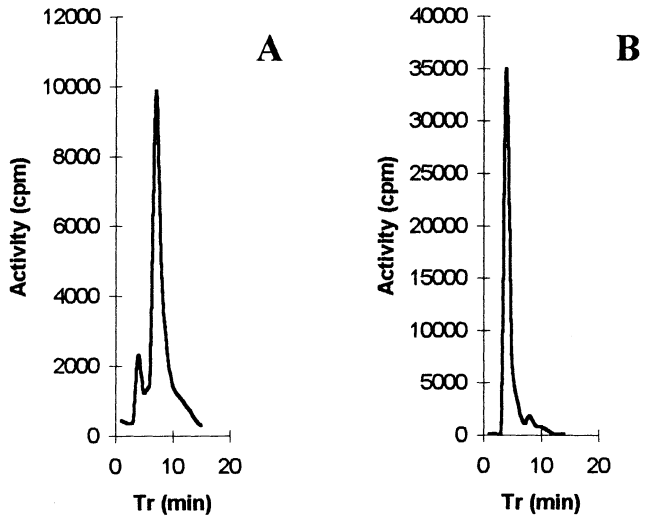


FIG. 5. Radiochromatographic profile obtained during the preparation of $^{153}SmH_1ETA$ ^{labelled} MoAb A) 10 min after incubation B) 24 h after incubation.

The specific activity of the ^{labelled} antibody was 111 MBq/mg (3 mCi/mg). Sm-153(III) is commercially available with specific activities up to 318.2 GBq/mg (Oak Ridge National Laboratory). Therefore, under the conditions described above ^{153}SmHETA ^{labelled} MoAb could be obtained with specific activity up to 1.14 GBq/mg (30.7 mCi/mg).

In order to establish the therapeutic possibilities for $^{153}SmH_1ETA$ ^{labelled} MoAb obtained in this study it will be necessary to perform studies in normal and tumour-bearing mice.

ACKNOWLEDGEMENTS

We thank Dr. Juan Padilla from chemical department of UAM-I Mexico for the EPR measurements.

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8. GENERAL

PIROCARBOTRAT™: A NEW RADIOPHARMACEUTICAL LABELLED WITH ^{32}P FOR THE TREATMENT OF SOLID TUMOURS. THERAPEUTIC ACTION AND RADIODOSIMETRIC CALCULATIONS

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Abstract. Pirocarbotrat™ is a gelatin protected charcoal suspension labelled with chromic [^{32}P] pyrophosphate. To evaluate its effectiveness as a therapeutic agent for the treatment of solid tumours, studies of therapeutic action and dose calculations, were carried out after an intratumoural single dose of this radiopharmaceutical. We used 28 female Sprague Dawley rats in which experimental mammary adenocarcinomas were induced. The tumours were injected with a single dose of 18.5 MBq. Once the experiment was finished, animals were sacrificed to extract their organs and the injected tumours, the activity of which were measured by the Bremsstrahlung photons of ^{32}P. Representative pieces of tissues from the treated and control tumours were selected for histolopathological examination. The results show that after 32 days of treatment, the per centage of activity found in the tumour was 84.50 ± 2.60%, while the per centage of activity found in the other evaluated organs was almost negligible. The therapeutic action was evaluated by the per centage of tumour regression (P.T.R.) which was 78.3%. The treated tumours showed closely packed black charcoal particles at the injection point, which are shown always in sharply demarcated big clusters, always associated with necrotic debris from the neoplastic tissue. The extension of the necrotic tissue in the tumour vicinity is variable, ranging from 1 to 4 mm. Radiodosimetric calculations, carried out according to the Medical Internal Radiation Dose Committee (MIRD) of the Society of Nuclear Medicine, demonstrate that the dose absorbed by the tumours was 6200 Gy. The dose absorbed by the rest of the organism is 0.533 Gy. The ratio dose to the tumour/dose to the rest of the organism is 1.17×10^4. We can conclude that Pirocarbotrat™, a non-sealed beta radiation source, behaves very closely to a sealed beta radiation source when it is intratumourally injected into solid tumours.

1. INTRODUCTION

The treatment of solid tumours is a big challenge for the physicians due to the variety of its histological patterns and its disorganized angiogenesis. Most of these kind of tumours do not respond to the conventional therapy [1, 2], therefore different alternative therapies are being studied.

Brachytherapy, that is the placement of radioactive sources into or near the tumour, is a valid alternative for the treatment of this kind of tumours [3]. In this way, a malignant lesion can be irradiated with a planed dose and with negligible or null irradiation to the rest of the organism if the source do not move from the injection point. Different radioisotopes such as ^{90}Y, ^{198}Au, ^{125}I and ^{32}P have been used for the treatment of several diseases [4–11].

Brachytherapy has demonstrated to be an effective method of treatment for this pathology [12]. Sealed sources of ^{125}I needles [13], metallic ^{198}Au seeds [10], ^{90}Y microspheres [14] among others, have been studied and some of them are still being used. This kind of radiation sources have the advantage of being immobilized at the point where they are placed. Because of construction

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characteristics of the ^{125}I needles and ^{198}Au seeds, only x and/or γ photons are used for *in situ* irradiation of the tumour with the disadvantage of irradiation of surrounding normal tissues [15]. Thus, sublethal doses must be administered in order to protect healthy tissue. For this reason, pure β^- sources for therapeutic purposes (betatherapy) have been developed.

It can be accepted that, ^{32}P has very good properties for this purpose, since it is a pure β^- emitter with a mean energy of 0.695 MeV, a half-life of 14.3 days and with a mean tissue penetration between 3–4 mm [16]. Several colloidal chromic [^{32}P] phosphate dispersions with different particles sizes were studied in order to decrease the migration of the colloidal particles [17–19]. However, in all cases, its radiopharmacological behaviour showed that a considerable migration from the place of injection after intracavitary administration takes place [19]. These studies demonstrated a direct relationship between the radiation dose absorbed by the tumour and the therapeutic effect of the radiopharmaceutical, as well as the convenience to use higher radiation doses or control the colloid migration in order to improve the treatment [18].

We studied several chromic [^{32}P] phosphate dispersions in rats for its use in brachytherapy, but results have been discouraging owing to the high mobilization of the radionuclide to the rest of the organism [20–22].

In the present work we studied Pirocarbotrat™, a gelatin-protected charcoal suspension labelled with chromic [^{32}P] pyrophosphate. The purpose of this study was to evaluate its therapeutic action, histopathology and radiodosimetric calculations in rats after a single intratumoural injection of the radiopharmaceutical.

2. MATERIALS AND METHODS

2.1. Radiopharmaceutical

2.1.1. Pirocarbotrat ™ (BACON Laboratories)

The preparation of the radiopharmaceutical was described elsewhere [23–24]. Its radiochemical purity was tested by ascending paper chromatography on Whatman nº 1 paper with 0.1 N HCl as solvent, according to Mitta and Robles [25].

2.2. Animals

We used 28 female Sprague Dawley rats in which experimental mammary adenocarcinomas were induced according to the method proposed by Gullino et al. [26] and modified by Rivera et al. [27]. The animals were placed in steel cages and were maintained with standard food and water *ad libitum* with cycles of 12 hours of light and darkness.

2.3. Administration of the radiopharmaceutical

The tumour size was measured with a caliper to localize the geometrical center of the tumour. The zone was thoroughly depilated. 18.5 MBq (0.5 mCi) were injected, using a fine needle to minimize tissue destruction. The injection was carried out very slowly and carefully in order to allow the collapse of the tissue and thereby avoid an eventual reflux of the dispersion.

2.4. Bioelimination studies

After the injection of the product, animals were placed in stainless steel metabolic cages that allow the separation of urine from feces. Rats were maintained with standard food and water *ad libitum*. Samples of urine and feces were collected in plastic flasks in order to standardize the measurement geometry.

2.5. Therapeutic action

The size of the injected and not-injected tumours was determined with a caliper along two axes as a function of time, and their mean diameters were compared. The not-injected tumours were used as controls in order to compare its evolution to that of the injected tumours in the same animal.

2.6. Biodistribution studies

Once the experiment was finished, animals were anaesthetized with diethyl-ether and sacrificed in order to extract their organs and the injected tumours. The organs and the tumours were disrupted and mineralized with sulfochromic mixture. The samples and the ^{32}P standard were taken to the same volume in plastic flasks of the same size to standardize the geometry of the radioactivity determinations.

2.7. Radioactive measurements

The activity concentration of the radiopharmaceutical was measured in an ionization chamber RADX model 255 Remote.

The radioactivity of the urine, feces, organs and tumour samples as well as a ^{32}P standard were measured in a monochannel gamma spectrometer with a 5 cm × 5 cm NaI(Tl) standard well crystal, using the Bremsstrahlung photons of ^{32}P, in optimal electronic conditions. A ^{32}P standard with an absolute activity of 18.5 MBq (500 μCi) was prepared and measured with the same geometry as that of the samples. Per centage of the retained activity in each organ as well as the per centage of elimination were referred to the ^{32}P standard.

2.8. Histolopathological studies

Representative pieces of tissues from the treated tumours as well as from the control tumours were selected for histopathological examination. They were fixed in buffered formalin and routinely processed for paraffin embedding. Sections were cut at 7 μm and stained with hematoxylin and eosin [28].

The histological findings were evaluated according the type and degree of local response to the radiopharmaceutical, concerning the neoplasia and the non-neoplastic surrounding tissue.

2.9. Dosimetric calculations

The dosimetric calculations were performed according to the Medical Internal Radiation Dose Committee (MIRD) of the Society of Nuclear Medicine [16]. To calculate the dose to the rest of the organism, a non-linear regression of the experimental data was performed by fitting each accumulated bioelimination curve to the following equation:

$$Y = Y_{max} (1 - e^{-k.t}) \quad (1)$$

where

Y is the per centage of total eliminated activity at time = t.
Y_{max} is the per centage of total eliminated activity at time = ∞.
k is the bioelimination constant.
t is the considered time.

2.10. Statistical studies

Results are given as mean ± SD. To test for differences, we evaluated the results by one-way analysis of variance (ANOVA) and Scheffé test, fixing a $p < 0.01$ as limit for the significance [29].

Tumour regression was compared by means of χ^2 test [30].

3. RESULTS AND DISCUSSION

3.1. Biological results

Bioelimination and biodistribution studies for Pirocarbotrat™ demonstrate that after 32 days of treatment, the total eliminated activity was 12.70 ± 3.90 %, distributed in urine (8.30 ± 1.80)% and in feces (4.40 ± 3.50)%. As it can be observed in Fig. 1, the per centage of activity found in the tumour was 84.50 ± 2.60%, while the per centage of activity found in organs which have reticuloendothelial system cells such as liver (0.40 ± 0.12)%, spleen (0.80 ± 0.15)% and lungs (0.08 ± 0.02)%, as well as in other organs, was almost negligible [23–24].

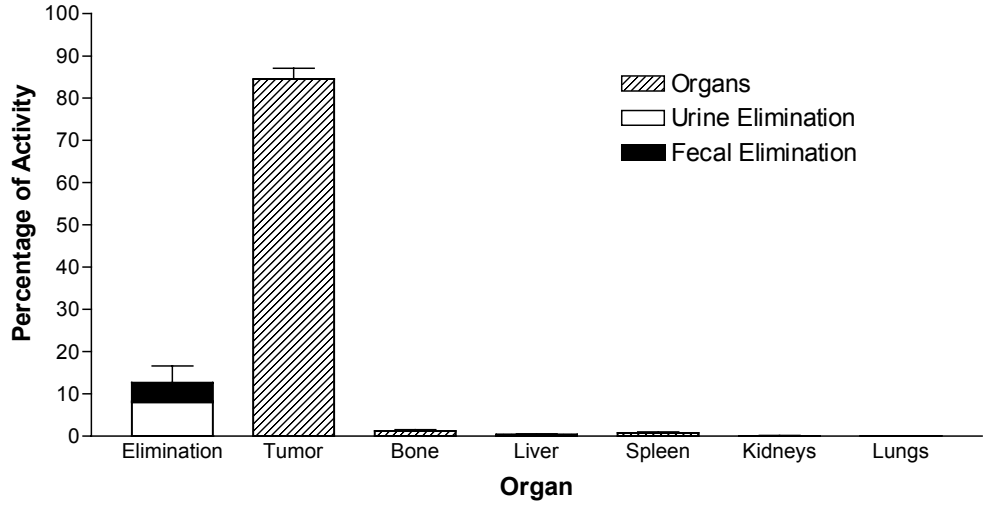

FIG. 1. Biological studies of Pirocarbotrat ™.

The therapeutic action was evaluated daily by the measurement of the tumour size with a caliper. The mean size ratio (M.S.R.), defined as the tumour size at the last day of life/tumour size at the day of the injection and the per centage of tumour regression (P.T.R.) are shown in Table 1.

TABLE I. MEAN SIZE RATIO (M.S.R.) AND PER CENTAGE OF TUMOUR REGRESION (P.T.R.) AT THE END OF THE EXPERIMENT OF THE NMU INDUCED TUMOURS IN FEMALE SPRAGUE DAWLEY RATS

TUMOURS TREATMENT	Nº of Tumours	M.S.R.[#]± S.D*	P.T.R.*
Controls (not injected)	81	4.9 ± 1.9	0.0
Pirocarbotrat™	28	0.6 ± 0.3	78.3

[#] *M.S.R.: tumour size at the last day of life (T = 32 days)/tumour size at the day of the injection (T = 0 days). At the beginning of the experiment, the M.S.R. was 1.0.*
**Differences are statistically significant.*

Histopathological studies were performed on either control and treated tumours. The histological findings for the control tumours showed that the 91.5% of the studied tumours were ductal carcinomas with an invasive pattern, while 8.5% were adenocarcinomas. In the case of the

treated tumours, radiopharmaceutical location was easy to demonstrate in histological sections because the black particles of charcoal remain closely packed at the point of injection, and the technical procedures for obtaining a microscopic section do not remove them. They are always in sharply demarcated big clusters and always associated with necrotic debris from the neoplastic tissue. Near the border of the cluster variable tumour necrosis is shown together with reparative changes arising from the surrounding normal tissues such as young fibroblasts, blood vessel growth, edema and lymphocytes. The extension of the necrotic tissue in the vicinity is variable, ranging from 1 to 4 mm. Reparative changes replace the necrotic areas progressively. In the reparative tissue there are changes related to radiation damage, such as large and stellate fibroblasts with atypical and bizarre nuclei and hyaline thickening of the walls of the blood vessels; the lumen is nearly or entirely occluded. The black charcoal particles which indicate the radiopharmaceutical location are seldom spilled out of the main cluster. In this case, the extent of the spreading was not more than 1–2 mm, and the changes related to radiation are striking in these sites.

3.2. Dosimetric calculations

Dosimetric calculations were performed according to the MIRD calculations, according to the following equation:

$$\overline{D} = \tilde{A}\, S \tag{2}$$

where $\overline{D}$ is the average (mean) absorbed dose in the target organ, $\tilde{A}$ is the cumulated activity in the source organ and S is a factor that relates $\overline{D}$ with $\tilde{A}$ for a particular radionuclide and a particular source-target organ pair.

Although tabulated S values are available for most of the organ pairs of interest in dosimetry, this is not the case for tumours or organs of abnormal size. In these cases it is often possible to use an expanded form of the absorbed dose formula.

$$\overline{D} = \tilde{A}\, \Sigma\, \Delta\, (\phi/m) \tag{3}$$

where Δ is the mean energy emitted per unit cumulated activity and Φ is the specific absorbed fraction, which depends on the geometrical relationship between the source and target organs and on the composition of the tissues or other material between each element of source and target volumes.

For this particular case, the pair source-target organs is tumour-tumour, that means that the dose is absorbed by the same tumour in which the radionuclide was administered. For β^- particles, $\phi = 1$ because the energy is totally absorbed by the target tissue. On the other hand, taking into account that in the case of ^{32}P, only one β^- particle is emitted per transition and its mean energy is E = 0.695 MeV.

$$\Delta_i = 2.13 \times 1 \times 0.695 \text{ cGy g/}\mu\text{Ci h} \tag{4}$$
$$\Delta_i = 1.480 \text{ cGy g/}\mu\text{Ci h}$$

The ^{32}P is 1.4799 cGy g/μCi h [16].

Taking into account that the tumour mass is 0.5 g, and that the administered activity is 0.5 mCi, we can write:

$$\overline{D} = \tilde{A}\, \Sigma\, \Delta\, (\phi/m) \tag{3}$$

$$\overline{D} = 500\ \mu\text{Ci} \times 1.480 \text{ cGy g/}\mu\text{Ci h} \times (1/0.5 \text{ g})$$

$$\overline{D} = 1480 \text{ cGy/h}$$

Taking into account that approximately 85% of the administered activity remains at the injection point:

$$\tilde{A}_{(0,\infty)} = A_0/\lambda \quad (5)$$

$$\tilde{A}_{(0,\infty)}/A_0 = 1/\lambda$$

$$\frac{\tilde{A}_{(0,\infty)}}{A_0} = \frac{14.3 \text{ d} \times 24 \text{ h/d}}{0.693} = 495 \text{ h}$$

$$\overline{D} = 1480 \text{ cGy/h} \times 495 \text{ h} \times 0.85$$

$$\overline{D} = 6227 \text{ Gy}$$

Due to the mobilization of 15.5% of the administered activity from the injection point, we calculated the dose delivered to the rest of the organism. For that purpose, we calculated the biological $T_{½}$ of the ^{32}P that is released from the Pirocarbotrat™, taking into account that the experimental data of the bioelimination studies were calculated against a ^{32}P standard to get independence of the physical $T_{½}$ of ^{32}P. The biological $T_{½}$ of the ^{32}P from the Pirocarbotrat™ was calculated by a non-linear regression of the experimental data, according to equation 1. The results are shown in Fig. 2.

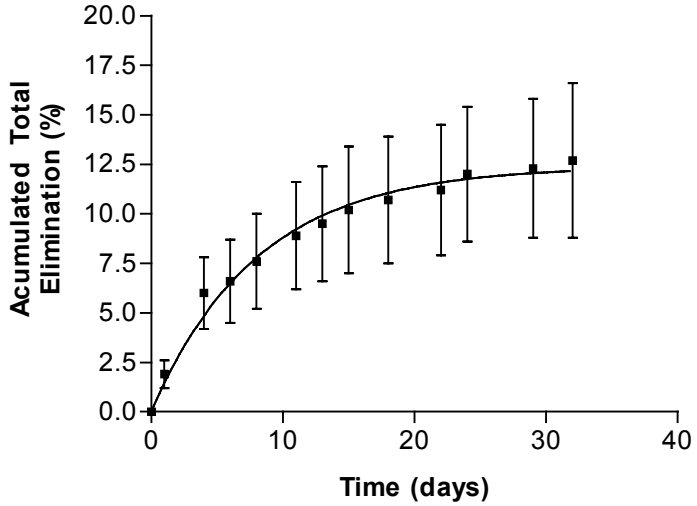

FIG. 2. Mean bioelimination kinetics of the ^{32}P of the Pirocarbotrat™.

Bioelimination curves for the ^{32}P of the Pirocarbotrat™ were fitted for each of the 28 animals treated with Pirocarbotrat™. The results demonstrate that the $T_{½b}$ was 5.555 ± 1.268 days, Y_{max} was 12.7 ± 3.9 % and k was 0.1248 ± 0.0280/day. The correlation coefficient between the experimental data (r^2) was 0.9811 ± 0.0128.

The dose absorbed by the rest of the organism can be estimated taking into account that the mean weight of the animals in this study was 300 g and that the biological half-life ($T_{½}$) for the ^{32}P was 5.6 days. The effective half-life is 4.02 days.

The initial dose rate delivered to the rest of the organism is 0.38 rad/h = 0.38 cGy/h.

Taking into account that at the end of the experiment (32 days after Pirocarbotrat™ administration) the mobilization of the product was 15.5%, the dose delivered to the rest of the organism was $5.33 \times 10–^5$ Mrad = 0.533 Gy.

Dosimetric calculations overestimate the dose that is delivered to the rest of the organism, because approximately 12.7% of the administered activity is eliminated with an exponential profile. However, the applied method assures that the tumour does not receive a lower dose and that the rest of the organism does not receive a higher dose than the calculated values. The ratio dose to the tumour/dose to the rest of the organism is 1.17×10^4.

Pirocarbotrat™, a gelatin-protected charcoal suspension labelled with chromic [^{32}P] pyrophosphate, remains at the injection point in the solid tumours. This behaviour allows the delivery of high doses to the tumour, with low irradiation to surrounding tissues and organs. Irradiation to the rest of the organism is negligible. The high concentration of the radiopharmaceutical in the tumour explains its efficiency. We can conclude that, even though Pirocarbotrat™ is a non-sealed beta radiation source, the radiopharmaceutical behaves very closely to a sealed beta radiation source for the treatment of solid tumours.

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THERAPEUTIC TRIALS TO CONTROL METASTATIC CANCER WITH ^{90}Y-DOTA-LANREOTIDE

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Abstract. As Somatostatin-analogue-scintigraphy using ^{111}In labelled ligands could demonstrate a high density of somatostatin-receptors in a variety of cancer types, we tried to use ^{90}Y-DOTA-Lanreotide (= "MAURITIUS") for therapy in patients with rapidly progressing metastatic disease in whom no other therapy had been effective after uptake of the molecule in metastases was assessed by scanning (including SPECT) with 184 MBq ^{111}In –DOTA-Lanreotide. Fifteen patients were treated so far (carcinoid tumours 8, thyroid cancer 5, oesophagus cancer 1, colon/prostate cancer 1) after dosimetry including estimates of radiation dose to tumour, whole body, marrow, urinary tract and liver. According to these data they received 740–15^{90} MBq ^{90}Y-Lanreotide at intervals of 2–6 weeks up to 6 times which gave 10–18 Gy to the tumour. Follow-up for up to 12 months showed complete/partial remissions in 2/15, stable disease in 6/15 and no effect in 6/15. A comparison of scan data between ^{111}In-Octreotide and ^{111}In-Lanreotide showed that binding of both tracers was different in 7/17 patients, showing either better Octreotide-uptake or better Lanreotide-uptake. Side effects were only transient thrombocytopenia (5/15) and moderate leukopenia (6/15). Obviously, even with low doses of ^{90}Y-Lanreotide some improvement in the management of patients with cancer types expressing somatostatin receptors can be achieved when rapid progression of metastases occurs. Modifications of the therapy protocol could perhaps improve our preliminary results.

1. INTRODUCTION

Research on binding of different somatostatin analogues has shown, that binding to different receptors can occur [1] and that Lanreotide binds to a variety of such receptors possibly also to VIP-receptors [2]. A high density of such receptors was observed in several cancer types [3] (e.g. carcinoid tumours, thyroid cancer, pancreas tumours, lymphoma, gastrinoma). With scanning using ^{111}In-labelled molecules the intense binding of these radiotracers to such tumours has been shown [4]. Consequently ^{90}Y-labelled somatostatin analogues were proposed for therapy in cases with inoperable metastases of such tumours [5]. We tried therefore to evaluate ^{90}Y-DOTA-Lanreotide in control of metastases of relevant cancer types and to compare binding data of ^{111}In-Octreotide with ^{111}In-Lanreotide.

2. MATERIAL AND METHODS

So far 15 patients have been treated (carcinoid tumours 8, thyroid cancer 5, oesophagus cancer 1, colon/prostate cancer 1). Admission criteria were proof of rapidly progressing inoperable metastases, intense uptake of ^{111}In-Lanreotide in lesions, in thyroid cancer no ^{131}I-uptake in metastases, no severe myelodepression, normal renal function, life expectancy >1 month, no local radiotherapy possible.

All patients had initially whole body scans 1 h, 24 h and 48 h after 148 MBq ^{111}In-Lanreotide ("Mauritius") provided by Forschungszentrum Seibersdorf, Austria using a double head y-camera

(Helix Elscint) with SPECT of body regions with possibly abnormal tracer uptake. SPECT data were analyzed on a Hermes workstation (NUD) providing data on tumour volume and regional tracer concentration. Serial blood and urine samples were obtained and measured for ^{111}In activity over 3 days to assess blood clearance, radiation dose to bone marrow and urinary tract. All data were incorporated in dosimetric calculations [6] with the aim to achieve a dose of approx. 3 Gy in one therapeutic application of ^{90}Y-Lanreotide. In 17 patients with similar tumours also whole body scans (and regional SPECT) were done before therapy with 148 MBq ^{111}In-Octreotide. These studies were performed and evaluated in the same manner as studies with ^{111}In-Lanreotide mentioned above. After these studies all patients gave informed consent to participate in the study which was approved by the local ethical committee. Patients then received 740–15^{90} MBq ^{90}Y-DOTA-Lanreotide with a radiochemical purity to > 90 % (tested by ITLC-SG in citrate buffer pH 5,2) in an infusion of saline and glucose over 2 h These infusions were repeated after 2–6 weeks up to 6 times, so that total applied activity was 1850–4400 MBq giving an estimated dose of 10–18 Gy to the tumour. Follow-up initially was performed every 2 weeks, then every month for up to 10 months with clinical exam, blood counts, renal and liver function parameters, urine analysis and imaging techniques (i.e. sonography, CT, MRT) to assess therapeutic effects and side effects of therapy.

3. RESULTS

Local uptake of ^{111}In-Octreotide and ^{111}In-Lanreotide was quite different in several cases (7/17): Sometimes a better uptake of ^{111}In-Lanreotide occurred, sometimes the opposite (Fig. 1). We could even observe in liver metastases of a carcinoid tumour, that several metastases could not be identified with ^{111}In-Lanreotide, while they concentrated ^{111}In-Octreotide. But one lesion without uptake of ^{111}In-Octreotide was "hot" with ^{111}In-Lanreotide (Fig. 2). These data are summarized in Table I. Even with the low radiation dose applied our preliminary results seem promising (Table II). Considering side effects thrombocytopenia occurred quite frequently (5/15) with values of < 50 k in 3 patients, while leukopenia occurred with a similar frequency (6/15) but was only moderate (WBC > 2000). No renal complications were observed so far.

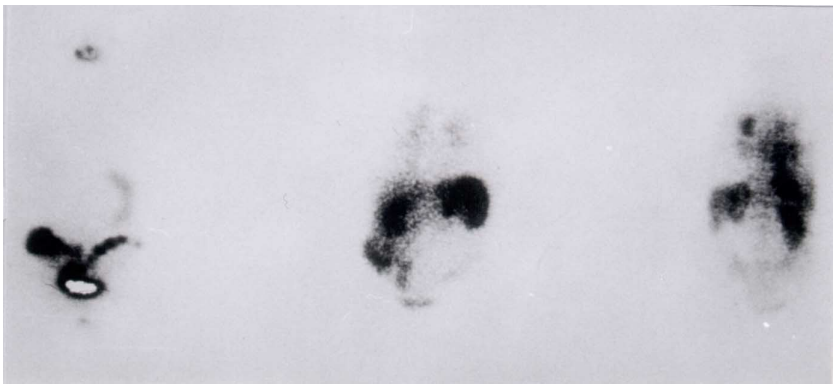

FIG. 1. Scan with ^{131}I (left), ^{111}In-Octreotide (middle) and ^{111}In-Lanreotide (right) in patient with multiple metastases of thyroid cancer. No uptake of ^{131}I, minimal uptake of ^{111}In-Oct., intense uptake of ^{111}In-Lan.

TABLE I. COMPARISON ^{111}IN-OCTREOTIDE AND ^{111}IN-"MAURITIUS"

Diagnosis	n	Same results	Octreotide better	Lanreotide better
Carcinoid	10	6	2	2
Colon, Oesophagus, Pancreas	3	2		1
Thyroid Carcinoma	4	2	1	1
Overall	17	10	3	4

TABLE II. RESULTS OF THERAPY WITH ^{90}Y-DOTA-LANREOTIDE

Tumour type	n	complete remission	partial remission	stable disease	progression
Carcinoid Tumours	8		1	5	2
Oesophagus Ca.	1				1
Colon + prostate Ca.	1				1
Thyroid Ca.	5	1	1	1	2
Overall	15	1	2	6	6

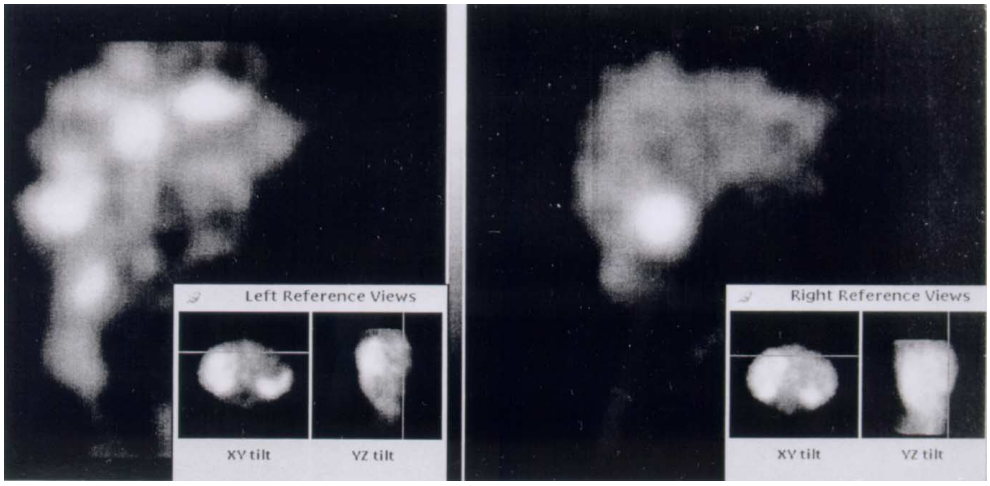

FIG. 2. ^{111}In-Octreotide scan (left) and ^{111}In-Lanreotide scan (right) of patient with multiple liver metastases of carcinoid tumour. With ^{111}In-Oct. several "hot" lesions, cold lesion on right lower liver margin. With ^{111}In-Lan. 1 hot lesion (cold with ^{111}In-Oct.!), other lesions cold.

4. DISCUSSION

There is obviously a discrepancy between estimated radiation doses to tumour tissue and clinical outcome. This could be due to inadequate dosimetric assumptions or to an inhomogenous uptake of the radiopharmaceutical in cancer foci [7]. Moreover the different uptake pattern of Octreotide and Lanreotide is striking: Metastases of the same tumour can either bind one ligand or the other or both with different intensity. Probably this is due to different receptor subtypes in tumours [8] and there is even the possibility that receptor type expression can change over time. While it is well known that somatostatin receptors are abundant in carcinoid tumours it seems important that by expression of such receptors in thyroid cancer without ^{131}I-uptake these neoplasms can possibly also be controlled by ^{90}Y-Lanreotide therapy. In the future it will be important to assess receptor expression in a variety of tumours (colon, lymphoma, thyroid, pancreas) perhaps with different ^{111}In-labelled somatostatin analogues and to decide then, which molecule labelled with ^{90}Y, should be used for therapy. Probably improvements in dosimetry and application of higher doses to tumour could further improve results when renal toxicity can be reduced by infusion of D-Lysine [9]. Radionuclide therapy with specific receptor ligands could so become an important progress in management of several forms of cancer in which other therapeutic modalities have failed.

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STUDIES IN RATS ON OCTREOTIDE LABELLED WITH Ga-67: A POTENTIAL RADIOPHARMACEUTICAL AGENT FOR THE TREATMENT OF SOMATOSTATIN RECEPTOR-POSITIVE TUMOURS

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Abstract. The paper presents the preparation, biodistribution and analysis of elimination mechanisms of ^{67}Ga-[DFO]-octreotide in rats. For labelling of the ligand with ^{67}Ga, desferrioxamine B (DFO) coupled to octreotide via the succinyl linker has been shown to form a stable chelating agent for binding of ^{67}Ga [1]. The radiopharmaceutical was prepared by direct chelating of $^{67}Ga^{3+}$ with [DFO]-octreotide in a slightly acidic reaction medium with high radiochemical purity. Pharmacokinetics of ^{67}Ga-[DFO]-octreotide of radioactivity in rats has shown relatively rapid elimination of the compound from the body with a long-term retention in the kidney and organs with high somatostatin receptor density. The agent was eliminated mostly by urine predominantly by the mechanism of glomerular filtration.

1. INTRODUCTION

Somatostatin, a natural tetradecapeptide, has been found to be an important endogenous antiproliferative agent. Somatostatin receptors are present in endocrine tumours, such as pituitary adenomas, islet cell tumours, carcinoids, paragangliomas, medullary thyroid carcinomas, small-cell lung cancers, and phaeochromocytomas [2]. In order to visualize somatostatin receptor-containing tumours, long acting somatostatin analogues labelled with a suitable radionuclide are required because the native somatostatin has a half-life of only 3 min due to its rapid enzymatic degradation. In recent years, various analogues of the native hormone have been developed as highly effective peptides acting in the management of a variety of endocrine tumours [3, 4]. Over the past few years, techniques have been developed to label these peptides with different radionuclides. Recently, octreotide (Sandostatin®), an octapeptide analogue of somatostatin, has been labelled with ^{111}In and the labelled compound has been shown to detect a variety of neuroendocrine tumours with high specificity and sensitivity [5]. ^{111}In-DTPA-octreotide is the only agent commercially available for somatostatin receptor scintigraphy, but some other somatostatin analogues have been reported to be very promising for detection of a variety of neuroendocrine tumours. The choice of the radioisotope is essentially based on its physical characteristics (half-life of disintegration, type of decay, energy of radiation), and also on its cost and availability. Labelling procedures have employed either direct labelling of the peptide with radionuclide or, more frequently, alternative labelling methods have been investigated (so called conjugation labelling). In these methods, strong chelating groups such as DTPA (dithylenetriaminepentaacetic acid), DOTA (tetraaza-cyclododecanetetraacetic acid) and DFO (desferrioxamine) are covalently attached to the peptide molecules so that they may be labelled with radiometal.

A new and fascinating application is the use of labelled octreotide analogues for radionuclide therapy since the labelled radionuclide could be changed to deliver beta radiation to ablate the malignant tumour. For the preparation of such receptor specific radiotherapeuticals, high purity and high specific activity of the radionuclide, *in vivo* stability of the labelled peptide and high receptor affinity and long residence time of labelled peptide in the target tissue are required.

Besides to beta-emitters, conversion and Auger electron emitters are radionuclides of choice as their *LET* (linear energy transfer) and consequently cell killing probability is larger than that for beta particles. One of the attractive radionuclides for radiotherapy which is chelated to octreotide by DFO

is ^{67}Ga. ^{67}Ga-[DFO]-octreotide was primarily proposed as the radiopharmaceutical having the potential to localize somatostatin receptor-positive tumours using gamma scintigraphy. The physical half-life of ^{67}Ga is 77.9 h and its decay mode is by an internal conversion. However, ^{67}Ga emits conversion and Auger electrons that deposit the radiation dose over a short range in tissues (maximal range of 90–110 μm) [4,6]. Emission of gamma radiation allows one to follow the fate of ^{67}Ga-[DFO]-octreotide *in vivo* and to estimate dosimetry in individual patients. This calculation can be made after administration of the agent with activity suitable for scintigraphic examination prior the application of relatively high therapeutic activity of ^{67}Ga-[DFO]-octreotide.

The present work evaluates biodistribution and analyzes the elimination mechanisms of ^{67}Ga-[DFO]-octreotide — a potential radiopharmaceutical for imaging and therapy of somatostatin receptor-positive tumours — in rats.

2. MATERIALS AND METHODS

2.1. Preparation and quality control of ^{67}Ga-[DFO]-octreotide.

The pharmaceutical was prepared by addition of 4 μl 1mM [DFO]-octreotide in 0.1% acetic acid into 100 μl of 0.1M ammonium acetate pH 5.6 together with 10 μl $^{67}Ga^{3+}$ (about 2 GBq/ml in 0.04 M HCl). Molar concentration ratio [DFO]-octreotide to no carrier added Ga^{3+} was approximately 30. After 60 min incubation 2 μl of the reaction mixture was diluted 100 times to 20mM ammonium acetate pH 4.5 and analyzed by HPLC on C-18 Supelcosil, a Tessek column 3 × 15 cm with gradient elution. As beginning solvent A 0.02 M ammonium acetate pH 4.5 served and solvent B was acetonitrile. The gradient was programmed as follows: 0–10 min 0% B, 10–15 min 0–45% B, 15–30 min 45% B.

An example of HPLC profile of ^{67}Ga-[DFO]-octreotide is presented in Figure 1. Radiochemical purity determined by HPLC analysis was over 99%.

For biological experiments the radiopharmaceutical was diluted 10 times with saline.

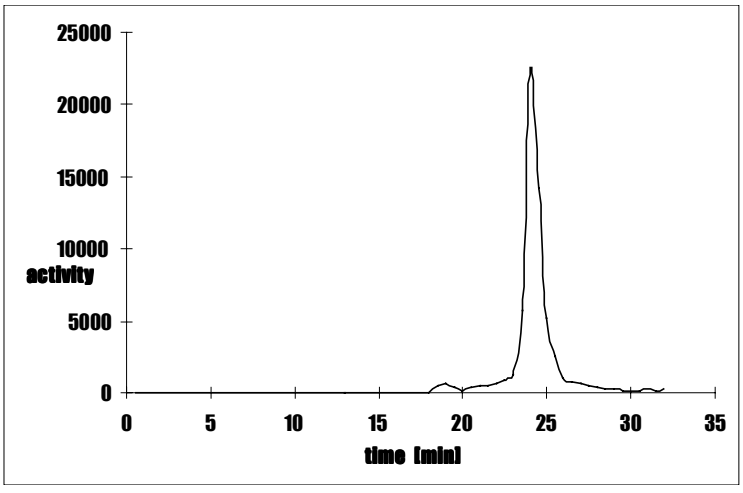

FIG.1. An example of HPLC analysis of ^{67}Ga-[DFO]-octreotide.

2.2. Biological experiments

Male Wistar rats weighing 190–250 g were used. The animals were fasted for 18–24 h before the experiment. ^{67}Ga-[DFO]-octreotide was administered to animals intravenously into the tail vein in

a volume of 0.2 ml. During the course of the experiment the rats were housed singly in cages. At time intervals 5 min, 1 h, and 48 h after dosing the carotid artery was exposed under ether anaesthesia and a blood sample was collected in a glass tube containing dry heparin. After exsanguination, selected organs and tissues were taken out to determine the distribution of the agent.

For elimination studies, ^{67}Ga-[DFO]-octreotide was administered to rats as described previously and the animals were placed singly in glass metabolic cages, the construction of which allowed reliable separation of urine from faeces. The rats had free access to a standard pellet diet and water. Two hours after administration of the tracer the rats were forced to empty their urinary bladders by handling (immobilization) and urine and faeces were collected. The animals were placed again into the same cages, and urine and faeces were again collected 24 h and 48 h after dosing.

Rat kidney perfusion studies were carried out by the method described previously [7].

All animal experiments were approved by the Ethical Committee of the Faculty of Pharmacy, Charles University.

2.3. Plasma protein binding of ^{67}Ga-[DFO]-octreotide

Binding of the compound under study to rat plasma proteins or to proteins of perfusion medium was determined by equilibrium dialysis at 37°C [8].

3. RESULTS AND DISCUSSION

Distribution of radioactivity after ^{67}Ga-[DFO]-octreotide administration to rats is presented in Table I. Whereas the radioactivity in blood and most organs and tissues decreased rapidly with time, the radioactivity in the kidneys and adrenals decreased very slowly and in later time intervals remained relatively unchanged. At the same time, the concentrations of radioactivity in the kidneys and adrenals at longer time intervals were mutually similar (about 10 per cent dose per 1% body weight). The kidney accumulation was probably due to a partial reabsorption of the labelled peptide by the cells of proximal tubules by means of pinocytosis [9]. In consequence of this process the peptide was transferred into lysosomes and digested by proteolytic enzymes. Resulting breakdown products (i.e. radiolabelled amino acids) remained in lysosomes of renal cells for a long postinjection period. The adrenals are an example of somatostatin receptor-rich tissue and high radioactivity concentration in this organ was evidently connected with the interaction of the agent with somatostatin receptors. The radioactivity determined in the bowels was mostly due to a partial elimination of the agent and/or its metabolites by bile.

^{67}Ga-[DFO]-octreotide was eliminated mostly by urine and the urinary excretion was relatively rapid (Table II). About 8% of the administered dose was excreted also by faeces.

An analysis of renal elimination mechanisms by employing the perfused rat kidney *in situ* showed that ^{67}Ga-[DFO]-octreotide was eliminated predominantly by the mechanism of glomerular filtration (Table III). As renal clearance of free (non-protein bound) ^{67}Ga-[DFO]-octreotide was slightly higher than glomerular filtration rate, a partial secretion of the agent by the renal tubules was also likely.

The binding of ^{67}Ga-[DFO]-octreotide to rat blood cells was negligible and about one third of the agent was bound to rat plasma proteins.

The results presented in this paper could contribute to the picture of pharmacokinetics of ^{67}Ga-[DFO]-octreotide in rats and explain some points of its biological behaviour.

TABLE I. DISTRIBUTION OF ^{67}GA-DFO-OCTREOTIDE IN RATS

	5 min	60 min	48 h
Per cent dose in whole organ			
Liver	4.26 ± 0.47	1.83 ± 0.28	0.95 ± 0.13
Adrenals	0.20 ± 0.04	0.29 ± 0.05	0.23 ± 0.05
Kidney	11.86 ± 3.85	8.88 ± 1.52	6.17 ± 0.38
Lung	1.34 ± 0.31	0.37 ± 0.12	0.02 ± 0.01
Heart	0.34 ± 0.04	0.09 ± 0.01	<0.01
Spleen	0.21 ± 0.04	0.06 ± 0.01	0.06 ± 0.01
Stomach	1.38 ± 0.89	1.20 ± 0.48	0.26 ± 0.07
Small int.	3.25 ± 1.12	2.25 ± 0.61	0.50 ± 0.02
Colon	1.16 ± 0.15	0.57 ± 0.21	1.42 ± 0.45
Testes	0.20 ± 0.05	0.13 ± 0.05	0.03 ± 0.01
Thyroid	0.07 ± 0.01	0.03 ± 0.02	<0.01
Brain	0.08 ± 0.02	0.02 ± 0.01	<0.01
Per cent dose per 1% body weight			
Blood	2.44 ± 0.22	0.55 ± 0.05	0.01 ± 0.01
Plasma	4.58 ± 0.31	1.08 ± 0.14	0.03 ± 0.01
Skin	1.11 ± 0.01	0.43 ± 0.11	0.03 ± 0.03
Muscle	0.47 ± 0.06	0.11 ± 0.03	0.01 ± 0.01
Fat	0.76 ± 0.03	0.34 ± 0.25	0.18 ± 0.11
Bone	±0.04	±0.03	±0.04
Adrenals	7.05 ± 0.82	11.19 ± 2.79	8.21 ± 0.85
Kidney	19.10 ± 1.68	13.28 ± 2.43	10.93 ± 1.32

TABLE II. CUMULATIVE EXCRETION OF RADIOACTIVITY AFTER ^{67}GA-DFO-OCTREOTIDE IN RATS

Time	Urine	Faeces
2 h	71.8 ± 11.2	-
24 h	81.0 ± 11.3	5.4 ± 1.2
48 h	83.5 ± 12.5	8.1 ± 1.1

TABLE III. HANDLING OF ^{67}GA-DFO-OCTREOTIDE IN PERFUSED RAT KIDNEY

Effective renal plasma flow (ml/min/g)	1.88 ± 0.36
Glomerular filtration rate (ml/min/g)	0.59 ± 0.15
Renal clearance of ^{67}Ga-octreotide (ml/min/g)	0.63 ± 0.12
Free fraction of ^{67}Ga-octreotide in perfusate	0.87 ± 0.08

ACKNOWLEDGEMENT

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THE DEVELOPMENT OF *META*-IODOBENZYLGUANIDINE ANALOGUES FOR THE THERAPY OF NEUROENDOCRINE AND OTHER TUMOURS*

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Abstract. Radioiodinated *meta*-iodobenzylguanidine (MIBG) has been extensively used in the diagnosis and therapy of neuroendocrine tumours such as neuroblastoma. We have developed a no-carrier-added synthesis (n.c.a.) for MIBG as well as other analogues which may improve clinical utility. In SK-N-SH human neuroblastoma cells *in vitro*, the uptake of n.c.a. [^{131}I]MIBG remained constant over a 2–3-log activity concentration range. In contrast, the uptake of [^{131}I]MIBG prepared by an exchange radioiodination (ex-[^{131}I]MIBG) steadily decreased over the same range demonstrating the saturability of uptake under these conditions. Similar differences in uptake were seen in normal mouse heart and adrenals, the normal target tissues for MIBG. While no advantage of n.c.a [^{131}I]MIBG over ex-[^{131}I]MIBG was seen in athymic mice hosting SK-N-SH neuroblastoma xenografts, higher tumour uptake and tumour-to-normal tissue ratios were observed when SK-N-BE(2C) xenografts were used. Since neuroblastoma is often associated with micrometastases, an MIBG analogue labelled with the -particle emitting ^{211}At could be advantageous. A method has been developed for the efficient synthesis of *meta*-[^{211}At]astatobenzylguanidine (MABG). A number of *in vitro* assays and tissue distribution studies showed that MABG is an excellent analogue of MIBG. From clonogenic assays using SK-N-SH neuroblastoma cells, it was calculated that the D_0 value for MABG (215 Bq/ml) was more than 1000-fold lower than that of n.c.a. [^{131}I]MIBG. A ^{18}F-labelled analogue of MIBG, 4-[^{18}F]fluoro-3-iodobenzylguanidine ([^{18}F]FIBG), has been prepared and is shown to have a higher uptake in SK-N-SH cells than MIBG. Because it may be an invaluable tool in combination with [^{18}F]FIBG, a method has been developed for the synthesis of its radioiodinated analogue, [^{131}I]FIBG. It was shown that SK-N-SH cells retained FIBG to a significantly higher degree than MIBG over a 3-day period, suggesting that [^{131}I]FIBG may deliver a higher integrated dose to the tumour than [^{131}I]MIBG.

1. INTRODUCTION

Although relatively rare, neuroblastoma is the most common among the solid malignant pediatric tumours; about 15% of all cancer deaths in children are due to neuroblastoma. Despite the use of intensive multimodal therapy regimens, which have increased remission rate and duration, the long term survival of stage IV disease has remained less than 15 per cent. Targeted radiotherapy is an alternative approach because it could allow the delivery of curative doses to tumour while minimizing normal tissue toxicities. Originally developed as an adrenomedullary imaging agent [1], radioiodinated *meta*-iodobenzylguanidine, MIBG (Figure 1) has found use in the diagnosis and treatment of a number of neuroendocrine tumours such as neuroblastoma, pheochromocytoma and carcinoid [2–5]. Although MIBG is an effective diagnostic agent for neuroblastoma and pheochromocytoma [6], its therapeutic efficacy is less than desired [7]. To address this problem, our laboratory is involved in the development of newer analogues of MIBG with the goal of improving its clinical usefulness. This paper describes some of our efforts in this area.

2. NO-CARRIER-ADDED MIBG

Radioiodinated MIBG used in the clinic is prepared by an exchange radioiodination (ex-MIBG) and thus contains a substantial amount of unlabelled carrier. Like norepinephrine, MIBG is taken up by the norepinephrine transporter by an active uptake-1 mechanism [8]. Although there are conflicting reports on the dependence of uptake on specific activity, one study has shown that at higher loading doses (lower specific activity), the uptake of [^{123}I]MIBG was reduced in rat hearts [9]. To investigate

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the effect of specific activity in detail, the availability of radioiodinated MIBG at a no-carrier-added (n.c.a.) level is advantageous. Towards this goal, a silicon precursor of MIBG, 3-trimethylsilylbenzylguanidine (TMSBG) was prepared starting from 3-bromotoluene in 5 steps. It was possible to prepare MIBG of very high specific activity and in >90% radiochemical yield by reacting TMSBG with radioiodine and *N*-chlorosuccinimide in trifluoroacetic acid for 5 min at room temperature.

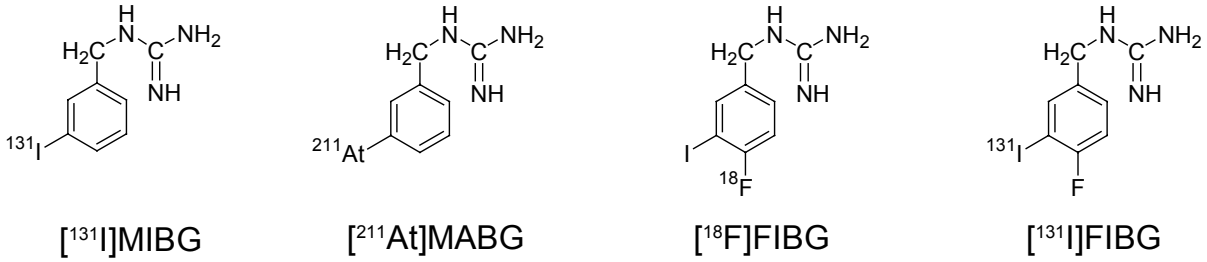

FIG. 1. Structures of [^{131}I]MIBG, [^{211}At]MABG, [^{18}F]FIBG and [^{131}I]FIBG.

2.1. Uptake of n.c.a.- and ex-[^{131}I]MIBG by SK-N-SH human neuroblastoma cells as a function of activity concentration

SK-N-SH human neuroblastoma cells (4–5 × 10^5 cells per well in 500 µl incubation medium) were incubated in quadruplicate with varying concentrations (1.2–233 nM) of ex-[^{131}I]MIBG (370 mBq/mg) in 24-well plates. In parallel, an equivalent activity range of n.c.a. [^{131}I]MIBG (10 000-2 000 000 cpm) was also incubated. After a 2-h incubation, the cell-associated activity was determined. Non-specific uptake was determined by repeating the experiment with another neuroblastoma cell line, SK-N-MC which does not take up MIBG. As shown in Table 1, the specific uptake of n.c.a. [^{131}I]MIBG remained fairly constant whereas that of ex-[^{131}I]MIBG steadily decreased over the 2–3-log activity concentration range. These data clearly demonstrate that the uptake of MIBG by SK-N-SH cells under these conditions is saturable.

TABLE I. UPTAKE OF N.C.A. [^{131}I]MIBG AND EX-[^{131}I]MIBG BY SK-N-SH CELLS AS A FUNCTION OF ACTIVITY CONCENTRATION

	Specific uptake (per cent of input) of [131I]MIBG	
Log Input (count per minute)	No-carrier-added	Exchange Preparation
4.0	48.9 ± 0.8	41.1 ± 1.1
5.0	48.0 ± 1.1	39.1 ± 0.4
5.4	47.3 ± 0.6	30.7 ± 1.5
5.7	45.1 ± 1.2	21.5 ± 1.6
6.0	43.5 ± 0.6	11.4 ± 0.6
6.3	44.3 ± 2.8	6.4 ± 0.6

2.2. Tissue distribution of n.c.a. [^{131}I]MIBG in normal mice

Sympathetically innervated tissues such as heart and adrenals sequester MIBG and can serve as valuable indicators of uptake-1 mediated targeting. To assess the uptake of n.c.a. [^{131}I]MIBG in normal tissues, especially the above, the tissue distribution of the n.c.a. [^{131}I]MIBG in normal mice was compared with that of ex-[^{131}I]MIBG over a period of 24 h. The myocardial uptake of n.c.a. preparation was significantly ($p < 0.05$) higher than that of ex-[^{131}I]MIBG at all time points studied. For example, the heart uptake of the n.c.a. preparation 1 h after injection was 26.4 ± 5.2% ID/g; in comparison, for ex-[^{131}I]MIBG it was 9.2 ± 1.3%, a 3-fold difference. Initially, the difference between

the adrenal uptake of the two preparations was not significant; however, by 24 h, the value for n.c.a. preparation was 4-fold higher than that for ex-[^{131}I]MIBG. These results suggest that n.c.a.[^{131}I]MIBG may be advantageous for clinical applications.

2.3. Effect of specific activity on uptake in vivo *in human neuroblastoma xenograft model*

2.3.1. SK-N-SH model

A comparative biodistribution of n.c.a.[^{131}I]MIBG and ex-[^{131}I]MIBG (2 :g of unlabelled MIBG per mouse) was carried out in separate groups of BALB/c *nu/nu* athymic mice hosting SK-N-SH xenografts. Over a period of 48 h, no significant difference in tumour uptake was seen between the two preparations. For example, at 4 h after administration, when maximum tumour accumulation was observed, the uptake was 3.2 $\pm$ 0.3%ID/g and 2.7 $\pm$ 1.0 %ID/g for the exchange and n.c.a. preparations, respectively. Selective targeting of highly innervated tissues such as heart and adrenals was seen; however, levels of uptake expressed as %ID/g were less than those seen in normal mice. In addition, specific activity did not have an effect on the myocardial or adrenal uptake. However, when the study was performed using non-tumour bearing athymic mice, the myocardial uptake was higher for the n.c.a. preparation. Yet another study was performed in which n.c.a. preparation was administered alone, or with varying amounts of unlabelled MIBG in athymic mice with xenografts. This was done to insure that differences in radiopharmaceutical quality did not obscure any specific activity effects. Again, no differences in tumour uptake were seen. However, at 4 h after administration, the heart uptake was reduced by a factor of 1.5 by the presence of carrier (3 g per mouse).

2.3.2. SK-N-BE(2C) model

Contrary to the above results with SK-N-SH model, higher uptake of n.c.a. [^{131}I]MIBG compared with ex-[^{131}I]MIBG was seen in tumour, heart and adrenals when the biodistribution was carried out in MF1 *nu*/*nu* athymic mice bearing SK-N-BE(2C) xenografts. In addition, tumour-to-normal tissue ratios were higher for the n.c.a. preparation. For example, the tumour-to-liver ratio for n.c.a. [^{131}I]MIBG was 4.4 $\pm$ 1.8 at 24 h, almost twice that of ex-[^{131}I]MIBG (2.3 $\pm$ 1.0; $p < 0.01$).

There are several factors that might have contributed to the differences observed between the two studies. For example, the strain of mice used for the two studies was different. As mentioned above, reduced heart and adrenal accumulation was seen between normal to athymic mice, suggesting that the uptake may be species-dependent. The most important difference between the two studies was the xenograft model itself. *In vitro* studies have shown that the uptake is saturable in both cell lines at a concentration of about 100 nM. Based on this, one would predict that the amount of unlabelled MIBG used in the SK-N-SH study would have been sufficient to saturate tumour uptake. A plausible explanation may be the differences in the NET. An inverse correlation between the expression of NET and tyrosine hydroxylase, the key regulatory enzyme of catecholamine synthesis, has been observed for SK-N-SH cells [10]. It may be possible that the expression of NET gene is diminished for SK-N-SH cells when implanted *in vivo*. Preliminary results have shown that receptor gene expression by SK-N-SH, but not SK-N-BE(2C) cells, is diminished when the cells are grown as xenografts in MF1 *nu*/*nu* mice. If this is true for BALB/c *nu*/*nu* mice also, then a considerable amount of [^{131}I]MIBG uptake by the specific uptake-1 mechanism would be compromised in the SK-N-SH model *in vivo*. Then, the majority of the uptake would be by passive diffusion, where added carrier could actually enhance the uptake.

3. META-[^{211}At]ASTATOBENZYLGUANIDINE

Neuroblastoma, the tumour most commonly treated with [^{131}I]MIBG, often is characterized by micrometastatic disease. Unfortunately, the physical properties of β-particles, such as those emitted by ^{131}I, are suboptimal for smaller tumours. The range in tissue of β-particles is of the order of

millimeters. As a result of this, the fraction of absorbed dose deposited in small tumours decreases as the tumour volume decreases, making [^{131}I]MIBG less than ideal for the therapy of micrometastatic tumours. Alpha particles, on the other hand, have ranges of 50–100 :m and hence their energy is fully absorbed within a few cell diameters. In addition, α-particles are radiations of high linear energy transfer (LET), and thus have higher relative biological effectiveness. Astatine-211 is a 7.2 h α□emitter of particular interest for endoradiotherapy. Being a halogen, it is generally easy to introduce ^{211}At onto organic molecules by adapting radioiodination chemistry. Furthermore, astatinated compounds often retain the biological characteristics of their iodinated counterparts [11]. Thus, an astatinated analogue of MIBG, *meta*-[^{211}At]astatobenzylguanidine (MABG; Figure 1) could be potentially useful for the treatment of micrometastatic neuroblastoma.

3.1. Synthesis of MABG

MABG was prepared by the astatination of TMSBG, the silicon precursor used for the preparation of n.c.a. [^{131}I]MIBG. Astatine-211 was produced by the cyclotron irradiation of natural bismuth metal targets by the ^{209}Bi(α, 2n)^{211}At nuclear reaction. Dry distillation was used to isolate ^{211}At from the target generally in chloroform. The ^{211}At activity from this solution was extracted into a small volume of 0.1 N NaOH and treated with *N*-chlorosuccinimide and the silicon precursor. Unlike radioiodination, a temperature of 50–70^{o}C was necessary for astatination. Under these conditions, more than 85% of radiochemical yields were obtained.

3.2. **In vitro** *uptake of MABG by SK-N-SH cells: Mechanistic studies*

To investigate whether substitution of ^{211}At for iodine compromised the molecular properties of MIBG, experiments were performed in SK-N-SH human neuroblastoma cells *in vitro* to see whether, like MIBG, MABG is taken up by an active uptake-1 mechanism. For this, the cells (5×10^5 per well per 0.5 ml medium) were preincubated with various uptake-1 inhibitors for 30 min in 24-well plates. The medium was removed, fresh medium containing MABG (2.8 kBq per well) was added and incubated at 37^{o}C for an additional 2 h, and cell-associated activity was determined. In addition, an experiment was done to determine the effect of lower temperature on uptake to see whether tracer accumulation was due to an energy-dependent process. For this, the cells as above were incubated with MABG at 4^{o}C.

Because MIBG is an analogue of the neuronal transmitter norepinephrine, the addition of norepinephrine can inhibit the uptake of MIBG by SK-N-SH cells. Indeed, the uptake of MABG was reduced to 84%, 13% and 4% of control values by 1, 10 and 1000 :M norepinephrine. The tricyclic antidepressant desipramine (DMI) is an inhibitor of the uptake-1 mechanism. When pretreated with 0.1, 0.5 and 1 :M DMI, the uptake of MABG was reduced to 21%, 12% and 11% of control values, respectively. To determine the energy-dependency of MABG uptake by SK-N-SH cells, three conditions were used. When MABG was preincubated with 1.5 mM dithionite, which depletes the oxygen from the medium, the uptake was reduced to 18% of control values. Ouabain (1mM), which inhibits ATPase and hence the uptake-1 mechanism, also reduced MABG uptake to 8% of controls. Finally, incubation at 4^{o}C also resulted in the reduction (to 8%) of MABG uptake. These data clearly demonstrate that MABG, like MIBG, is transported via an active uptake-1 mechanism in SK-N-SH neuroblastoma cells.

3.3. Biodistribution in normal mice

The biological similarity of MIBG and MABG was further investigated by performing tissue distributions in normal mice. As shown in Figure 2, the accumulation of both tracers in normal mouse

tissues was quite similar over a 24 h period. Differences in the uptake in adrenals, one of the target tissues for MIBG were not statistically significant at any time points. The heart uptake of MABG was 80–83% of that seen for n.c.a. [^{131}I]MIBG at 1 and 4h; however, the difference was statistically

significant only at 4 h (13.0 ± 1.6% vs 16.1 ± 2.6%; $p < 0.05$). By 24 h, the value for MABG was about 1.3-fold that for [^{131}I]MIBG, but the difference was not statistically significant. The uptake of MABG in adrenals and heart was reduced to 50% and 33%, respectively, of control values when the mice were pretreated with the uptake-1 inhibitor DMI. This suggests that the accumulation of ^{211}At these tissues was mediated by a specific uptake mechanism. A potential concern with using astatinated radiopharmaceuticals is their *in vivo* instability. Thyroid uptake, an indicator of *in vivo* dehalogenation, was similar for both tracers suggesting a low degree of dehalogenation for both compounds. However, it is important to note that the uptake of astatide in mouse thyroid is only 10–50% that of iodide over the time course of this study [12]. In addition, spleen and lungs are two organs with relative selectivity for astatide about 10 times that seen for iodide. The above results show similar values of uptake for both tracers in these tissues, suggesting that MABG may be reasonably stable *in vivo*.

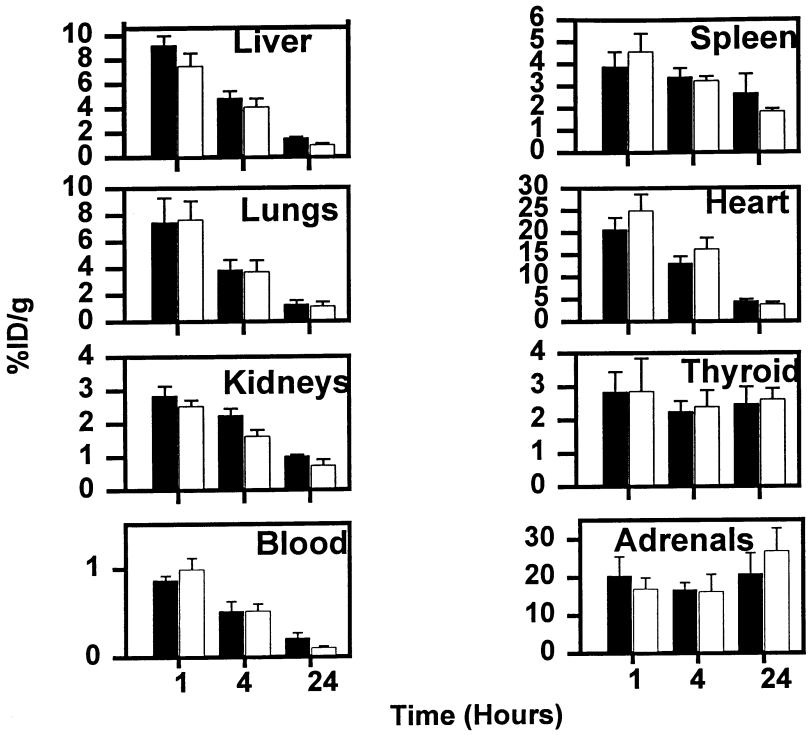

FIG. 2. Tissue distribution of n.c.a. [^{131}I]MIBG and MABG in normal mice.

3.4. Tissue distribution in athymic mouse neuroblastoma xenograft model

The potential usefulness of MABG for targeted radiotherapy was further evaluated by doing a paired-label tissue distribution of MABG and n.c.a. [^{131}I]MIBG in athymic mice bearing SK-N-SH neuroblastoma xenografts. As shown in Figure 3, at 8 h after injection, the tumour uptake of MABG and n.c.a. [^{131}I]MIBG was 3.8 ± 0.8%ID/g and 3.1 ± 0.7%ID/g, respectively, and the difference was statistically significant ($p < 0.05$). At all time points the tumour uptake of MABG was higher than that of n.c.a. [^{131}I]MIBG. Pretreatment of mice with DMI reduced the tumour uptake of MABG by 43%, suggesting that its accumulation was related to a specific uptake-1 mechanism. The uptake of MABG in other tissues was generally higher than that of n.c.a. [^{131}I]MIBG, and the difference in uptake between the two tracers increased with time. Although uptake in thyroid was similar for both tracers, uptake in lung, spleen and stomach was higher for ^{211}At, suggesting that MABG has a greater susceptibility to dehalogenation in this model than seen in normal mice. This, and the probable higher lipophilicity of MABG, may be the reasons why tissue uptake of MABG was generally higher in all tissues.

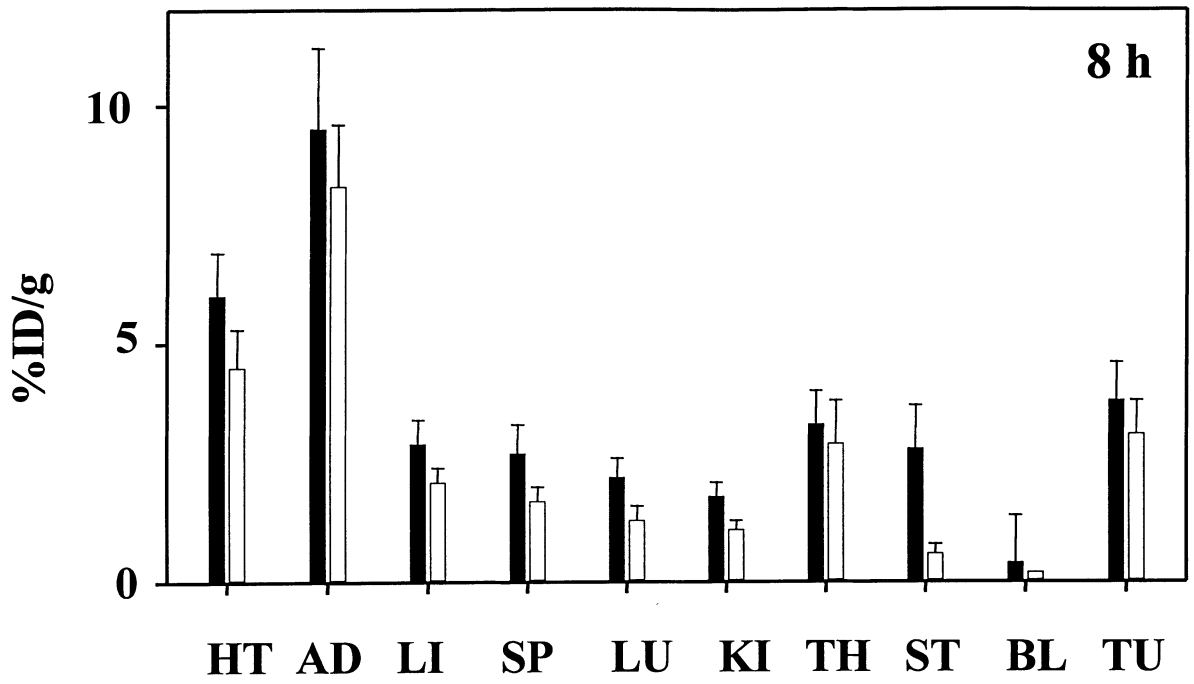

FIG. 3. Paired-label tissue distribution of MABG and n.c.a. [^{131}I]MIBG in athymic mice hosting SK-N-SH xenograft 8 h after injection.

3.5. Cytotoxicity

3.5.1. Inhibition of [^{3}H]thymidine uptake

SK-N-SH cells were initially treated with varying concentrations of ex- [^{131}I]MIBG, n.c.a. [^{131}I]MIBG and MABG for 30 min. After washing, the cells were incubated with thymidine-deficient medium for 24 h. Subsequently, the ability of cells to incorporate thymidine was determined by incubating them with [^{3}H]thymidine for 30 min. The amount of thymidine incorporated was reduced to less than 50% of the control level with as little as 118 Bq of MABG. No significant reduction in thymidine uptake was seen even with 3 kBq of n.c.a. [^{131}I]MIBG. The thymidine uptake was completely impaired with about 370 kBq of n.c.a. [^{131}I]MIBG, whereas at this level more than 50% of thymidine incorporation was seen with ex-[^{131}I]MIBG.

3.5.2. Clonogenic survival

The proliferative capacity of untreated SK-N-SH cells and those treated with MABG, [^{211}At]astatide and n.c.a. [^{131}I]MIBG was determined using a limiting dilution clonogenic assay [13]. The D_0 values, the amount of initial radioactivity concentration necessary to reduce the survival to 37%, were calculated from these data. A D_0 value of 215 Bq/ml was calculated for [^{211}At]MABG. In comparison, the value for n.c.a. [^{131}I]MIBG was 384 kBq/ml implying a more than 1,000-fold higher cytotoxicity for the α-particle emitting analogue. That the exquisite cytotoxicity of [^{211}At]MABG is indeed due to its specific uptake and retention in SK-N-SH cells was demonstrated by the fact that the D_0 for [^{211}At]astatide, 17.8 kBq/ml, was more than 80-fold higher than that for [^{211}At]MABG.

4. 4-FLUORO-3-[^{131}I]IODOBENZYLGUANIDINE

Positron emission tomography (PET) is a superior imaging technique. An MIBG analogue labelled with a positron emitter would be attractive for diagnostic oncology. Towards this goal, we have prepared 4-[^{18}F]fluoro-3-iodobenzylguanidine ([^{18}F]FIBG; Figure 1) [14]. Preliminary results

have indicated that [^{18}F]FIBG is a suitable analogue of MIBG and may find application in the PET imaging of neuroendocrine tumours and the myocardium.

With regard to the oncologic strategy of using PET as a prelude to radionuclide therapy, utilization of radionuclides of the same element for both diagnosis and treatment would be advantageous, particularly if PET is to be used for dosimetry planning. For example, ^{124}I and ^{131}I, while not ideal, may be a useful pair of radionuclides for labeling MIBG or an MIBG analogue such as FIBG with increased retention in neuroblastoma cells. Another strategy is to have a molecule which can be labelled with either the therapeutic or positron-emitting nuclide. FIBG presents such an opportunity since it contains both fluorine and iodine. Because our results with [^{18}F]FIBG suggest that 4-fluoro-3-[^{131}I]iodobenzylguanidine([^{131}I]FIBG; Figure 1) may offer higher binding to neuroblastoma cells than MIBG itself, we developed a no-carrier-added synthesis of [^{131}I]FIBG from a silicon precursor and evaluated its potential usefulness.

4.1. Synthesis of [^{131}I]FIBG

Since n.c.a. [^{131}I]MIBG could be prepared in excellent radiochemical yield from a silicon precursor, we decided to follow the same strategy for the preparation of radioiodinated FIBG. Towards this end, a silicon precursor, 4-fluoro-3-(trimethylsilyl) benzylguanidine (FTMSBG) was prepared in 5 steps. When the conditions used for the conversion of TMSBG to MIBG were applied, only 60–65% of [^{131}I]FIBG was obtained from FTMSBG. A radiolabelled byproduct, in an amount roughly equal to half of [^{131}I]FIBG, was also formed. Its HPLC behaviour and *in vitro* binding to SK-N-SH cells indicate that this compound is MIBG. Radiochemical yields of 75–80% for [^{131}I]FIBG were obtained, however, when FTMSBG was radioiodinated using hydrogen peroxide as the oxidant in aqueous acidic conditions at 50^{o}C.

4.2. In vitro evaluation

When performed in a paired-label format, the specific binding of [^{131}I]FIBG to SK-N-SH cells remained fairly constant (45–60%) over a 2–3-log activity range, and was 11–14% higher ($p < 0.05$) than that of [^{125}I]MIBG. The uptake of [^{131}I]FIBG was blocked to varying degrees by several interventional agents, and by performing the incubation at 4^{o}C. The uptake-1 inhibitor DMI (1.5 :M) reduced the binding of [^{131}I]FIBG to 13% of the control value. Ouabain (1 mM) and incubation at 4^{o}C reduced the uptake to 31% and 8% of the control value, respectively, suggesting that the uptake of [^{131}I]FIBG in this cell line was energy-dependent. The specificity of [^{131}I]FIBG uptake was further demonstrated by the reduction of its binding to 8%, 6% and 5% of the control value by 50 :M norepinephrine, 10:M MIBG and 10 :M FIBG, respectively. These results suggest that uptake of [^{131}I]FIBG by this cell line is specific and is mediated through an active uptake-1 mechanism.

In addition to higher uptake, retention of a radiotherapeutic agent by the tumour for time period compatible with the physical half-life of the radionuclide is important for its efficacy. The ability of SK-N-SH cells to retain [^{131}I]FIBG and [^{125}I]MIBG was determined in a paired-label format. After incubating the cells with both tracers for a period of 2 h, the cells were washed to remove unincorporated activity. Subsequently, cells were incubated with fresh medium without and with desipramine. The cell-associated activity at various intervals was determined. As shown in Table 2, 76% of the originally bound [^{131}I]FIBG activity was retained in SK-N-SH cells after 3 days compared with 30% for [^{125}I]MIBG. Using these binding data, time-activity curves were constructed assuming that both tracers were labelled with ^{131}I. The area under the FIBG time-activity curve extrapolated to infinity was about twice that for MIBG, suggesting a significant advantage in radiation absorbed dose to this cell line might be achievable with [^{131}I]FIBG. Further, it was demonstrated that DMI enhanced the washout of initially bound [^{125}I]MIBG and [^{131}I]FIBG, indicating that tracer retention is mediated by the re-uptake of released activity.

TABLE II. PAIRED-LABEL RETENTION OF [^{131}I]FIBG AND [^{125}I]MIBG BY SK-N-SH CELLS AS A FUNCTION OF TIME.

	Cell-associated activity (Per cent of Input)			
Time (Hours)	[^{131}I]FIBG	[^{131}I]FIBG + DMI	[^{125}I]MIBG	[^{125}I]MIBG + DMI
0	74.1 ± 3.1	74.1 ± 3.1	66.6 ± 3.7	66.6 ± 3.7
2	78.4 ± 0.6	53.0 ± 1.2	67.0 ± 0.4	40.6 ± 1.0
4	77.3 ± 2.3	38.5 ± 3.2	64.2 ± 2.6	25.8 ± 2.2
8	69.8 ± 4.3	27.3 ± 0.9	54.9 ± 3.3	14.5 ± 0.5
24	72.9 ± 2.6	23.5 ± 2.0	45.8 ± 4.6	10.8 ± 0.7
48	64.5 ± 5.0	25.6 ± 0.9	34.9 ± 2.0	12.7 ± 0.4
72	50.6 ± 5.0	29.6 ± 3.1	17.3 ± 2.5	14.9 ± 1.4
96	25.7 ± 5.7	26.7 ± 3.3	7.7 ± 1.3	12.6 ± 1.4

5.1. Biodistribution in normal mice

A paired-label tissue distribution of [^{131}I]FIBG and [^{125}I]MIBG was performed in normal mice over a period of 7 days. High uptake of [^{131}I]FIBG was seen in both heart and adrenals. The [^{131}I]FIBG/[^{125}I]MIBG myocardial uptake ratio increased from about 1 at 1 h to 1.3, 3.8 and 12.2 at 4 h, 1 d and 3 d, respectively. Adrenal uptake was similar for both tracers up to 2 days; however, a 1.4–2-fold higher retention of [^{131}I]FIBG was seen from 3 to 7 d. One hour after injection, the heart and adrenal uptake of [^{131}I]FIBG in DMI-treated mice was reduced to 48% and 60% of the control values, respectively confirming the specificity of uptake in these tissues.

Retention of [^{131}I]FIBG was higher in most other tissues also. Thyroid was a notable exception. For example, at 24 h, the thyroid uptake of ^{131}I (1.9 ± 0.4%ID/g) was half that of ^{125}I (3.7 ± 1.7%ID/g; $p < 0.05$) and this difference increased with time. This suggests that FIBG is less susceptible towards deiodination.

6. CONCLUSIONS

A method has been developed to prepare radioiodinated MIBG at a no-carrier-added level. *In vitro* studies and some *in vivo* studies indicate that n.c.a. [^{131}I]MIBG may be advantageous for clinical applications. It was possible to prepare MABG in high radiochemical yields and it retained the molecular properties of MIBG to a considerable degree. MABG was shown to be extremely cytotoxic and should find applications in the treatment of metastatic neuroblastoma. It has been shown that MIBG is taken up by several medulloblastoma cell lines and that MABG is cytotoxic to some of these as a result of the specific uptake of MABG (data not given). Since neoplastic meningitis, a disease that should be amenable to α-particle therapy, is often associated with medulloblastoma, MABG could be a suitable endoradiotherapeutic agent for this type of cancer. Fluorine substitution in MIBG resulted in a molecule which was retained to a higher degree by the tumour cells. Thus therapeutic efficiency of [^{131}I]FIBG can be anticipated to be higher than that of [^{131}I]MIBG. Hopefully, clinical investigations with some of these more potent MIBG analogues will be initiated in the near future.

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PREPARATION AND EVALUATION OF VARIOUS ^{32}P SOURCES FOR INTRAVASCULAR BRACHYTHERAPY

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Abstract. A relatively high per cent of restenoses, being a long-term complication of percutaneous transluminal coronary angioplasty (PTCA), can be significantly reduced by short-range ionizing radiation applied locally, immediately after PTCA. In search for dosimetrically favourable and easy to handle radiation sources for this purpose, we tried a pure β^- emitter ^{32}P ($t_{1/2}$=14.3 days). Ways of preparation of ^{32}P sources were the following: (1) Neutron activation of ^{31}P layers implanted into metallic surfaces by ionic methods; (2) Conversion coating of metallic surfaces in aqueous solutions containing $^{32}PO_4^{3-}$ ions; (3) Direct application of $Na_2H^{32}PO_4$ solutions in the angioplasty balloon. It was shown that: (1) ^{32}P sources obtained by ^{31}P ion implantation followed by neutron activation can be useful, but only if activation of the support material by thermal neutrons is negligible; (2) Phosphate layers on stainless steel surface exhibit rather poor adhesion. Similar layers on titanium require further studies; (3) Liquid ^{32}P sources ensure very good radial dose distribution but only utmost care in filling the balloon can give a reliable activity-dose dependence. Dosimetry of liquid sources, performed in a PMMA phantom by thermoluminescence method showed that ^{32}P sources of radioactive concentration of 200 MBq/cm^3 can deposit therapeutic dose during about 12 min of exposition. TL detectors manufactured for this purpose in our laboratory show very good spatial resolution and can be recommended for similar studies.

1. INTRODUCTION

1.1. General

Percutaneous transluminal coronary angioplasty (PTCA) is a powerful means for treatment of coronary disease. Short-term efficiency of this method reaches 95% but its long-term complication is a relatively high per cent of restenoses (30–50% of cases during 6 months), which may lead to repetitive treatment. Metallic stents can reduce the number of restenoses by ca 50% simply by acting against the elastic component of the wall narrowing, but they cannot prevent metabolic transformations or proliferation of the arterial wall cells, which is the main factor responsible for restenosis. The search for ways of preventing this effect revealed that radiation doses of 10–30 Gy play a positive role in remodelling of the arterial wall [1]-[7] which in mid-nineties initiated the practice of post-PTCA intravascular brachytherapy (IVBT). Nuclear properties of the nuclides suggested for IVBT [8] are compared in Table I. Among these three, ^{32}P has advantages such as convenient half-life, decay by pure β^- emission and favourable range of the emitted particles. It is important to note that, contrary to the oncological brachytherapy, nuclides emitting γ radiation should be avoided in IVBT because of the very short distances in the tissue at which the radiation energy should be deposited.

Physical form of the source determines the treatment fractionation and technique (high- or low dose rate). Angioplasty balloons and thrust wires, which are in brief contact with the tissue, are most suitable as supports for radionuclides in the high dose rate techniques whereas stents, remaining in the organism for ever, are natural carriers for radioactivity in the low dose rate techniques. To choose a method for preparation of IVBT sources, one has to take into account physical and chemical properties of stent or thrust wire materials which can be: pure titanium, Ti-Ni alloy or stainless steel. For animal studies we are going to use stainless steel stents which are shaped on-site by one of us [9].

The objective of this work was to compare several methods of preparation of ^{32}P sources and their dosimetry as a first step towards choosing sources for high-dose-rate or low-dose-rate treatment. In preliminary experiments on solid source preparation we used either stainless steel or pure titanium supports, in the form of plates, foils, or wires, depending on the parameters to be measured

afterwards. We tried both ion implantation and chemical conversion, to compare the quality of ^{32}P-containing coatings and possibilities of preparing sources under conditions when the ion implanter is not available. Apart from solid ^{32}P sources, we worked with water solution of ^{32}P phosphate which can be introduced directly into the angioplasty balloon. This liquid source, tempting from the viewpoint of dose uniformity, availability of registered ^{32}P radiopharmaceuticals and possibility of using standard angioplasty balloons for IVBT, was also evaluated.

TABLE I. NUCLEAR PROPERTIES OF SELECTED NUCLIDES SUGGESTED FOR IVBT

Nuclide	$t_{1/2}$ (days)	Gamma energy and intensity (keV (%))	Maximum beta energy (MeV)	Average beta energy (MeV)	Average beta range in water (mm)
^{32}P	**14,3 d**	**-**	**1,7**	**0,695**	**7,5**
^{90}Y	2,7 d	-	2,3	0,934	10
^{192}Ir	74,0 d	67 (4) 296 (28) 308 (29) 317 (83) 468 (48) 588 (4) 604 (8)	0,7	0,171	~2

1.2. Implantation of phosphorus onto metallic surfaces

Beams of ions are very suitable for doping thin surface layers of solid materials by stable or radioactive isotopes, or for creation of hard complex coating layers with very good adhesion and friction resistance [10]. A rather easy way of source preparation is implantation of stable ^{31}P into bulk material, especially into titanium. If titanium is really of high chemical purity, the implanted ^{31}P can be later activated with thermal neutrons to ^{32}P, without much risk of activating the supporting material. In case of stainless steel or nickel-titanium alloys, ion beam modification of the surfaces requires direct implantation of radioactive ^{32}P. Otherwise, contrary to titanium of highest purity, thermal neutron bombardment would activate unwanted long-lived gamma-emitting nuclides in these materials. The disadvantage of implantation of radioactive ^{32}P into stent or thrust wire material is the necessity of using radioactive ^{32}P as the ion source. In the geometry of our ion implanter this can severly contaminate the machine and rule it out from other uses for long periods.

1.3. Conversion coatings

Aiming at an easy method of preparation of ^{32}P source, possibly alternative to implantation of radioactive ^{32}P, we looked for simple ways of chemical modification of metallic surfaces, bearing in mind that biocompatibility of orthopedic or dental titanium implants is due to formation of passive oxide film on metal surface [9]. Recent investigations brought evidence that this film does not remain intact under physiologic conditions but in prolonged periods incorporates spontaneously mineral ions, especially Ca^{2+} and PO_4^{3-}, from biological fluids [12]-[15].

Another question was about the behaviour of stainless steel under physiologic or laboratory conditions in solutions containing various concentrations of PO_4^{3-} ions. Formation of phosphate conversion coatings on mild steels is a widely used method of corrosion protection, and our question was whether and how phosphates would precipitate on surfaces of chromium-rich stainless steels from which most stents are made. To look at possibilities of chemical modification of such passive surfaces, we performed another set of experiments.

1.4. Dosimetric considerations

The main problem in dosimetry of ^{32}P radiation are high dose-rate gradients occurring at distances of a few millimetres of tissue. Therefore, it is necessary to use in measurements detectors exhibiting very good spatial resolution. One of possible solutions are thermoluminescent (TL) detectors, and as our laboratory has a long experience in preparation and application of TL detectors, we decided to use this technique for dosimetric measurements. To obtain sufficiently good spatial resolution it was necessary to develop a completely new type of miniature TL detectors particularly for this purpose.

2. MATERIALS AND METHODS

2.1. Ion implantation

Our dual beam 75 kV ion implanter, used either for implantation or for the IBAD* technique, was described in detail in Ref. [16]. Its main features are listed in Table 3 in the Appendix.

Stainless steel stents and pure titanium wires were mounted on an Al sheet (purity 99.99%). The same sheet served as support for a Ti pellet (∅ 5 mm d = 1 mm, Goodfellow, purity 99.999%). The samples were implanted by 25 keV/10 μA ^{31}P ions, focused to form a 8 mm × 70 mm trace on the sheet. To improve implantation uniformity, the whole target was oscillating in the plane perpendicular to the beam. The overall ^{31}P dose, measured by the Faraday cup, was 10^{17} of ^{31}P ions/cm^2. The range of 25 keV ^{31}P in Ti, calculated using the SRIM program, was 25 nm. In the described experiments, only one side of each sample was implanted. The samples were sent for activation with thermal neutrons to the reactor in POLATOM, Swierk, Poland.

The efficiency of the implantation process was checked by PIXE and RBS methods. To do this it was sufficient to measure ^{31}P content in the Al support and in the Ti pellet. The depth distribution of ^{31}P was determined from energetic spectrum of back-scattered 2040 keV He^+ ions bombarding the doped surfaces, perpendicularly or at 45^o angle. The scattered He^+ ions were detected at 168^o angle and 10 cm distance, using a ∅ 8 mm particle detector of 12 keV resolution. Simultaneously, He^+ - induced X-ray spectra were registered at detection angle of 90^o respective to the He^+ beam.

2.2. Chemical conversion coatings

Preliminary experiments were performed using ^{31}P phosphates. Metallic support samples were either of the following: (a) 3 mm × 5 mm × 10 mm plates of austenitic chromium-nickel steel (AISI 316L), (b) stainless steel wire (AISI 316, ∅ = 0.15 mm), (c) 3 mm × 25 mm pieces of 0.3 thick mm titanium foil (Johnson-Matthey, 99.7+% purity), (d) titanium wire (Goodfellow, 99.6+% purity, ∅ = 0.4 mm).

All metallic samples were ultrasonically degreased with CCl_4, acetone, and ethanol, for 15 min each, and rinsed with deionized water in between. Additionally, before degreasing, the stainless steel plates were mechanically polished with 500, 1200, and 1500 grit silicon carbide paper. The merely degreased samples served as control. Some stainless steel samples were additionally pickled in 20% HCl at 20^{o}C for 3 min [17], and part of the Ti ones in 40% HNO_3 at 20^{o}C for 30 min (cf. Ref. 14).

All phosphating baths were kept at 85^{o}C. These for stainless steel were: (a) 0.15 M (15 g/dm^3) H_3PO_4, (b) 1.5 × 10–^{5} M (1.5 mg/dm^3) H_3PO_4, (c) 0.15 M (18 g/dm^3) NaH_2PO_4 , (d) 1.5 × 10–^{5} M (1.8 mg/dm^3) NaH_2PO_4, with or without the addition of 0.01 M H_2O_2, (e) 3.7 MBq/cm^3 (40–400 GBq/mg P) $Na_2H^{32}PO_4$ (POLATOM, Œwierk, PL). The baths for Ti were: (f) 0.001 M

* IBAD = Ion-Beam Assisted Deposition, PIXE = Particle-Induced X-ray Emission, RBS = Rutherford Back-Scattering.

(0.12 g/dm^3) Na_2HPO_4, (g) same as (f) with addition of H_2O_2 up to 0.1 M concentration. Stainless steel samples were just immersed in phosphating baths, whereas Ti samples were polarized to anodic potential of 0.5 V relative to standard calomel electrode (SCE). Phosphate deposition on Ti was observed by measuring the current between the immersed metal and the auxiliary Pt electrode.

2.3. Liquid ^{32}P sources

Liquid ^{32}P sources were prepared from commercial $Na_2H^{32}PO_4$ (POLATOM, Œwierk, Poland). Radioactive concentration of stock solution was 355 MBq/cm^3 at the calibration date. Aliquots of this solution were diluted with water/75%Uropolinum** mixture (1:1 v/v) to desired radioactive concentrations, and introduced into angioplasty balloons (Schneider AG, Europe). To fill a 25 mm long, ∅ 3 mm balloon along with its 1350 mm long cathether we needed ca 0.5 cm^3 of radioactive solution, of which only about 0.15 cm^3 (less than 30%) was in the balloon itself. The radioactive concentrations of ^{32}P solutions used in dosimetric measurements ranged between 1.37 MBq/cm^3 and 3.6 MBq/cm^3.

2.4. Dosimetric measurements

The manufactured TL detectors have shape of circular pellets with diameter of 2 mm and effective thickness of ca. 0.05 mm (overall thickness was 0.5 mm). They were constructed with use of the two-layer technique [18], which enables making the radiation-sensitive part of the detector extremely thin. The thermoluminescent material used for preparation of detectors was lithium fluoride activated with magnesium, copper and phosphorus (LiF:Mg,Cu,P; trade name MCP-N), which characterises nearly tissue equivalence and very good sensitivity. Calibration of the detectors was performed with ^{137}Cs 661 keV gamma rays in terms of absorbed dose in water.

(a) phantom body *(b) insert with a TL detector*

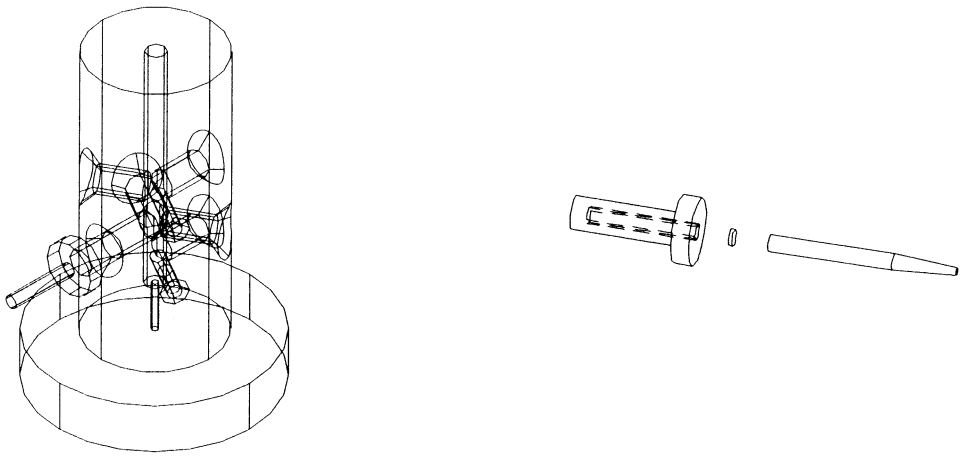


FIG. 1. Phantom used in dosimetric measurements.

To mimic real conditions of radiation interaction with human tissue, measurements were performed within a PMMA phantom. A sketch of the phantom is presented in Fig. 1. It has cylindrical shape with a hole drilled along the central axis in which the balloon was placed. Perpendicularly to the axis a few channels were drilled. In these channels the inserts containing TL detectors were placed. Head parts of the inserts (made also of PMMA) were of different thickness, ranging from 0.15 mm to 4.00 mm. In this way it was possible to measure doses at different distances from the balloon surface. The "zero distance" measurements were performed using TL detectors wrapped in

** Uropolinum®, Polpharma, PL — iodine containing contrast medium, necessary to control the degree of ballon filling during PTCA. 75%Uropolinum contains 380 mg I/cm3.

1mg/cm^2 thick foil (food wrapping foil, Ulith, Germany), placed directly on the balloon surface. Balloon background radiation was measured under the same conditions for the air-inflated balloon. Laboratory background was measured far from all sources, using the same set of TL detectors.

3. RESULTS AND DISCUSSION

3.1. Ion-implanted sources

Overall dose of implanted phosphorus, its depth distribution and concentration of trace elements was determined from PIXE and RBS spectra, whose examples are shown in Fig. 2 and Fig. 3. It can be seen (Fig. 2) that trace elements content was below the detection limit. The RBS spectrum confirms the dose 10^{17} of ^{31}P atoms/cm^2 implanted into the target and allows to determine depth distribution of ^{31}P in Al. Since implantation conditions were the same for Al and Ti targets, the ^{31}P dose determined initially for Al can be used for interpretation of both RBS and PIXE spectra for Ti, especially for depth distribution of ^{31}P in Ti. The latter parameter will be the crucial one in optimizing ^{31}P implantation conditions and in calculating corrections for shielding of ^{32}P β^- emission in metal surface layers. A program for such calculations is developed in our laboratory.

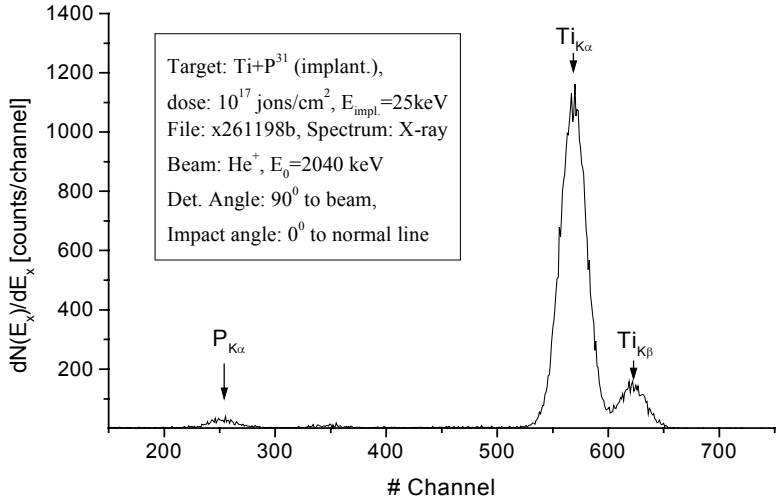


FIG. 2. PIXE spectrum of a Ti plate implanted by ^{31}P ions.

3.2. Conversion coatings

It seems evident that immersion method of ^{32}P source preparation cannot be applied to stainless steel because of very poor adhesion of phosphate coatings: simple wipe tests on treated stainless steel wires showed significant loss of ^{32}P radioactivity. At the same time it is a confirmation of chemical resistance of the material used.

Time necessary for reaching equilibrium, checked by observing potential changes after metal immersion, was about 60 min for stainless steel and about 20 min for Ti samples. Anodic potential applied to metallic samples significantly increases the phosphating rate. Typical current plots for Ti are represented in Fig. 4. Shift between two series of curves seems to evidence the increase of active surface of metal after acid etching. As titanium phosphates are rather active inorganic ion exchangers, it is very desirable to further study such coatings in the context of possible applications not only in IVBT but also in implant surgery. Influence of H_2O_2 and of other chemical species on Ti behaviour in phosphating baths is still under study in our laboratory.

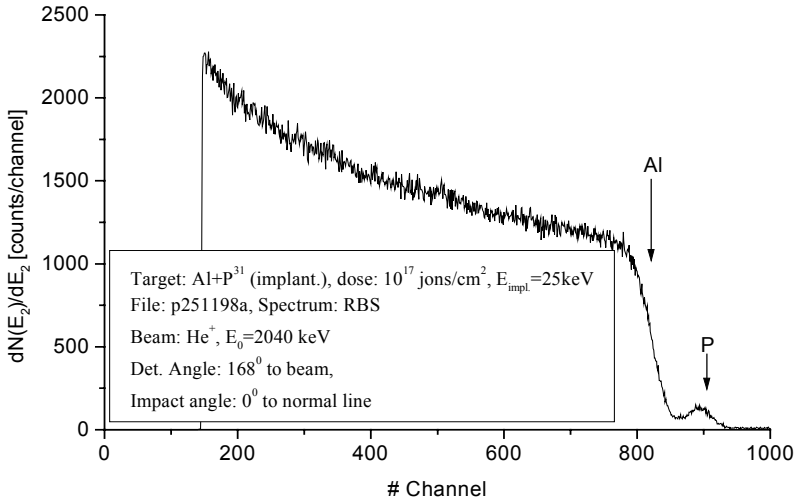
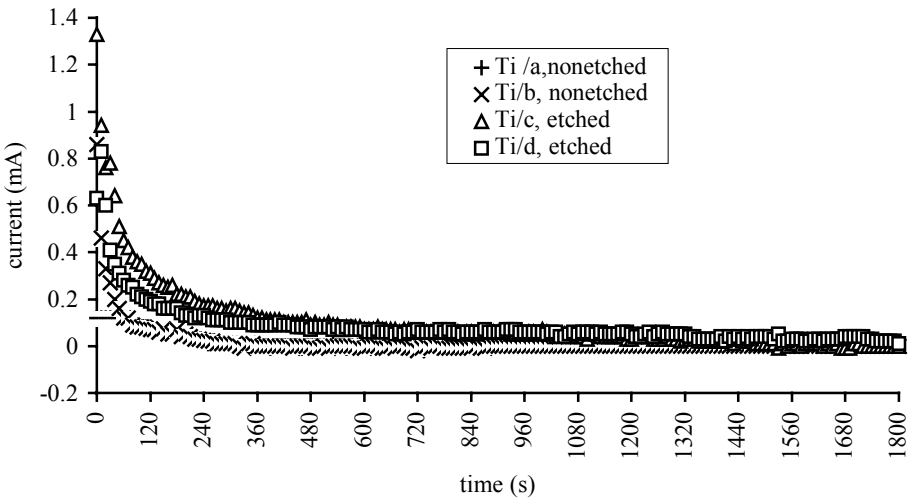


FIG. 3. RBS spectrum for Al support implanted by ^{31}P ions.



Note: Data for samples Ti/a and Ti/b practically overlap. Horizontal line for Ti/a between 0 s and 60 s denotes lack of data.

FIG. 4. Phosphating rate of Ti wire.

3.3. Dosimetry of ^{32}P liquid sources

The measured radial dose-rate distribution around a ∅ 3mm balloon is presented in Fig. 5. Each data point represents an average of a larger number of measurements (different for each distance, ranging between 4 and 25). The solid line represents an empirical fit with exponential function: $f(r) = 4.55 \times exp\ (-1.7 \times r)$, where r is a distance from the balloon surface. The pre-exponential factor represents a coefficient of proportionality between radioactive concentration inside the balloon and a dose-rate on its surface. Using this formula it is possible to calculate dose rates averaged over different depths inside the phantom, what is presented in the Table 2. The dose-rate drops rapidly with distance, which is very favourable from the viewpoint of radiation protection of healthy tissue.

TABLE II. CALCULATED DOSE-RATES, AVERAGED FOR VARIOUS "SHELLS" SURROUNDING A BALLOON FILLED WITH ^{32}P/UROPOLINUM SOLUTION.

Shell thickness (mm)	Average dose-rate (mGy/min)/(MBq/cm^3)	Dose-rate for effective 200 MBq/cm^3 ^{32}P (Gy/min)
0.25	3.71	0.742
0.50	3.07	0.614
1.00	2.19	0.438
2.00	1.30	0.260

Commercial ^{32}P solutions from POLATOM can have maximum radioactive concentration of 400 MBq/cm^3. Assuming that dilution with Uropolinum reduces this concentration to effective 200 MBq/cm^3, it can be seen that this ensures dose rate of ca. 0.75 Gy/min near the balloon surface. This would mean that deposition of minimum therapeutic dose (10 Gy) within first 0.25 mm "shell" around the balloon surface would require at least 12 min exposition. Since during PTCA the balloon cannot block the artery for longer than 2 min, at least 6 consecutive filling and emptying of the balloon would be necessary. Our laboratory practice shows that Schneider AG balloons resist very well such manipulations, probably because pressures necessary to fill the balloon with radioactive solution are much lower than those necessary for angioplasty. Occasionally, we had some problems with tightness of stopcocks and valves connecting the catheter with Luer-lock syringe supplying the radioactive solution.

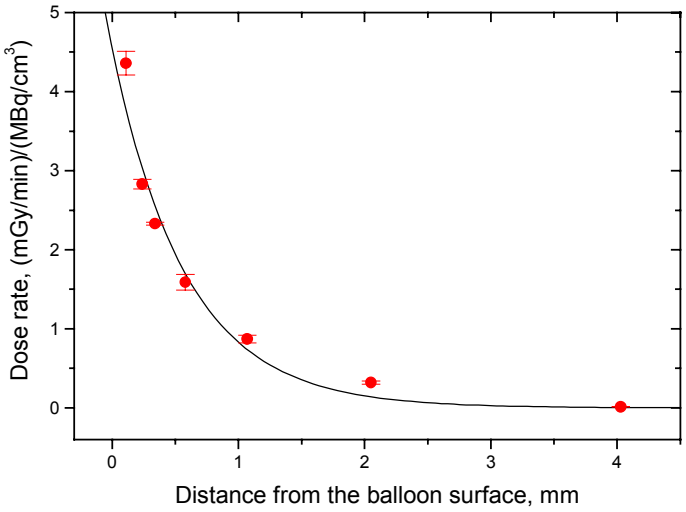


FIG. 5. Measured and fitted dose rates at various distances from the balloon surface.

4. CONCLUSIONS

Ionic methods are recommendable for deposition of phosphorus into metal surface layer. Their advantage is that they produce very hard, friction- and chemically resistant surfaces which can be considered as sealed sources. Its limitation is the risk of radioactive contamination of our multipurpose apparatus during implantation of ^{32}P. Neutron activation of ^{31}P-implanted stents or thrust wires is recommendable only for those from purest Ti. Activation of long-lived gamma emitting nuclides in medical alloys such as Nitinol (Ni-Ti) or stainless steel may cause dosimetric problems, especially in stent-based techniques of IVBT.

Attempts to produce ^{32}P conversion coatings on stainless steel were not successful. Phosphate coatings on Ti seem very interesting but require further studies.

Use of liquid sources may be a relatively simple method for medium-to-high dose-rate technique, especially in cases when ^{32}P phosphate *pro inj.* solutions are readily available. The measured dose distribution around the balloon filled with ^{32}P/contrast mixture shows very steep drop with distance, indicating that the range of ^{32}P β^- particles is just as needed for this type of treatment.

The TL detectors, elaborated for this research, have very good spatial resolution and can be recommended for other similar studies.

ACKNOWLEDGEMENTS

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APPENDIX

TABLE III. PARAMETERS OF THE ION IMPLANTER USED

- The main ion beam line:

Ion species — almost all the elements (with the exception of e.g. heavy platinum metals)
Hot-cathode, arc discharge in a magnetic field ion source:
- discharge volume — 15 cm^3
- pressure — ~ 10^{-2} mm Hg
- arc voltage — up to 30 V
- arc current intensity — up to 50 A
- magnetic field intensity — up to 0.08 T
- temperature — up to 1600°C
- material feeding — gases, liquids, solid state material
- emission area — 1.8 mm^2
- ion current intensity — up to ~5 mA
- power supply — anode voltage, cathode filament, electromagnet, arc chamber heater, oven heater I, oven heater II
- total power — up to 4 kW
- cooling — water
- service life — up to ~ 40 h

Accelerating voltage — from 15 kV to 45 kV
Beam focusing — electrostatic lens
Mass separation of ions (Q/M):
- electromagnetic analyser — 60° sector of homogeneous magnetic field
- magnetic field intensity — up to 1.25 T
- height of deflection chamber — 60 mm
- mean radius of ion trajectories — 50 cm
- energy of mass separated ions — from 15 keV to 45 keV for singly charged ions (for multiple ionized atoms multiplied by the charge state number)
- mass resolution $M/\Delta M$ — ~ 350
- focal plane linear dispersion — 5 mm/mass %

Vacuum in the ion beam line — ~ 10^{-5} mm Hg

- The additional ion beam line:

Ion species — noble or reactive gases
Hollow — cold — cathode ion source:
- discharge volume — 0.5 cm^3
- pressure — > 10^{-2} mm Hg
- arc voltage — up to 1 kV
- arc current intensity — up to 500 mA
- temperature — up to 1700 °C
- material feeding — gases
- gas flow rate — from 20 cm^3/h to 40 cm^3/h
- ion current intensity — up to ~ 1 mA
- power supply — anode voltage
- total power — ~ 250 W
- cooling — water
- service life — up to 40 h

Accelerating voltage — from 5 kV to 45 kV
Beam focusing — electrostatic lens
Size of the ion beam — ∅ 15 mm
Vacuum in the ion beam line — ~ 10^{-5} mm Hg

DOSIMETRICAL CONSIDERATIONS IN ASTATINE-211 RADIOIMMUNOTHERAPY*

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Abstract. Several dosimetrical quantities have been suggested for use in alpha-particle dosimetry. To evaluate the expected biological effect when using these quantities, a Monte Carlo program was set to register the single-event distribution of both specific energy and alpha-particle track length to a cell nucleus (r = 5.6 μm). Distributions were acquired for both "bound" (simulating the effect of ^{211}At-labelled antibodies bound to antigens on cell surfaces (r = 7.0 μm)) as well as "non-bound" (simulating ^{211}At-labelled antibodies that have not bound to a cell) astatine-211. From these distributions, various theoretical cell survival curves were established for 3 different dosimetrical quantities, i.e. specific energy, number of alpha-particle hits and total track length. The survival curves for all quantities are presented for the corresponding mean absorbed dose in order to facilitate comparisons of the expected effects of using the 3 different quantities for both distributions of ^{211}At decays. The theoretical survival curves presented here could, combined with experiments using "bound" and "non-bound" ^{211}At in a single-cell suspension, reveal which dosimetrical quantity is most suitable for ^{211}At-radioimmunotherapy.

1. INTRODUCTION

With the increasing interest in the use of the alpha-particle-emitter ^{211}At for targeted radiotherapy [1–4], various theoretical models of its biological effect have been presented. A traditional investigation into this biological effect would involve a comparison with the effect of a control low-linear-energy-transfer, (often ^{60}Co) radiation. An experiment to study the biological effect (often "cell death", e.g. loss of proliferation capability) should be linked with a calculation of the required specific energy for a specific target (e.g. the cell nucleus). This procedure should be performed for the two radiation qualities. This would then allow an estimate of the relative biological effect (RBE) between these radiation qualities.

Other approaches include attempts to correlate cell death with calculations of the number of alpha-particle traversals through the cell nuclei [5], as well as the more elaborate idea of including a variation in the biological effect with the varying linear-energy-transfer (LET) of the alpha-particle track [6].

Common for the above approaches is the expectancy of an exponential correlation between the biological effect of high-LET radiation and the dosimetric "quantity", whether this quantity is absorbed dose (or specific energy), number of traversals, total track length, or something else. Apart from being of general interest for the understanding of the biological impact of high-LET radiation, a parameter suitable for describing the radiation quantity is necessary for the thorough evaluation of the potential of ^{211}At-radioimmunotherapy.

Ideally, a study aimed at identifying a useful dosimetric quantity would consist of at least two parts. First, a fully controlled biological experiment. This would include knowledge of all cell and cell nuclei shapes and volumes, as well as the distribution of all radiation energy and all cells in the volume concerned in the experiment. Second, using the above information, a suitable theoretical treatment of the impact of the radiation on the biological target (e.g. cell nuclei) would allow coupling of a dosimetric quantity to the biological effect of this radiation.

The aim of this study was to clarify the dosimetrical implications of the various theoretical approaches, and to recommend biological studies that could lead to the identification of the most correct dosimetrical method. This would primarily include establishing true (i.e. not theoretical) cell

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survival curves based on the various theoretical assumptions discussed in this work, which could then prove valuable in finding a suitable dosimetrical quantity applicable for the clinical use of ^{211}At-radioimmunotherapy.

2. MATERIAL AND METHODS

2.1. Concept

A method of testing the accuracy of a certain theoretical approach in linking a specific dosimetric quantity to an expected biological effect would be to use the theory to predict "dose-response" curves. Such curves could then be compared with those established from experiments. The theory adopted in this study was used to predict cell survival curves when using 2 distributions for the origin of the ^{211}At decays, and 3 dosimetric quantities for the associated alpha particles. The "dose" in the "dose-response" curves will include either specific energy, number of alpha-particle hits, or total track length; all to the cell nuclei. The "response" could be any biological response due to the irradiation, but is here taken to be cell survival.

Two distributions for the origin of ^{211}At decays, both of importance for radioimmunotherapy, were considered in this study. One assumes the origin of the alpha particles to be homogeneously distributed over a cell surface, thereby simulating the effect of ^{211}At-labelled antibodies bound to antigens on cell surfaces. This distribution will be referred to as "bound ^{211}At". The other distribution mimics ^{211}At-labelled antibodies that are not bound to a cell. Here, the origin of the alpha-particle tracks will be homogeneously distributed in the space outside the cells. This distribution will be referred to as "non-bound ^{211}At". Both distributions are for single cells and should simulate *in vitro* experiments with well-dispersed single cells in suspension (i.e. sufficiently dispersed to ensure that the probability of an alpha particle hitting more than one cell nuclei is negligible).

Assuming that the correct quantity leads to identical survival curves for the two distributions discussed, there will necessarily be a separation of the curves when the "wrong" quantity is used. If, for example, specific energy is the correct quantity, then the dose-response curves would be identical for both "non-bound" and "bound" ^{211}At, provided that the specific energy to the nucleus can be determined accurately for both cases.

2.1.1. Absorbed dose

The most common way to present a cell-survival curve following irradiation of any kind is to relate the survival of the cells to the absorbed dose to these cells. For high-LET radiation, the biological response is expected to adhere to equation (1) [7]

$$S_1 = e^{-D/D_0} \tag{1}$$

where

S_1 is the cell survival (%),
D is the mean absorbed dose to the biological target (often the cell nuclei) (Gy),
and D_0 is the mean absorbed dose required for 37% cell survival.

Accepting the concept of autonomous cells, i.e. not accounting for a possible by-stander effect [8], allows a more detailed treatment of single cells

$$S_2 = \sum_{n=0}^{\infty} F_n e^{-Dn/D_0} \tag{2}$$

where

S_2 is the cell survival (%),
F_n is the fraction of cells being hit n times,
and D_n is the mean absorbed dose to the target when hit n times.

Finally, taking into account the total energy deposited in individual cells yields

$$S_3 = \sum_{n=0}^{\infty} F_n \int_{z=0}^{\infty} f_n(z) e^{-z/z_0} dz \quad (3)$$

where

S_3 is the cell survival (%),
$f_n(z)$ is the single- or multi- (n > 1) event distribution of specific energy to the target when hit n times,
z is the specific energy to the target (Gy),
and z_0 is the specific energy required for 37% cell survival (Gy).

2.1.2. Number of hits

Considering the high LET values of alpha particles, a simplified theory connecting the number of alpha-particle traversals through the biological target, i.e. a cell nucleus, could prove relevant. For this concept, two survival curves can be established. The first one would be

$$S_4 = e^{-H/H_0} \quad (4)$$

where

S_4 is the cell survival (%),
H is the mean number of hits on the cell nuclei,
and H_0 is the mean number of hits required for 37% cell survival.

The other survival curve would be more detailed

$$S_5 = \sum_{0}^{\infty} F_n e^{-n/H_0} \quad (5)$$

where

S_5 is the cell survival (%),
and n is the number of times a target has been hit.

2.2.3. Track length

This formalism will follow that of absorbed dose. The first expression is based on the mean track length

$$S_6 = e^{-C/C_0} \quad (6)$$

where

S_6 is the cell survival (%),
C is the mean alpha-particle track length through the target (μm),
and C_0 is the required track length for 37% cell survival (μm).

Following the same formalism, we obtain

$$S_7 = \sum_{n=0}^{\infty} F_n \int_{c=0}^{\infty} f_n(c) e^{-c/c_0} dc \qquad (7)$$

where

S_7 is the cell survival,
c is the specific alpha-particle track length through the target (μm),
$f_n(c)$ is the single- or multi- (n > 1) event distribution of specific track length when hit n times,
and c_0 is the required track length for 37% cell survival (μm).

2.2. Computer program

A Monte Carlo program was developed in the language C and run on a PC. A spherical cell (radius 7 μm) with a central nucleus (radius 5.6 μm) was simulated in a 3-dimensional co-ordinate system. The origin of the decays was randomly distributed throughout the space surrounding the cell ("non-bound ^{211}At"), or on the cell surface ("bound ^{211}At"). The two main alpha-particle energies were taken into consideration, i.e. 5.867 MeV (41.7% probability per ^{211}At decay) in the ^{211}At decay, and 7.450 MeV (58.3%) in the daughter, ^{211}Po, decay. The spatial directions of the alpha-particle tracks were set using a random number generator for two angles in spherical co-ordinates.

Range and stopping-power values for alpha particles in liquid water were taken from ICRU 49 [9]. For each 0.01 μm along the track, the program was set to register the tracks entering the cell nucleus. Once inside, the energy deposition along each 0.01 μm segment was calculated by multiplying the corresponding stopping-power value by the length of this segment. Finally, the total track length through the nucleus, as well as the energy imparted, was registered.

The program was tested by comparing the results with the analytically expected (i) energy imparted ("non-bound ^{211}At"), (ii) mean chord length ("bound ^{211}At"), and (iii) number of hits per decay ("bound ^{211}At"). For all runs, the deviation in any of these from the expected values was less than 0.2%.

3. RESULTS

3.1. Distribution of specific energy and track lengths

The Monte Carlo program was designed to consider only one cell in order to simulate a well-dispersed single-cell suspension. It was run until 500,000 hits to the cell nucleus were recorded for both distributions, i.e. bound and non-bound astatine. The energy imparted, as well as the alpha-particle track length, for each hit was recorded. This information was then used to establish the single-event distribution of specific energy (Fig. 1) and track length (Fig. 2) for the two distributions studied.

3.2. Dose-response curves

The dose-response curves will naturally be dependent on the single-cell radiosensitivity. For clarity, the "dose" required for 37% probability of survival for a single cell was set to the mean "dose" from a single alpha-particle traversal through the cell nucleus, originating from a homogeneous distribution of decays outside the cell.

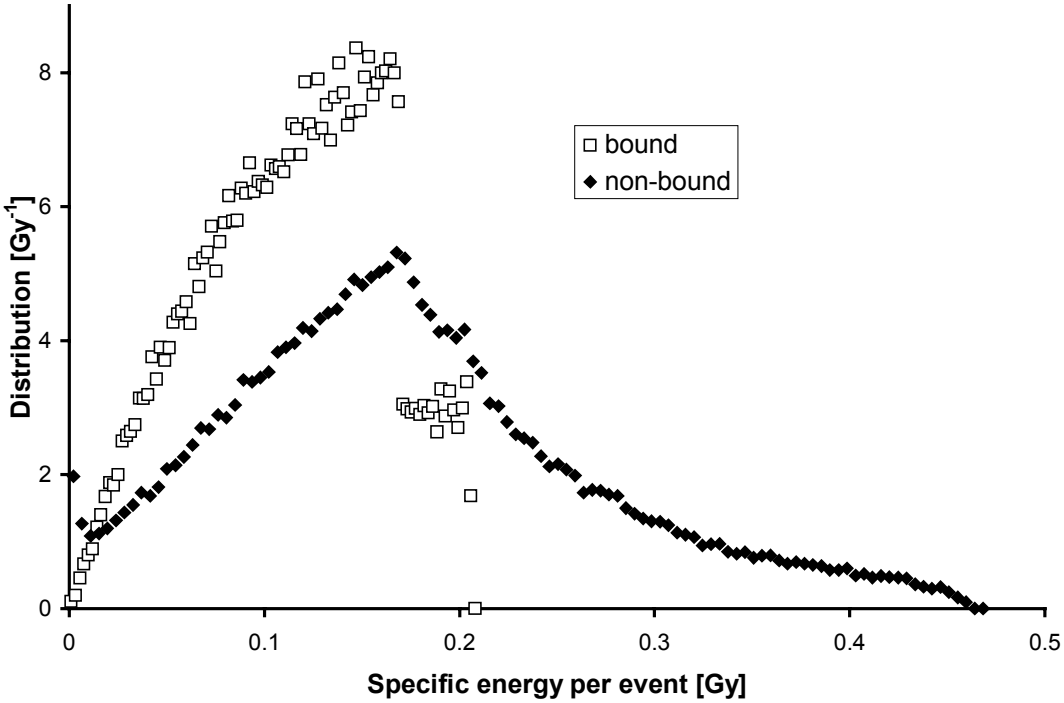

FIG. 1. Monte Carlo simulation of the single-event distribution of specific energy to a spherical cell nucleus (r = 5.6 μm) for alpha particles emanating from ^{211}At decays originating from "non-bound" ^{211}At (i.e. a homogeneous distribution outside the spherical cell (r = 7.0 μm)), and "bound" ^{211}At (i.e. a homogeneous distribution over the spherical cell (r = 7.0 μm)).

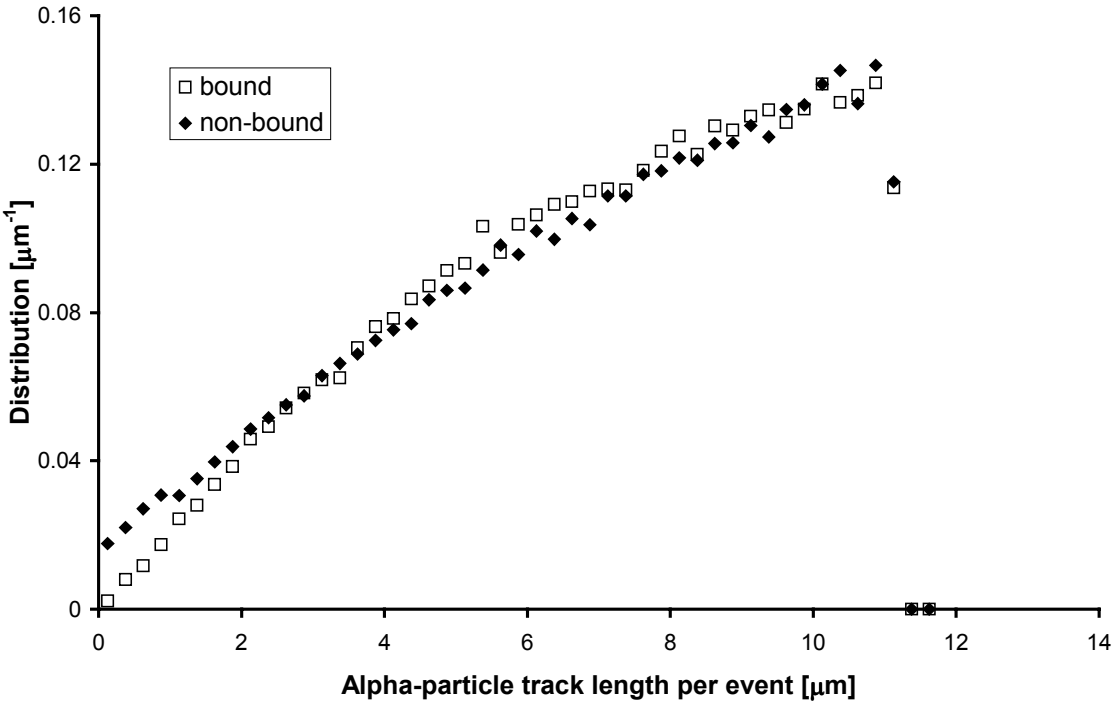

FIG. 2. Monte Carlo simulation of the single-event distribution of alpha-particle track length through a spherical cell nucleus (r = 5.6 μm) for alpha particles emanating from ^{211}At decays originating from "non-bound" and "bound" ^{211}At.

The mean absorbed dose required to reduce the cell survival to 37% was defined as being equal to the frequency-mean specific energy per event from non-bound decays. For a spherical cell (radius = 7.0 μm) with a central nucleus (r = 5.6 μm), the single-cell sensitivity was set to

- $D_0 = z_0 = 0.174$ (Gy),
- $H_0 = 1$ (hit), or
- $C_0 = 6.97$ (μm)

It should be noted that these prerequisites will, in themselves, be of importance when comparing the expected cell-survival curves established for each of the above dosimetric quantities. The requirement of a higher value of H_0 (i.e. several alpha-particle hits required for 37% cell survival) would, for example, gradually bring the cell-survival curve established according to Equation (3), closer to the curve established according to Equation (1). A single-cell alpha-particle sensitivity of H_0=1 is, however, a reasonable estimate [4].

The theoretical dose-response curves presented here (Figs 3–5) have been calculated according to Eqns (1–7) and then normalised for the corresponding mean absorbed dose to the cell nuclei. Using the mean absorbed dose to the cell nucleus for all the curves facilitates the comparison of the effects of each quantity (D, z, H, C, and c) on the expected cell survival. In addition, the mean absorbed dose has the advantage of being linearly related to the amount of ^{211}At involved in the irradiation of the cells. The use of mean absorbed dose is hence also advantageous as it reflects the situation in experiments where different levels of bound and non-bound ^{211}At radioactivity are added to the cells in order to establish cell-survival curves for both geometries.

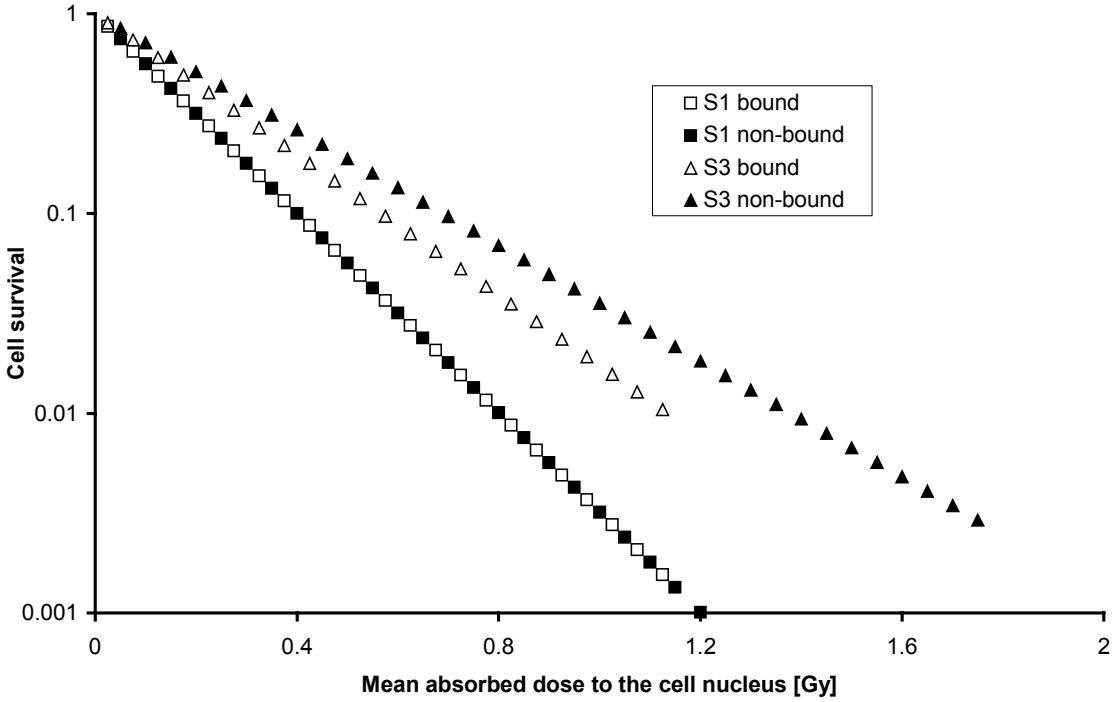

FIG. 3. Expected cell survival curves for ^{211}At decays originating from "non-bound" and "bound" ^{211}At when the dosimetric quantities from Equations (1) (S_1) and (3) (S_3) are used.

3.2.1. Dose-response curves if absorbed dose is the correct parameter for "dose"

When the calculations were corrected for mean absorbed dose it becomes apparent that the theoretical cell survival curves for S_3 deviated substantially from those of S_1 (Fig. 3).

For S_3, separate frequency distributions of specific energy, $f_n(z)$, were acquired for cell nuclei hit by 1 alpha-particle (n = 1), 2 particles (n = 2), etc. For an average of 1 hit to each cell nucleus from non-bound decays, the nuclei being hit 3 times will have an average distance to the origin of the decays about 1 μm shorter than those nuclei being hit only once. As the stopping power of an alpha-particle changes, this will affect the distribution of specific energy. The multi-hit distributions used for establishing the cell-survival curves in Fig. 3 were, however, simulated by sampling consecutive hits in order to save time. Like for those that have employed folding of the single-event distribution [10], this results in slightly incorrect multi-hit distributions.

The difference in cell survival curves for S_1 and S_3 is, however, mostly due to a stochastic variation in the number of cells being hit. One must remember that for an average of 1 hit per cell in a single-cell suspension, Poisson statistics mean 37% of the cells will not be hit at all, while 18% will be hit twice, and so on. These effects will "lift" the dose-response curves for both bound and non-bound ^{211}At.

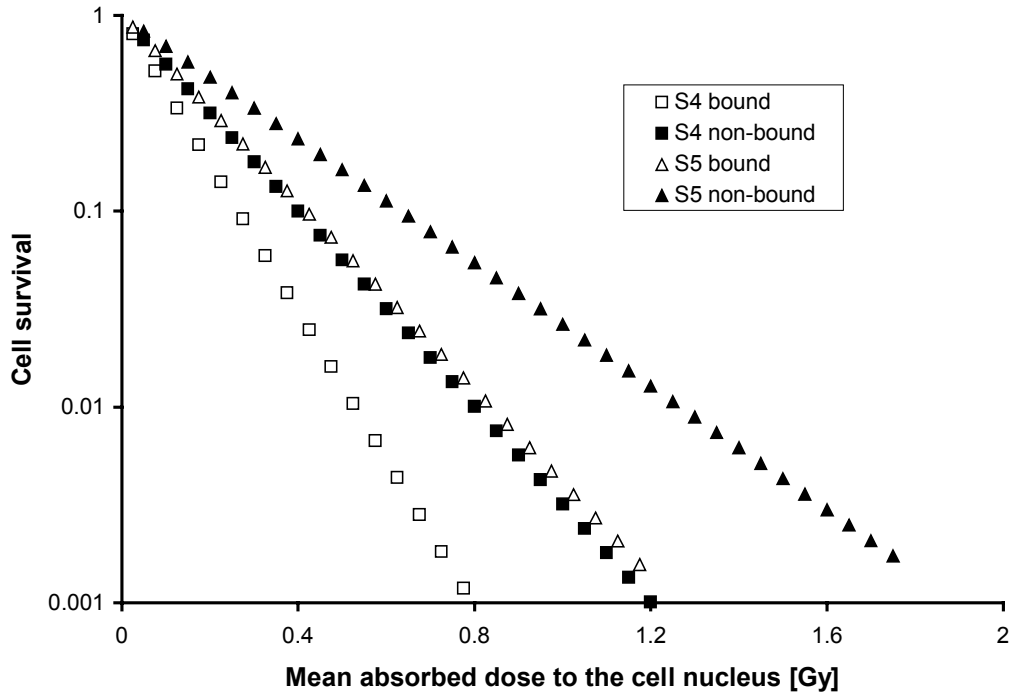

FIG. 4. Expected cell survival curves for ^{211}At decays originating from "non-bound" and "bound" ^{211}At when the dosimetric quantities from Equations (4) (S_4) and (5) (S_5) are used.

3.2.2. Dose-response curves if the number of alpha-particle traversals is the correct parameter for "dose"

With the set values (D_0 = 0.174 Gy, H_0 = 1 hit, etc.), the survival curve for non-bound ^{211}At according to S_4 is identical to that of S_1 (Fig 4). Again, for an average of 1 hit per cell nucleus, Poisson statistics predict that 37% of the cell nuclei will not be traversed by an alpha particle. As this is accounted for in S_5, its corresponding curve will be less steep.

For the same mean absorbed dose, more traversals are required from bound ^{211}At than from non-bound. This can be seen from the differences in the respective frequency distribution of specific energy per event (Fig. 1) for both bound and non-bound ^{211}At. Accounting for this effect results in steeper dose-response curves for bound ^{211}At.

3.2.3. Dose-response curves if the alpha-particle track length is the correct parameter

The idea of linking the length of an individual alpha-particle track through the cell nucleus to the biological effect could prove important as an intermediate between the two concepts discussed above. Dose-response curves established within this concept require knowledge of the distribution of track lengths through the target. The differences in this distribution for bound and non-bound ^{211}At (Fig. 2) will result in different dose-response curves for the two distributions.

3.3. Relative dosimetrical effect

The cell-survival curves discussed above can be used for comparisons of the required mean absorbed doses for 37% cell survival when calculated according to Eqns (1–7) (Table I). A comparison is made possible with the other methods (S_3–S_7) when S_1 is set at 0.174 Gy (the mean absorbed dose to the cell nuclei when hit, on average, once by alpha particles originating from a homogeneous distribution outside the cells).

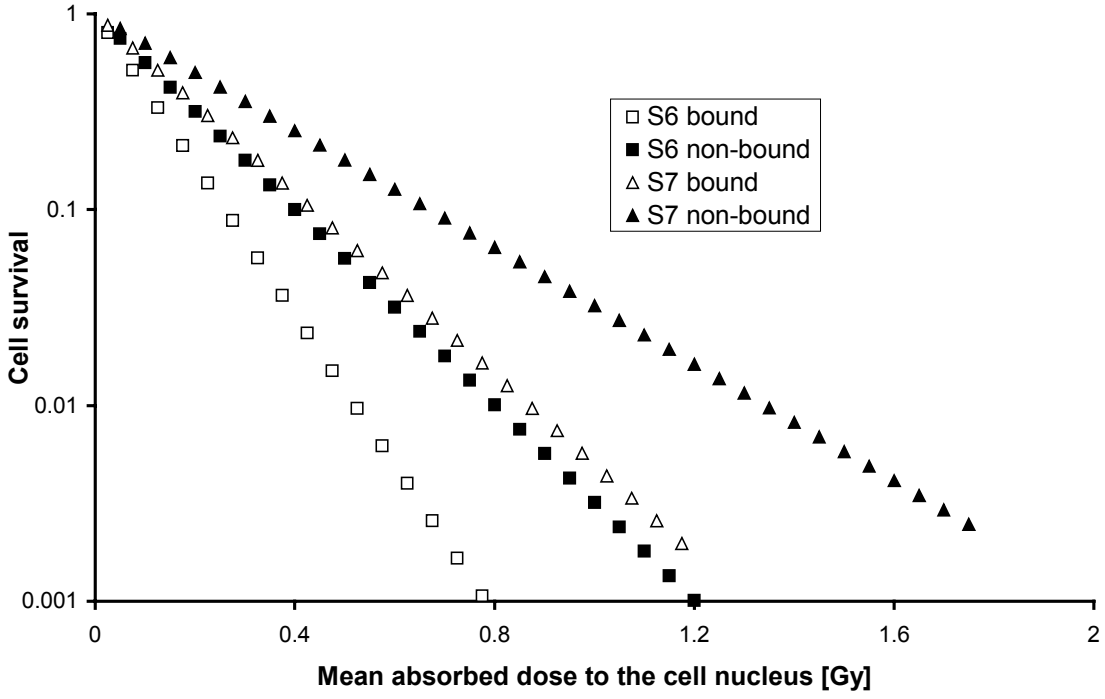

FIG. 5. Expected cell survival curves for ^{211}At decays originating from "non-bound" and "bound" ^{211}At when the dosimetric quantities from Equations (6) (S_6) and (7) (S_7) are used.

3.3.1. Absorbed dose

The specific energy deposited in an individual cell nucleus required for a 37% cell survival probability was set to 0.174 Gy. The mean absorbed dose from alpha particles emanating from cell-bound ^{211}At to all cell nuclei would then have to be 0.240 Gy (if calculated according to S_3) for 37% cell survival. With a homogeneous distribution of ^{211}At outside the cells, the same effect would require 0.300 Gy. The difference in required mean absorbed dose for the same biological effect can be explained by the difference in the frequency distribution of the specific energy per event for the two distributions of alpha-particle decay (Fig. 1).

3.3.2. Number of hits

With the value of H_0 set to 1 hit, it is possible to compare the mean absorbed doses required for 37% cell survival when using S_4 and S_5 to calculate the biological effect. For both S_4 and S_5, a lower mean absorbed dose to the cell nuclei is required for cell-bound ^{211}At than for a homogeneous distribution of non-bound ^{211}At outside the cells. This is due to the lower stopping-power values of alpha-particles of relatively high energy, as is the case when the cell nuclei are hit by alpha particles emanating from the cell surface, resulting in a lower mean absorbed dose per hit (Fig. 1). Taking into account the distribution of the true number of hits to individual cell nuclei (S_5) for each mean number of hits to all cell nuclei will result in a higher mean absorbed dose being required for 37% cell survival. This is mainly due to the inclusion of cell nuclei that will not have experienced an alpha-particle hit at all.

TABLE I. REQUIRED MEAN ABSORBED DOSES [Gy] TO CELL NUCLEI (r = 5.6 μm) FROM ASTATINE-211 DECAYS ORIGINATING FROM "NON-BOUND" At-211 (i.e. A HOMOGENEOUS DISTRIBUTION OUTSIDE SPHERICAL CELLS (r = 7.0 μm)), OR "BOUND" At-211 (i.e. A HOMOGENEOUS DISTRIBUTION OVER SPHERICAL CELLS (r = 7.0 μm)), FOR 37% CELL SURVIVAL. CALCULATIONS ACCORDING TO EQUATIONS 1–7

Position of decay	S_1 [a]	S_3 [a]	S_4 [b]	S_5 [b]	S_6 [c]	S_7 [c]
Bound ^{211}At	0.174	0.240	0.115	0.182	0.113	0.188
Non-bound ^{211}At	0.174	0.300	0.174	0.277	0.174	0.291

[a] Provided $D_0=z_0=0.174$ Gy.
[b] Provided $H_0=1$ hit.
[c] Provided $C_0=6.97$ μm.

3.3.3. Alpha-particle track length

For the calculation of cell survival according to S_6 and S_7, the mean alpha-particle track length through the cell nuclei from non-bound ^{211}At decay, i.e. 6.97 μm, was used as C_0. For non-bound ^{211}At, it is then apparent that the mean absorbed dose to cell nuclei required for a 37% cell survival according to S_6 will have to be the same as the required absorbed dose when using S_4. In this case, the mean number of hits is directly correlated to the mean track length through the cell nuclei. S_4 and S_6 will, however, not be correlated for bound ^{211}At. This is due to the slightly higher mean track length for this configuration. A mean value of 1 hit will result in a mean track length of 7.08 μm which will result in a higher biological effect than the set value of 6.97 μm. Hence, a somewhat lower absorbed dose, than when using S_4, will be required for 37% cell survival.

4. DISCUSSION

The results presented here can be used to identify a suitable dosimetric quantity for use in alpha-particle radiotherapy. Discrepancies between the biological effect of ^{211}At bound to various compounds and the effect of free ^{211}At or alpha particles from accelerator beams could be due to the use of a misleading dosimetric quantity. An experiment comparing the biological effect (e.g. cell death) of alpha-particle irradiation emanating either from bound and non-bound decay would be the starting point for this search.

In order to simplify the comparison of such experiments with the theory presented in this study, all cell-survival curves presented here are based on the mean absorbed dose to the nucleus. This quantity increases linearly with increasing ^{211}At activity, making a comparison with the theoretical curves possible without having detailed knowledge of all dosimetrical aspects.

Naturally, it would be best to compare the results from an experiment directly with each proposed dosimetric quantity. With the correct quantity, the dose-response curves would then be identical for both bound as well as non-bound decays of ^{211}At. This would, however, require full

knowledge of the true number of alpha-particle hits, the resulting specific energy, track length through the cell nucleus, etc. for many levels of "dose" for both bound and non-bound decays.

In this study, the target was assumed to be the whole nucleus of the cell. Many other studies have involved the search for a "primary target". Whether this is the cell, its nucleus, or the DNA (and one or more, more or less complicated lesions within) was not examined in this study.

Another concern is the delay between hits. In this study, no correction was made for a possible difference in biological effect for a certain energy imparted, if the energy was imparted immediately or in two or more fractions. Even if one takes into account a Poisson-like distribution of several hits for a cell survival curve (for autonomous cells), the impact of two time-separated alpha particles (e.g. 1 MeV imparted energy + 0.7 MeV) may not have the same biological effect as one event depositing 1.7 MeV.

It is also important to remember that the results presented here are only valid for the set values of the quantities discussed (cell size, shape, D_0, z_0, H_0, and C_0). Other attempts to predict the biological response to some irradiation include a variation of the biological effect of alpha-particle track segments with the different LETs of different segments [6]. The processes involved in the transfer of radiation energy to a cell, and the subsequent possibility of cell death is probably so complex that it is unlikely that a "true" and simple parameter exists. It would be more appropriate to search for a useful parameter, especially with reference to radioimmunotherapy and other targeting therapies using ^{211}At in the clinic. The results presented in this study should not be seen as the final treatment of all possible parameters, but serve as a warning signal when attempting to compare the biological effect of ^{211}At irradiation with some "dose" of this irradiation.

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MICRODOSIMETRY OF ASTATINE-211 AND COMPARISON WITH THAT OF IODINE-125

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Abstract. ^{211}At is an alpha and Auger emitter radionuclide and has been frequently used for labeling of different kind of chemical agents. ^{125}I is also known as an effective Auger emitter. The radionuclides which emit short range and high LET radiations such as alpha particles and Auger electrons have high radiotoxic effectiveness on the living systems. The microdosimetric data are suitable to clarify the real radiotoxic effectiveness and to get the detail of diagnostic and therapeutic application principles of these radionuclides. In this study, the energy and dose absorptions by cell nucleus from alpha particles and Auger electrons emitted by ^{211}At have been calculated using a Monte Carlo calculation program (code: UNMOC). For these calculations two different model corresponding to the cell nucleus have been used and the data obtained were compared with the data earlier obtained for ^{125}I. As a result, the radiotoxicity of ^{211}At is in the competition with ^{125}I. In the case of a specific agent labelled with ^{211}At or ^{125}I is incorporated into the cell or cell nucleus, but non-bound to DNA or not found very close to it, ^{211}At should considerably be much more radiotoxic than ^{125}I, but in the case of the labelled agent is bound to DNA or take a place very close to it, the radiotoxicity of ^{125}I should considerably be higher than ^{211}At.

1. INTRODUCTION

^{211}At as an alpha and Auger emitter radionuclide has an extremely high potential application in cancer therapy; particularly, in molecular radiotherapy. In the literature ^{211}At has been frequently used for labeling of different kind chemical agents. As some interesting examples, the chemical agents such as Cholesterol[1], tyrosine[2], 2-methyl-1,4-naphtoquinol bis(disodium phosphate) known as synkavit[3], tamoxifen[4], deoxyuridine[5] and others have been labelled with ^{211}At. According to the chemical characters of these carrying agents ^{211}At can be directed into the different localization zones in the living systems such as intracellular, cellular or nuclear zones (for a review see Ref. 6). As is known well, the radionuclides which emit short range and high LET radiations such as alpha and/or Auger electrons should be incorporated into the nucleus of cancer cells for resulting high radiotoxic effectiveness. Especially, it is experimentally observed by several authors that high radiotoxicity of ^{125}I as an effective Auger emitter was observed when it was incorporated into the structure of DNA or found very close to it [7–9]. This means that the dose distribution within the cell nucleus at the microscopic scale is very important for comparison of real differences between the radiotoxic effectiveness of different radionuclides. It is also important to outline that the microscopic dose calculation methods should consider the real cell nucleus composition. For this reason, such a calculation method should use the cell nucleus models having practically equal elemental composition. In the literature, the water models are generally used for these calculations. In reality, the cell nucleus differs from water in the lower oxygen content, which is replaced principally by carbon and nitogen. In this context, šnak[10] recently developed a dose calculation program for Auger electrons of ^{125}I within the cell nucleus (code: UNAKNUC). In this study, the same program has been adapted to the Auger electrons and alpha particles emitted by ^{211}At.

Figure-1 shows the decay scheme of ^{211}At. According to the data given by Stepanek et al.[8], 6.3 Auger electrons and 1 alpha particle are emitted by ^{211}At per a single decay. In reality, four different alpha particles of 5.98 MeV, 7.45 MeV, 6.89 MeV, 6.57 MeV with intensities of 0.418, 0.576, 0.0033, 0.0031, respectively, are emitted by ^{211}At and its very short half-lived daughter radionuclide ^{211}Po. The Auger electron spectrum of ^{211}At has been also given by the same authors.

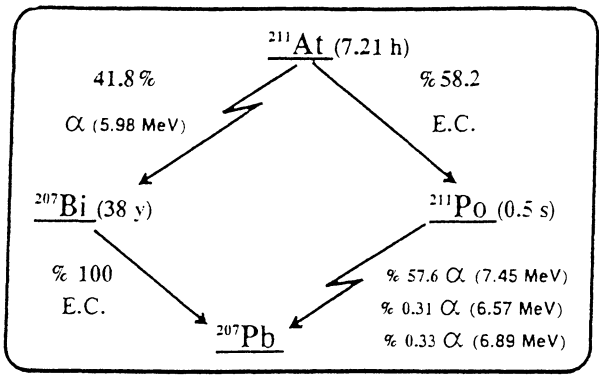

FIG. 1. Decay scheme of ^{211}At.

In this study, two different models of cell nucleus have been used for microdosimetric calculations of ^{211}At and these were compared with that of ^{125}I.

2. CALCULATION METHOD

2.1. Total energy absorption per a single decay of ^{211}At

The basic calculation method for microscopic dose distribution from Auger electrons in a cell nucleus per a single decay of ^{125}I placed at the nucleus center was earlier described in detail by šnak [10]. In that method a calculation program coded as UNAKNUK was used. The same calculation method has been also used in this study for the Auger electrons emitted during the decay of ^{211}At, and this method has been also adapted to the microscopic dose calculations from alpha particles. Finally, the total energy absorption by a cell nucleus per a single decay of ^{211}At could similarly be calculated. The principle of this method was based on the use of the real chemical compositions of radiation absorbing media. For this reason, the dose absorption differences between similar absorbing media, but having a little chemical composition difference can easily be distinguished by this calculation method. So, the energy absorption by a cell nucleus model representing the approximate elemental composition can be distinguished than that of a water model of cell nucleus. In the first model, a sphere of 4000 nm radius filled with a chemical material having approximately the real chemical composition of cell nucleus was used. In the second model, the same sphere was considered as filled only with water (Fig.-2).

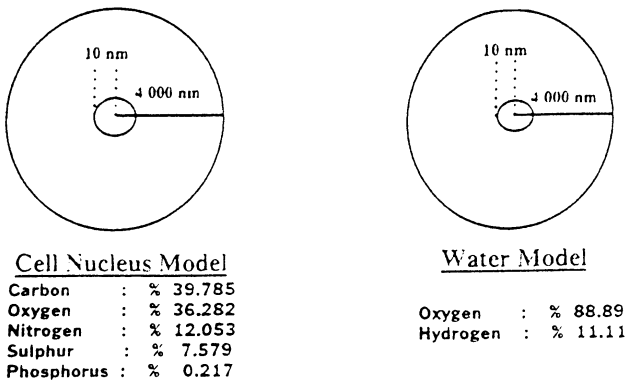

FIG. 2. Schematic representation of the cell nucleus models used in this study.

The Auger electron spectra given by Stepanek et al.[11] and Charlton and Booz [12] were used for ^{211}At and ^{125}I, respectively.

2.2 Monte Carlo calculations

Starting from the single radionuclide decay, a Monte Carlo calculation program (code:UNMOC) has been developed for cumulative dose absorption calculations by cell nucleus as a function of radionuclide decay time and radionuclide activity. The basic principle of these calculations is randomly distribution of radionuclides within the cell nucleus, and randomly determination of radionuclides decayed within the decay period considered. The number of radionuclides randomly distributed within the cell nucleus is, of course, depended on the corresponding radionuclide activity incorporated directly into the cell nucleus. For a given activity of ^{211}At and ^{125}I the dose absorptions as function of decay times have been calculated. For these calculations the position of each radionuclide within the nucleus has been randomly determined by the Monte Carlo program. The decayed radionuclides have been also randomly chosen by the same program. Then, the emission directions of each alpha particle or Auger electron have been again randomly determined and the distance covered by each particle within the nucleus has been found. So, the partial energy absorption per decay and the total absorption corresponding to the number of decayed radionuclides by the nucleus in a decay time have been calculated.

The accuracy of Monte Carlo data is, of course, depended on the number of the probability events repeated during the calculation procedures and the most accurate results can only be obtained when the number of probability events is as high as possible. For this reason, in this study the number of probability events was fixed as about 1000. Nevertheless, as is seen in Fig. 3, the data have practically been stabilized after about 300–400 events, and became sufficiently reliable.

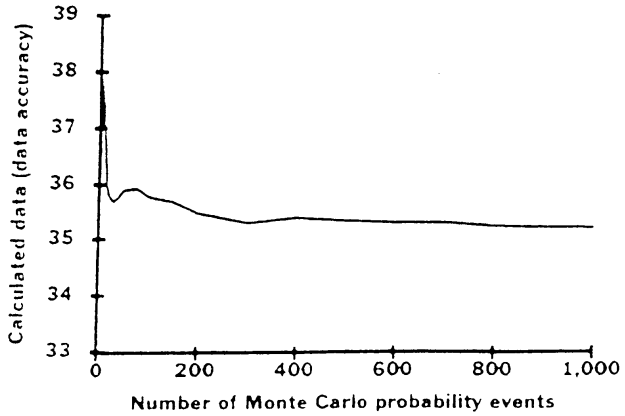

FIG. 3. Data accuracy as a function of Monte Carlo probability events repeated during the calculation procedures.

3. RESULTS AND DISCUSSION

3.1 Comparison of energy and dose absorptions by a cell nucleus per a single decay of ^{211}At or ^{125}I

Figure-4 shows the dose absorption in a cell nucleus as a function of distance from the decay center of a single ^{211}At or ^{125}I radionuclide just placed at the center of the nucleus. As is seen well, the dose absorption within the cell nucleus is not homogeneous and is very high at the decay vicinity, but rapidly decreases with the distance from the decay center. The absorbed dose by a spherical volume having the radius of about 10 nm that means at the DNA scale, is about 19382 Gy per a single decay of ^{211}At; but, this is much more higher for ^{125}I as being about 27042 Gy. Contrarily, the total energy absorption by the whole nucleus is 27.62 mGy for ^{211}At while this is about 5.65 mGy for ^{125}I.

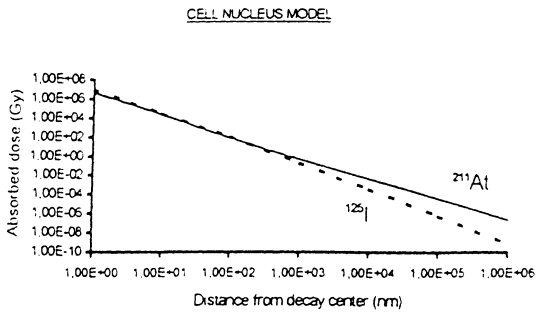

FIG. 4. Dose absorption variations as a function of distance from decay center of ^{211}At or ^{125}I radionuclides.

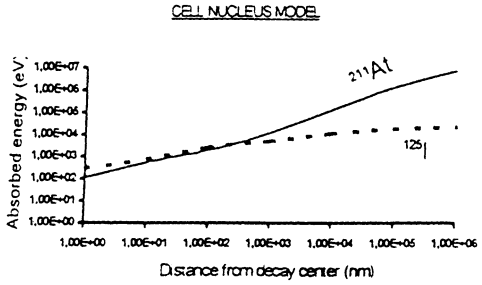

FIG. 5. Energy absorption variations as a function of distance from decay center of ^{211}At and ^{125}I radionuclides.

Figure 5 shows the similar energy absorptions from ^{211}At and ^{125}I. The total energy absorbed by the whole nucleus is about 50.84 keV for ^{211}At and 10.40 keV for ^{125}I. At the DNA scale these are 557 eV and 777 eV for ^{211}At and ^{125}I, respectively.

3.2 Comparison of the partial influence of alpha particles and Auger electrons of ^{211}At

The data given in Figs 4 and 5 are the total energy and dose values of ^{211}At. During the decay of this radionuclide alpha particles and Auger electrons are emitted simultaneously. Either alpha particles or Auger electrons are short range and high LET radiations. For this reason, it is difficult to judge which is more effective on the radiotoxicity of ^{211}At. Figs 6 and 7 show the individual influence of alpha particles and Auger electrons of ^{211}At. As is seen, the alpha particles are much more effective than the Auger electrons for whole cell nucleus; but contrarily, the influence of Auger electrons are more effective than alpha particles at the DNA scale.

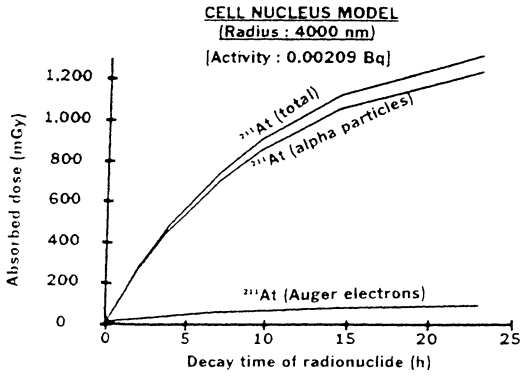

FIG. 6. Partial and total dose absorptions from alpha particles and Auger electrons of ^{211}At in a cell nucleus of a radius 4000 nm.

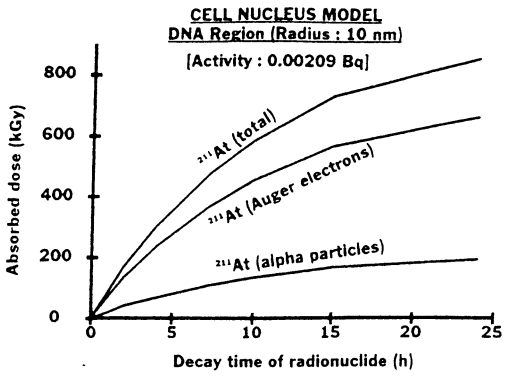

FIG. 7. Partial and total dose absorptions from alpha particles and Auger electrons of ^{211}At in a spherical volume of 10 nm radius corresponding to the DNA region.

3.3 Dose absorption data as a function of decay time of radionuclide

In practical applications many radionuclides can simultaneously be incorporated into the cell nucleus and are, of course, non-homogeneously distributed within the nucleus depending on the chemical properties of carrying radiopharmaceuticals. For this reason, the total dose absorption as a function of decay time or activity of the radionuclide is important for diagnostic and therapeutic applications. Figs 8 and 9 show the variations of dose absorption for having the equal activity of

0.00209 Bq in the first 24 h for whole nucleus and DNA scale. This activity corresponds to the ^{125}I activity which has been given by Humm and Charlton [13] as the initial activity per cell, to reduce the cell population to 37 % survival, and produces an average 100 single strand break (dsb) within two cell divisions (48 h). As is seen, the dose absorption increases rapidly as a function of decay time either for ^{211}At or ^{125}I, but after 24 h dose absorption rate is considerably slowing down for ^{211}At, while it is continuing to increase for ^{125}I. It is also important to outline that at the DNA scale, i.e. in a sphere of 10 nm radius the dose absorption from ^{125}I is considerably higher than that of ^{211}At.

3.4 Comparison of data for cell nucleus and water models

The energy absorption per a single decay of ^{211}At or ^{125}I, and the dose absorption as a function of decay time have been also repeated using the water model corresponding to a cell nucleus. As is noted in the introduction section, the water vapor or liquid water has been generally used in the literature as a radiation absorbing medium corresponding to cell or cell nucleus structure (for a short review of these microdosimetric approaches see the introduction section of Ref. 10). The comparison of our cell nucleus model with the water model was earlier done by Ünak [10] for ^{125}I. In this study the similar comparison was also repeated for the dose absorption calculations of ^{211}At. Figs 10 and 11 show these comparisons. Briefly, the chemical structure of water has the ability of higher energy absorption from the radiation than the real cell nucleus structure, while its density is slowly lower than that of a cell nucleus structure. Of course, this is a result of a lower oxygen content of cell nucleus than water, which is replaced principally by carbon and nitrogen.

4. CONCLUSION

The data obtained in this study show clearly that the radiotoxicity of ^{211}At as known as an alpha and Auger emitter radionuclide is in the competition with that of ^{125}I. In the case of a specific agent labelled with ^{211}At or ^{125}I is incorporated into the cell or cell nucleus, but non-bound to DNA or not found very close to it, ^{211}At should considerably be much more radiotoxic than ^{125}I, but in the case of the labelled agent with ^{211}At or ^{125}I is bound to DNA or take a place very close to it, the radiotoxicity of ^{125}I should considerably be higher than that of ^{211}At (see Figs 8 and 9).

On the other hand, in practical diagnostic and therapeutic applications of a radionuclide the critical dose calculations should be done using as really as possible chemical compositions of the target materials of body, if not, the serious mistakes between the real and calculated doses will be inevitable.

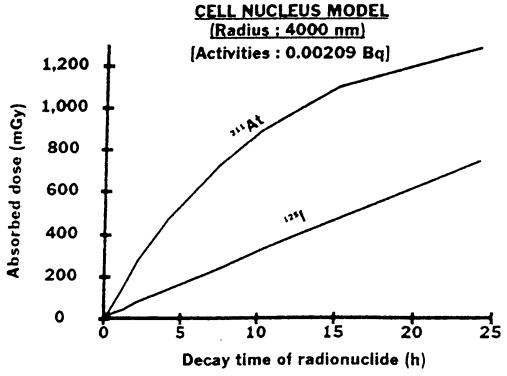

FIG. 8. Comparison of dose absorptions from ^{211}At and ^{125}I in a cell nucleus of 4000 nm adius.

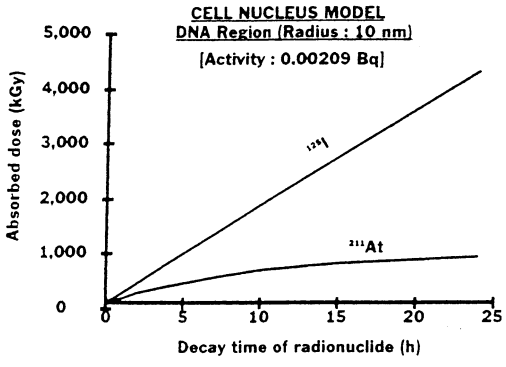

FIG. 9. Comparison of the dose absorptions from ^{211}At and ^{125}I in a spherical volume of 10 nm radius corresponding to the DNA region.

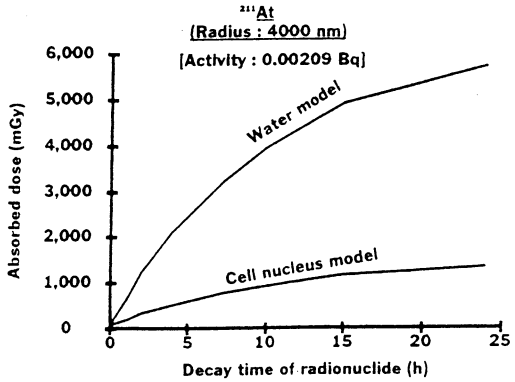

FIG. 10. Comparison of dose absorptions from ^{211}At in a cell nucleus of 4000 nm radius for two different cell nucleus models.

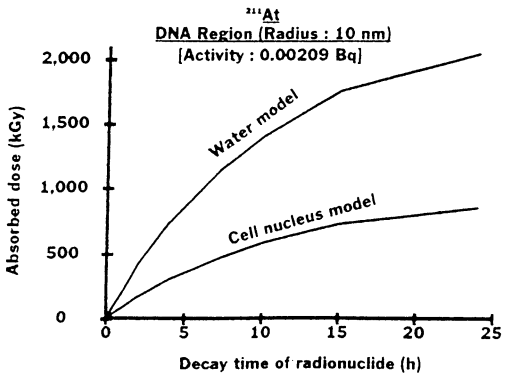

FIG. 11. Comparison of dose absorptions from ^{211}At in a spherical volume of 10 nm radius corresponding to the DNA region for two different cell nucleus models.

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ASTATINATED RADIOPHARMACEUTICALS FOR TARGETED ALPHA PARTICLE RADIOTHERAPY*

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Abstract. The radionuclides generally used for targeted radiotherapy such as ^{131}I and ^{90}Y, emit ∃-particles whose range in tissue is of the order of several millimeters. Alpha particles, on the other hand, traverse only a few cell diameters; thus, targeted alpha particle therapy would be ideally suited for micrometastases, tumours of circulation such as lymphoma, and compartmental-grown tumours such as cystic brain tumour, ovarian cancer and neoplastic meningitis. For example, it has been calculated that the absorbed fraction ratio for ^{211}At alpha particles, compared with ^{90}Y ∃-particles, increases from 9:1 for 1-mm diameter tumours to 33:1 for 0.2 mm diameter ones. Although a number of ∀-particle-emitting radionuclides exist, the properties of ^{211}At make it perhaps the most attractive candidate ∀-emitter for radiotherapy. Because ^{211}At is a halogen, radioiodination chemistry can be adapted for astatination. This paper describes the production of ^{211}At, as well as the preparation and evaluation of astatinated radiopharmaceuticals such as monoclonal antibodies and the 5-iododeoxyuridine analogue AUdR.

1. INTRODUCTION

An important point in selection of an appropriate therapeutic radionuclide is the spatial configuration (size and geometry) of the tumour. Iodine-131 and ^{90}Y are the most commonly used radionuclides for targeted radiotherapy. These emit high energy ∃-particles, the mean range in tissue of which is several millimeters. Alpha particles on the other hand, would traverse only a few cells. Theoretical calculations have shown that absorbed fraction ratio for ^{211}At ∀-particles, compared with ^{90}Y ∃-particles, increases from 9:1 to 33:1 as tumour diameter decreases from 1mm to 0.2 mm [1]. Thus, targeted alpha particle therapy could be ideally suited for micrometastases, tumours of circulation such as lymphoma and tumours with sheet-like geometry such as cystic brain tumours, ovarian cancer and neoplastic meningitis. Alpha particles have several radiobiological advantages such as high linear energy transfer (LET) and relative biological effectiveness (RBE), marginal dependence on dose rate, and an oxygen enhancement ratio close to unity. Although more than 100 ∀-particle emitting radionuclides exist, only ^{212}Bi, ^{213}Bi and ^{211}At have received serious attention. Astatine-211 decays by a double branched pathway resulting in the emission of one alpha particle per disintegration having an average energy of 6.8 MeV and 55–70 :m range. The LET of ^{211}At μ-particles is about 100 keV/:m at which maximum RBE occurs. Recently it has been shown that it is possible to externally image tissue distribution of ^{211}At by nuclear medicine techniques including SPECT [2]. This capability will be valuable for optimizing treatment strategies as well as determining tumour and normal tissue radiation dosimetry. Being a halogen, more often than not, radioiodination chemistry is adaptable for astatination. These facts have led to the development of a number of astatinated radiopharmaceuticals as potential agents for targeted radiotherapy [3, 4].

2. PRODUCTION OF ^{211}At

Astatine-211 is produced by the cyclotron bombardment of natural bismuth metal targets with 28 MeV α-particles using the ^{209}Bi(α,2n)^{211}At reaction. Until recently, an external target was used; however, the yields were not sufficient for clinical investigations. Using an internal target, ^{211}At could be produced in high yields and purity [5]. Production efficiency of 41 ± 7 MBq/:A≅h has been obtained routinely with this target. Up to 4 GBq of ^{211}At has been produced to date, a level that has permitted initiation of a clinical trial with ^{211}At at our institution.

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3. ASTATINATED MONOCLONAL ANTIBODIES

3.1. Astatination of mAbs

Combining the tumour cell specificity of monoclonal antibodies (mAbs) with ∀-particle-emitting radionuclides is an area which has received serious consideration in targeted therapy. Unlike radioiodination, it is not possible to astatinate proteins and antibodies in a stable form by direct electrophilic substitution. The first successful attempt to astatinate mAbs was made using *para*-[^{211}At]astatobenzoic acid (PABA) which was coupled to proteins via a mixed anhydride route [6]. Although this method resulted in astatinated mAbs with improved *in vivo* stability, radiochemical yields and specific activities were less than desired; the preparation of PABA itself was cumbersome. At Duke, a method was developed a decade ago to radioiodinate mAbs with excellent *in vivo* stability [7]. This utilized a conjugation agent, *N*-succinimidyl 3-[$^{125/131}$I]iodobenzoate (SIB) which was prepared from a tin precursor. It was possible to prepare the astato analogue, *N*-succinimidyl 3-[^{211}At]astatobenzoate (SAB) starting from the same tin precursor in excellent yields [8]. Monoclonal antibodies could be astatinated with [^{211}At]SAB under very mild conditions in excellent yields and specific activities [9] (Figure 1). The resultant astatinated mAbs retained affinity and immunoreactivity and were stable *in vivo*.

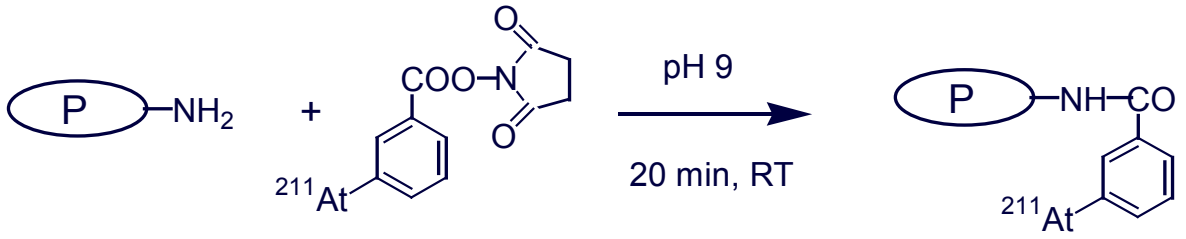

FIG. 1. Conjugation of mAbs with [^{211}At]SAB.

3.2. Tissue distribution of labelled mAbs

To investigate the potential utility of astatinated mAbs, chimeric mAb 81C6 was astatinated using [^{211}At]SAB. Murine 81C6 is an IgG_{2b} mAb which reacts with the extracellular matrix antigen tenascin that is present on gliomas and other tumors, but not on normal brain tissues. The human/mouse chimeric 81C6 mAb was constructed by linking the variable regions of murine 81C6 to human IgG_2 constant regions [10]. A paired-label tissue distribution of chimeric mAb 81C6 labelled with [^{211}At]SAB and [^{131}I]SIB was performed in athymic mice bearing D54-MG human glioma xenografts. The tumour uptake of ^{211}At increased from about 5% ID/g at 0.5 h to about 20% ID/g at 16 h, and remained constant thereafter (Figure 2). Up to 16 h, the tumour uptake of both ^{131}I and ^{211}At was similar. Tumour uptake of ^{131}I from 16 through 48 h was 10–39% higher ($p < 0.05$) than that of ^{211}At. Uptake of both nuclides in blood and other normal tissues decreased gradually. With the exception of the spleen and stomach, ^{211}At- and ^{131}I-labelled mAbs had similar uptake in all normal tissues over 48 h period. The ^{211}At/^{131}I ratios seen in this study were similar to those reported for another mAb in normal mice [11]. Taken together, these results suggest that astatinated intact mAbs behave, to a considerable degree, like their radioiodinated analogues.

3.3. Radioimmunotherapy

As mentioned above, targeted α-particle therapy should be well-suited for the treatment of neoplastic meningitis, a disease characterized by the leptomeningial spread of a variety of tumours within the cerebrospinal fluid compartment. Therapy experiments were carried out using the astatinated murine 81C6. A rat model was used for this study. Neoplastic meningitis was initiated by injecting TE-671 human rhabdomyosarcoma cells via a subarachanoid catheter [12]. Treatment was initiated 8 days after implantation of $5 \cdot 10^5$ cells, or 4 days after implantation of $6 \cdot 10^6$ cells. Three experiments — 2 eight days after and 1 four days after — were done with all reagents given via the

indwelling catheter. In the first experiment, groups of 9–10 rats were treated with saline or a single dose of 148 kBq, 259 kBq, and 481 kBq of ^{211}At-labelled 81C6. In the second experiment, animals received either saline, 444 kBq of ^{211}At-labelled nonspecific mAb, 45.6, 444 kBq or 666 kBq of ^{211}At-labelled 81C6. The third experiment, performed after 4 days of implantation, included both a saline control group and groups given 666 kBq of ^{211}At-labelled 81C6 or ^{211}At-labelled 45.6. Statistical analysis of survival data was performed using the Wilcoxon rank sum test, and $p < 0.05$ was considered to be significant.

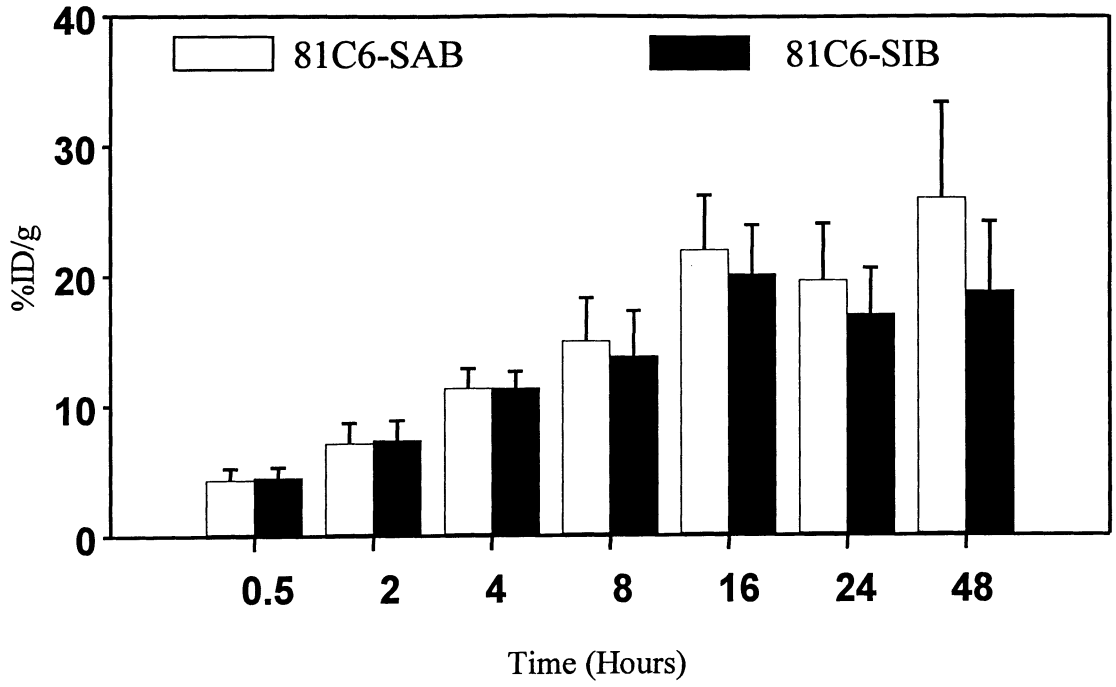

FIG. 2. Paired-label tumour uptake of chimeric 81C6 labelled with [^{131}I]SIB and [^{211}At]SAB in athymic mice hosting D-54 MG human glioma xenografts

In the first experiment, the median survival for the saline control group was 22.5 days compared with 30, 29, and 34 days for 148, 259, and 481 kBq, respectively. The prolongation in median survival for all doses was statistically significant ($p = 0.004$–0.02) compared to saline control; but difference between 148 and 259 kBq was not. Two rats from 481 kBq group and one from 259 kBq group was alive at 190 days with no evidence of disease. Results from the second study showed that, although compared with saline treatment there was a 33% (32 days vs 23.5 days) increase in the median survival for control mAb, the difference was not statistically significant. In contrast, treatment with the same amount of ^{211}At-labelled 81C6 increased median survival by 113%, which was significant compared to both saline and nonspecific mAb. With 666 kBq of ^{211}At-labelled 81C6, the median survival was 84 days, a 357% survival prolongation. In comparison, even with 11.1 MBq of ^{131}I-labelled Mel-14 F(ab')$_2$ fragment (another tumour reactive mAb), only 12% survival prolongation was noticed in this model [13]. There were 1 of 10, 0 of 9, 3 of 9, and 5 of 10 long-term survivors in the saline, 444 kBq 45.6, 444 kBq 81C6, and 666 kBq 81C6 groups, respectively, when the experiment was terminated on day 295. In the experiment with higher tumour burden, the median survival was 15 days for the saline control and 19 and 23 days for 666 kBq or 45.6 and 81C6, respectively. The increase in median survival observed with 81C6 was statistically significant ($p < 0.001$) compared with both the saline and nonspecific mAb controls. Taken together, these results

indicate that the therapeutic benefit of ^{211}At-labelled 81C6 is specific and is considerably more effective.

4. META-[^{211}At]ASTATOBENZYLGUANIDINE (MABG)

Radioiodinated *meta*-iodobenzylguanidine (MIBG), an analogue of the neurotransmitter norepinephrine, has been used for the detection and therapy of neuroendocrine tumours such as neuroblastoma. Since micrometastases are often associated with neuroblastoma, an astatinated analogue of MIBG should be advantageous for the treatment of such diseases. We have developed a synthetic method for the preparation of MABG and have evaluated its potential as a therapeutic agent. Details can be found in an accompanying paper in these proceedings.

5. 5-[^{211}AT]ASTATO-2'-DEOXYURIDINE

Being a thymidine analogue, 5-iodo-2'-deoxyuridine (IUdR) can be incorporated into the DNA of rapidly dividing cells. When labelled with very short range, high LET Auger electron-emitting ^{123}I or ^{125}I, IUdR is extremely cytotoxic to cells undergoing division. However, the strength of [^{125}I]IUdR as a radiotherapeutic agent, its specificity for rapidly dividing cells, is also its most severe limitation: those tumour cells not undergoing DNA synthesis are not subjected to its cytotoxic effects. It is thus desirable to have an agent which could act with high LET effects not only on the cells incorporating the agent into DNA, but also to those adjacent ones not in S-phase.

As a consequence of its α-particle emission, ^{211}At also emits another type of high LET radiation, short-range α-particle recoil nuclei. The mean range of these is 0.092 :m, and their mean LET is about 8 times higher than that of their α-particles. It was hypothesized that an astatinated IUdR analogue might be lethal not only to cells undergoing DNA synthesis, but also to those not in S-phase due to bystander killing. It has been predicted that the D_0 level of cell kill with an astatinated analogue of IUdR would be achieved with as low as 1 decay/cell [14]. To investigate this, an efficient method for the synthesis of 5-[^{211}At]astatodeoxyuridine (AUdR) was first developed and then the cytotoxicity of this potential therapeutic agent was studied.

5.1. Synthesis of AUdR

Two methods have been reported for the synthesis of AUdR. In the first, AUdR was prepared via a diazonium intermediate [15]. The radiochemical yield was unacceptably low. Although higher yields were obtained starting with a mercury precursor, the preparation involved iodine carrier [16]. Recently, radioiodinated IUdR has been prepared from a tin precursor [17]. It was possible to convert the same tin precursor to AUdR in 85–90% radiochemical yield by sonicating it for less than a minute with hydrogen peroxide/acetic acid mixture (Figure 3). AUdR was isolated by reverse-phase HPLC in high chemical and radiochemical purity.

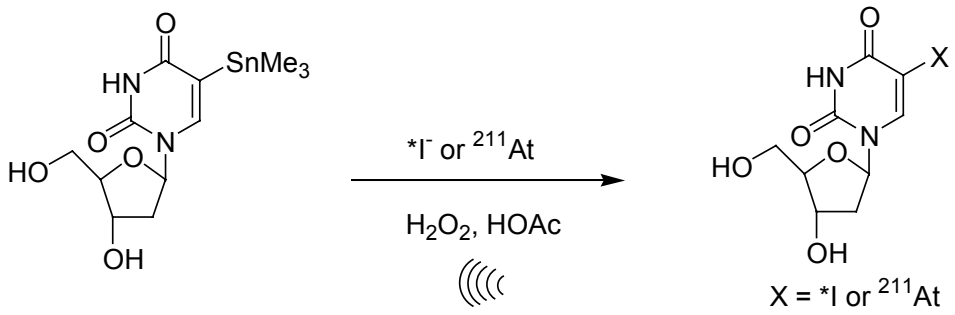

*FIG. 3. No-carrier-added synthesis of [*I]IUdR and AudR.*

5.2. In vitro stability of AUdR

The lack of stability of astatinated radiopharmaceuticals is a major concern. With the exception of ^{211}At-labelled intact mAbs, most astatinated compounds exhibit less than ideal stability in tissue culture and animal models. The intrinsic bond strength of carbon-astatine bond is the least of all carbon-halogen bonds. In addition, due to the high LET nature of ∀-particles, radiolytic degradation is another problem to be reckoned with. IUdR itself is not very stable *in vivo*, with half-life of the order of minutes. This is as a result of the cleavage of its *N*-glycosidic bond by nucleoside phosphorylases. We compared the *in vitro* stability of AUdR with that of [^{125}I]IUdR in tissue culture medium at 37^{o}C as a function of time. Purity at various time points was determined using HPLC. The per centage of ^{125}I and ^{211}At present as intact tracer declined with time. Catabolism appeared to be exclusively due to direct dehalogenation as no 5-halouracil was detected. AUdR was less stable than [^{125}I]IUdR. For example, when normalized to initial purity, 94% and 87% of [^{125}I]IUdR and AUdR, respectively, was present as intact tracer after a 6 h incubation. A similar trend was seen in serum. At 24 h after incubation, 91% of [^{125}I]IUdR was present as intact tracer; in comparison, the amount of AUdR present in intact form at this time was 87%. In this case, 5-halouracil was detected as a catabolite. Although AUdR appears to be slightly less stable than IUdR *in vitro*, the difference in stability between the two was not high.

5.3. Cellular uptake and DNA incorporation

IUdR and its bromo analogue, 5-bromo-2'-deoxyuridine (BUdR) are derivatives of thymidine obtained by replacing the methyl group of thymidine by iodine and bromine, respectively. Both IUdR and BUdR behave remarkably like thymidine. This is probably because the van der Waal's radii, and thus the size, of iodine and bromine are similar to that of the methyl group (2.15 Å and 1.95Å, respectively versus 2.0 Å) that they have replaced. The van der Waal radius of astatine has been estimated to be 2.3. This suggests that ^{211}At for methyl group substitution may alter the molecular properties in AUdR. To investigate this, the cellular uptake and DNA incorporation of IUdR and AUdR was determined *in vitro* in a paired-label format as a function of radioactivity concentration. Because the treatment of brain tumours is one of the most likely applications of AUdR, a human glioma cell line, D-247 MG, was used for these studies. Briefly, exponentially growing cells were incubated with varying activity concentrations of both [^{131}I]IUdR and AUdR for a period of 24 h. To determine the nonspecific uptake, a parallel assay was performed wherein the cells were co-incubated with 10 :M unlabelled IUdR. The cell-associated activity was determined at the end of the incubation period. Simultaneously, the per centage of cell-associated activity incorporated into DNA was determined following a literature protocol [18].

The uptake of both tracers increased linearly with increasing initial activity concentration. Furthermore, the presence of unlabelled IUdR decreased their uptake considerably, indicating competitive uptake has occurred. These results are qualitatively similar to those reported previously for [$^{123/125}$I]IUdR in V79 cells [19]; the magnitude of uptake, however, was less than reported for V79 cells. This discrepancy could be due to a number of factors. Since these tracers are taken up by cells in the S-phase, the differences in properties such as doubling time of the two cell lines (20 h vs 9 h) are critical. The assay conditions were also different (monolayer vs single-cell suspension). The level of DNA incorporation of [^{131}I]IUdR and AUdR was found to be similar. For example, at an activity concentration of 7.5 kBq/ml, the per centage of cell-associated activity incorporated into DNA was 55 $\pm$ 21% and 55 $\pm$ 13% for [^{131}I]IUdR and AUdR, respectively. These results indicate that ^{211}At for iodine substitution did not result in a significant alteration in cell uptake and DNA incorporation.

5.4. In vitro cytotoxicity

Cytotoxicity experiments were carried out by treating exponentially growing D-247 MG glioma and SK-MEL-28 melanoma cells (5×10^6 cells per flask) in 25-cm^2 flasks with varying concentrations of AUdR, [^{211}At]astatide or [^{125}I]IUdR for 2 or 20 h [20, 21]. At the end of incubation, cells were

trypsinized and washed and plated in triplicates. After 10 days, colony formation was determined. Colonies with more than 50 cells were scored as survival. Clonogenic survival was plotted as a function of the activity concentration present in the medium at the beginning of the incubation. Regression fits and 95% confidence intervals were determined using the Sigma Plot computer program.

The D_0 values calculated for the treatment of D-247 MG glioma and SK-MEL-28 melanoma cells for 2 and 20 h with both free [^{211}At]astatide and AUdR are shown in Table 1. From 20 h treatment it was shown that for both cell lines, the D_0 was about 2-fold higher for [^{211}At]astatide than for AUdR. For the 20 h treatment with AUdR, the D_0 calculated for D-247 MG cells was significantly lower than that for SK-MEL-28 cells. The number of cell-associated ^{211}At atoms needed for reduction in survival to 37% for both cell lines was about 2, which is equivalent to about one DNA-incorporated ^{211}At atom per cell.

TABLE I. D_0 VALUES CALCULATED FROM THE TREATMENT OF D-247 MG GLIOMA AND SK-MEL-28 MELANOMA CELLS WITH AUdR AND [^{211}At]ASTATIDE FOR 2 AND 24 h

Tracer	D_0 (kBq/ml)[a]			
	D-247 MG		SK-MEL-28	
	2 h	20 h	2 h	20 h
AUdR	33 (28–43)	15 (13–16)	132 (109–176)	17 (16–19)
^{211}At$^-$	132 (109–76)	28 (26–29)	125 (83–191)	29 (22–26)

[a]Mean with 95% confidence interval.

Since the majority of ^{211}At decay occurred during the incubation period and the range of its ∀-particles is more than a cell diameter, cytotoxicity related to the α-particles should be similar for [^{211}At]astatide and AUdR. Thus, the enhanced cytotoxicity of AUdR compared with [^{211}At]astatide may be related in part to sub-cellular range ∀-particle recoil radiations hitting the cell nucleus as a result of DNA-incorporation of AUdR. For the 2 h treatment, the data indicate that there is a significant difference in survival after treatment with AUdR compared with [^{211}At]astatide for the D-247 MG but not for the SK-MEL-28 cell line.

The clonogenic survival of D-247 MG and SK-MEL-28 cell lines after a 20-h treatment with [^{125}I]IUdR resulted in biphasic curves with little increase in cell kill with >150–200 kBq/ml. The D_0 values determined were 115 and 310 kBq/ml for D-247 MG and SK-MEL-28 cell lines, respectively, corresponding to about 3700 and 5000 ^{125}I bound atoms per cell. No significant reduction in survival was seen when the two cell lines were exposed to up to 200 kBq/ml of Na[^{125}I]I suggesting low cytotoxicity of extracellularly distributed ^{125}I activity. These results suggest that AUdR is more cytotoxic *in vitro* to these two cell lines than [^{125}I]IUdR.

AUdR was also found to be extremely cytotoxic to Chinese hamster V79 cells [22]. The extracellular concentration of AUdR (30 min exposure) causing reduction in survival to 37% was 7.3 kBq/ml. Since exposure of these cells to similar concentrations of ^{211}At$^-$ did not lead to any significant reduction in survival, the decrease in survival as a result of exposure to AUdR can be ascribed solely to the effects of DNA-incorporated activity. The cytotoxicity of AUdR in V79 cells was accompanied by considerable DNA damage as demonstrated by measurement of DNA double strand breaks (DSBs). Approximately 10-fold more DNA DSBs are produced per decay when cells are labelled with ^{211}At than when they are labelled with ^{125}I, further demonstrating the exquisite cytotoxicity of AUdR.

5.5. Tissue distribution of AUdR and [^{125}I]IUdR

It is known that following i.v. administration, [^{125}I]IUdR is rapidly degraded, mainly to free iodide, limiting its usefulness for applications where compartmental or intratumoural delivery is possible [23]. To determine whether AUdR, like [^{125}I]IUdR, deiodinated rapidly *in vivo*, paired-label

tissue distribution of [^{131}I]IUdR and AUdR was determined in normal mice. The uptake of ^{131}I and ^{211}At over a period of 24 h for selected tissues is shown in Table 2. The retention of ^{211}At was significantly ($p < 0.05$) higher in most tissues except intestines and thyroid. This tissue distribution pattern was qualitatively similar to that seen for free [^{211}At]astatide and [^{131}I]iodide [11] suggesting that both halouridines dehalogenated extensively *in vivo*. This behaviour, in combination with the short physical half-life of ^{211}At, emphasizes the need for confining the use of AUdR to therapeutic applications where rapid tumour uptake and limited exposure to normal tissues can be achieved, *i.e.*, with intratumoural or compartmental delivery.

6. CONCLUSION

It is possible to produce ^{211}At in sufficient quantities and acceptable purity for clinical applications. Generally, using conditions of radioiodinations, it is possible to introduce ^{211}At onto various radiopharmaceuticals. For certain applications, targeted α-particle therapy using astatinated radiopharmaceuticals may be advantageous. Clinical trials using ^{211}At-labeled 81C6 are under way at Duke for the treatment of surgically created glioma resection cavities. The outcome will help elucidate the potential usefulness of astatinated mAbs and other agents for the therapy of otherwise untreatable neoplasms.

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PRODUCTION AND QUALITY ASSURANCE OF I-131 CAPSULES

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Abstract. The Radiochemistry department produces two radioactive products for human use: ^{131}I-Sodium Iodide as a solution and as capsules dosage forms which are used for the diagnosis and treatment of various thyroid disorders including toxic nodules, thyroid hypofunction, carcinoma of the thyroid gland and hyperthyroidism, for nearly 50 years. As such, the Radiochemistry department is considered as a "mini" pharmaceutical enterprise, and therefore has to obey, under law, the rules of the Israeli ministry of Health. GMP requirements have been implemented in all our production and packaging units. A computerized system which is now an integral part of the whole process, starting from the stage of requested orders and ending with delivery to the hospitals, was developed and its features are presented here along with quality assurance policy.

1. INTRODUCTION

Radioiodine (^{131}I) hard-gelatine capsules are widely used for the diagnosis and treatment of various thyroid disorders including toxic nodules, thyroid hypofunction, carcinoma of the thyroid gland and hyperthyroidism.

These radiopharmaceuticals are produced on a very small scale basis and are indicated for "hospitals use only". Each preparation (for example a capsule) is designated for one patient only. Due to the unique safety problems and quality control procedures which are not encountered in the standard capsules' production in the conventional pharmaceutical industry, we have developed a self made computerized production facility. The following arrangements have been conducted in order to ensure that products will be at the quality required for their intended use, and that their quality will be consistent and reproducible. Standard operating procedures (SOPs) have been created to describe every segment of the department activities. All data, documents and records of every batch are retained for at least one year after expiration dates.

As a general rule, the Radiochemistry department adopts the guidelines suggested by FDA's CFR 21 (Code of Federal Regulations) and the quality control regime consists of the analysis of all inactive raw materials which are purchased from approved suppliers, who have, at least, ISO or preferably GMP certificates.

Hard gelatin capsules are purchased from the FDA-approved "Capsugel" Belgium. The existence of "Drug Master Files" and GMP certificates were verified. The dyes of the hard gelatin capsule are only those which are approved for use by the Israeli ministry of Health.

Some of the batches of I-131 Sodium Iodide, the principal active material, are occasionally sampled for γ-Spectrum analysis and thin layer chromatography for radiochemical purity. Every batch is counted using Dose Calibrators to verify the radioactive concentration.

The flow chart of the whole process is presented in Fig. 1

2. PROCEDURE

The nuclear medicine departments of all the medical centers in Israel make their orders of capsules through our marketing unit. Every capsule is intended for one patient and the amount of radioactivity for any day of treatment is decided by the physician according to the status of the patient.

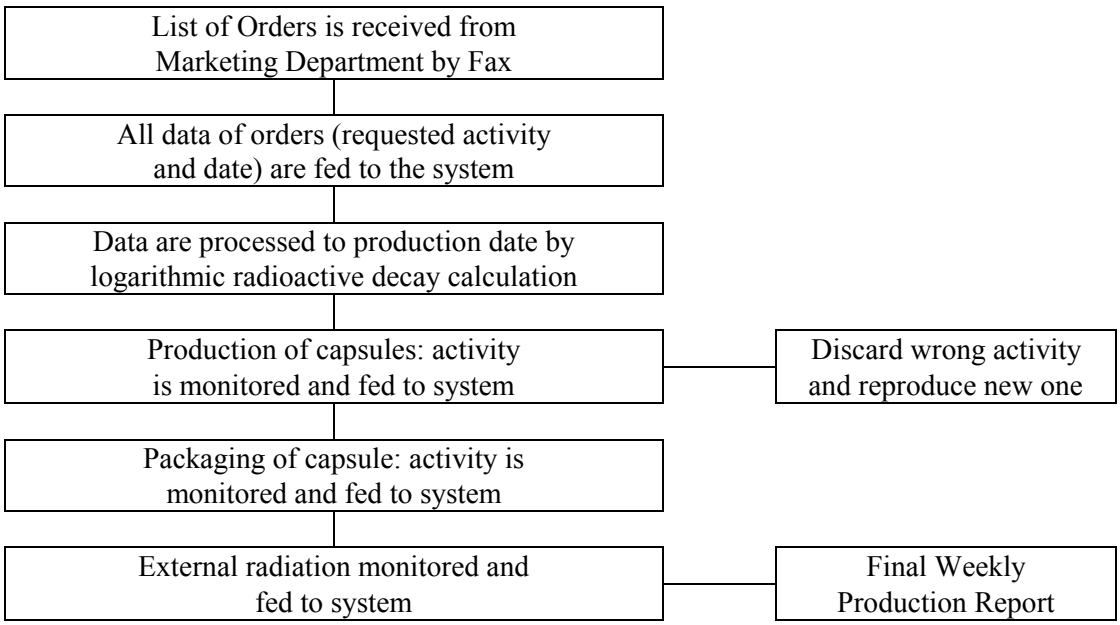

FIG. 1. Flow chart of production process.

A cumulative list of orders is transferred by fax to the production unit where all orders are fed into the system by entering the main menu (Fig. 2) and opening the icon of "ordering". In this screen (Fig 3) one types the order number, the institute and the date for which the capsule will be calibrated, namely the day when it will be given to the designated patient. The ordering number accompanies the capsule from the step of the marketing unit through production, packaging, external radiation field monitoring and finally delivery accompanied by a delivery note.

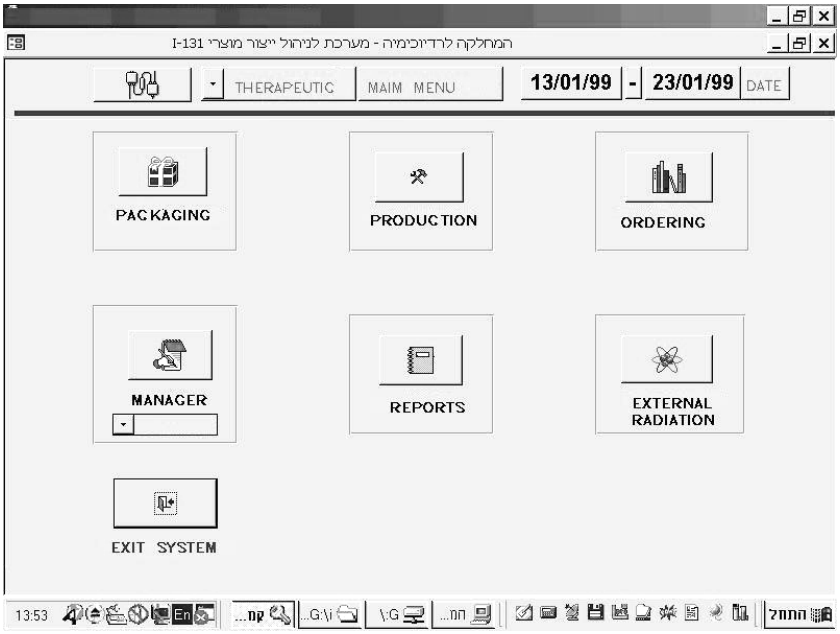

FIG. 2. Main menu screen.

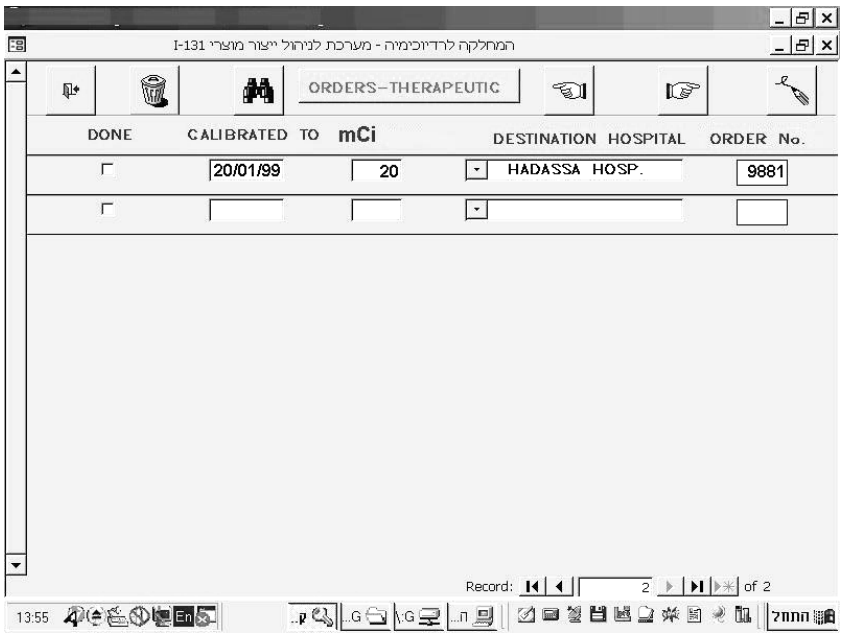

FIG. 3. Ordering screen.

Upon opening the icon of "production" in the main menu one gets the screen of production data (Fig 4) with the computer calculated amount of activity that should be produced on the day of production so that on calibration date the patient will get the exact amount decided for him by his physician.

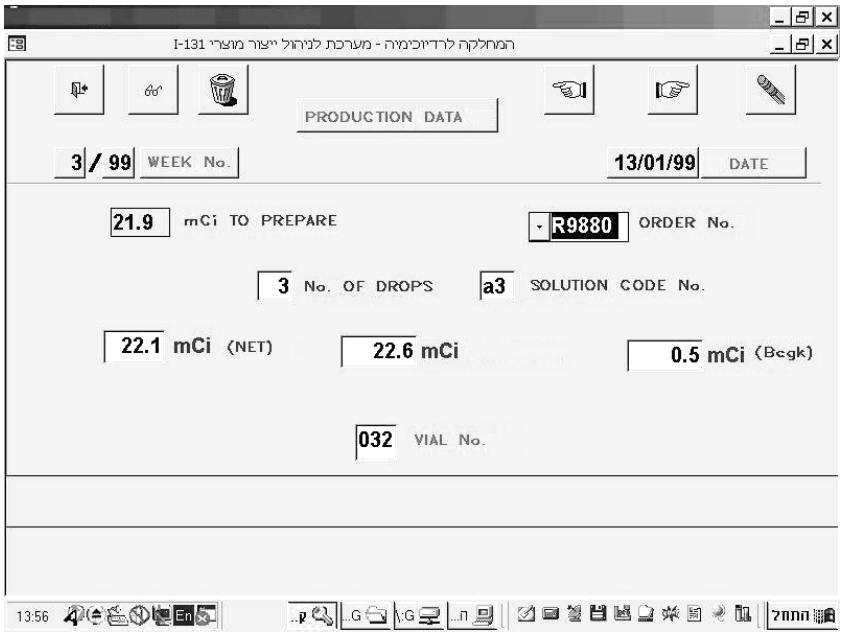

FIG. 4. Production data screen.

In contrast to the conventional capsules' production processes encountered in the pharmaceutical industry, where the active ingredient is mixed with an inactive excipient and the whole mixture is punched into the lower part of an empty hard gelatin capsule, such a procedure cannot be performed while operating radioactive ingredient due to high radioactive air contamination.

Due to this unique safety problem, we have developed a process and self- made production facility which is placed in a hot cell and is operated by remote control or tongues for a wide range of

capsules. A stock solution of highly concentrated I-131 Sodium Iodide (~4 Ci/ml) is diluted in such a manner so that the amount of activity which is needed for any capsule will be present in not more than 5 drops.

The production process starts, of course, from the highest activity and then by a series of dilutions one prepares the whole batch of a weekly list of order. Each capsule is placed in a numbered container and its radioactivity is monitored using a "Capintec" dose calibrator. All the data are typed into this screen and the system allows a tolerance of ±10% which is in agreement with the pharmacopoeia rules.

In the next step every capsule which is placed in the numbered container is transferred to the packaging area where it is monitored once again and the data are recorded in the screen shown in Fig. 5. In this screen one gets also the calculated result for the calibration date. This is also the step where the label for this capsule will be issued. The label contains the order number, the amount of radioactivity calculated for the future date when it will be dished to the patient. Each such capsule is placed in a lead shield which is then put into a metal container.

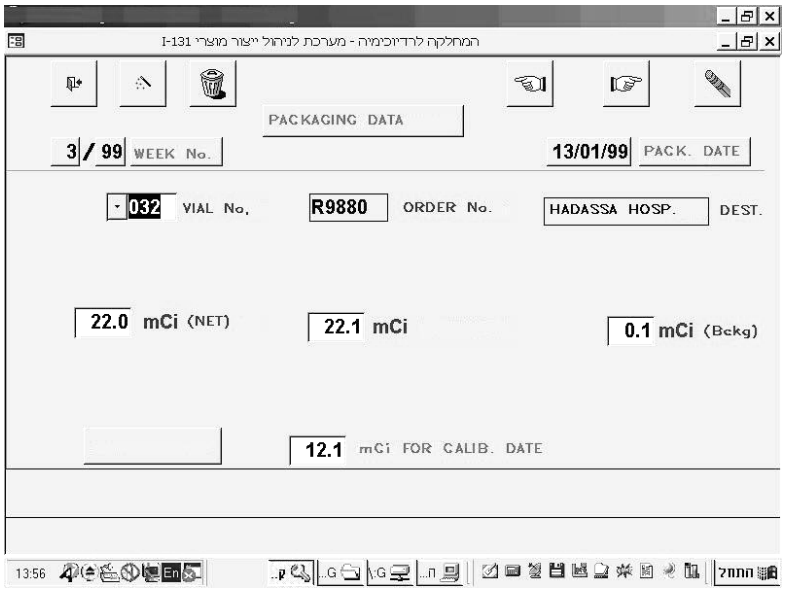

FIG. 5. Packaging data screen

The external radiation field of each such container is monitored and the data are recorded in the screen of external radiation (Fig 6).

A cumulative final report which contains all the data for every capsule which was produced during one week of production is issued and kept in the documentation center. One can, of course, issue any type of report for each step of the process as can be seen in Fig 7.

3. SOFTWARE FEATURES

The main advantage of the computerized system that was developed in our department, is that it replaces the cumbersome and inaccurate calculations which were based on the radioactive decay charts taking into consideration only half days decay, whereas with this software the system updates itself to any time that one segment of the whole process is being performed. Moreover, we have created a limitation factor of ±10% so that the system does not accept any deviations beyond this limit which is in agreement with pharmacopoeia rules. Any capsule which will be produced and its radioactivity value will be beyond these borders, the system will discard it and a notification will appear on the display to produce a new capsule.

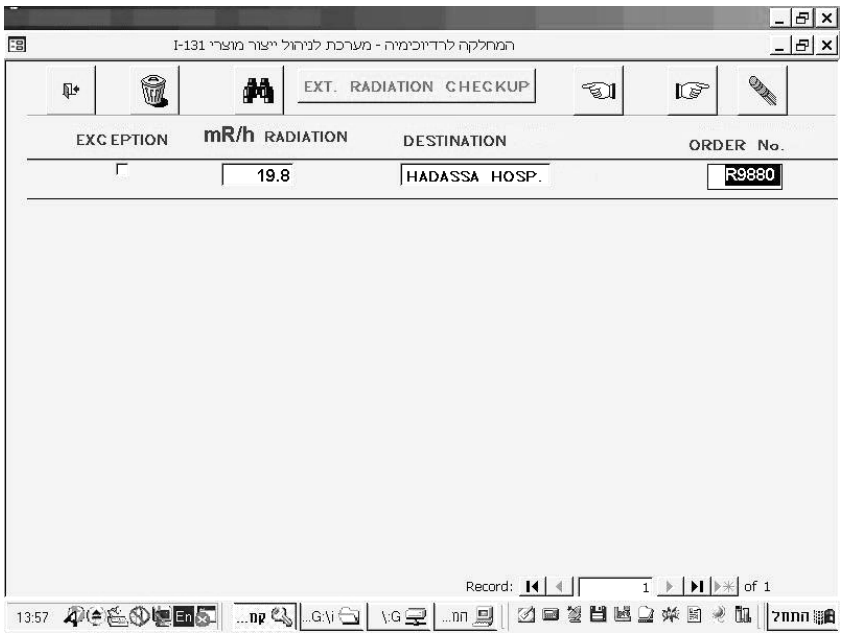

FIG. 6. External radiation data screen.

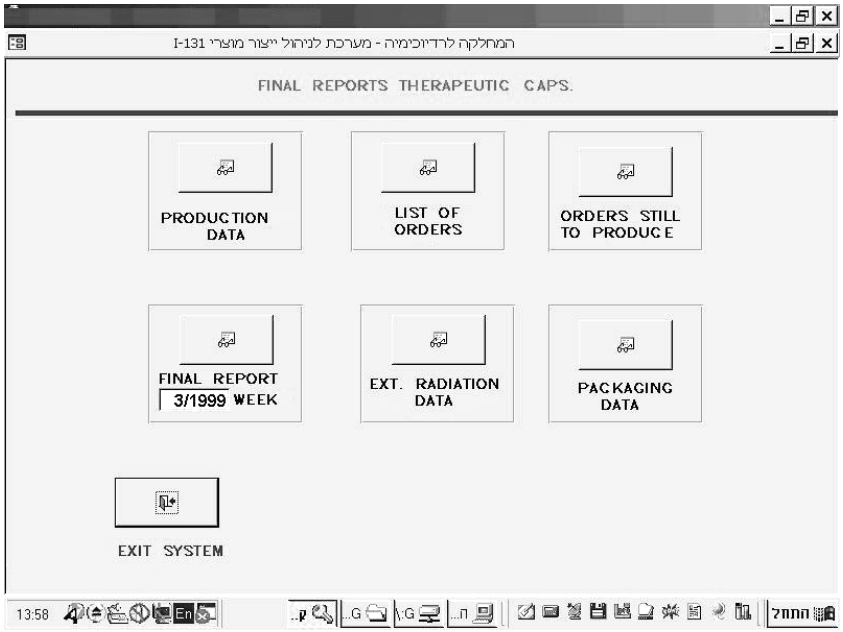

FIG. 7. Final report screen.

By this system we have achieved a high degree of accuracy and a possibility of following the "history" of capsules which have been produced in the past no matter how long ago. The system also excludes the possibility of a mistake in producing twice the same capsule, since once it has been manufactured, one cannot produce it again under the same order number. Actually one cannot even feed the system twice with the same order number.

4. CONCLUSION

We have presented here a computerized system which accompanies the production, packaging and delivery of radioiodine capsules. The system enables easy and very accurate procedure for production of I-131 capsule for therapeutic use.

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