Send Orders for Reprints to reprints@benthamscience.ae

Theranostic Applications of Lutetium-177 in Radionuclide Therapy

Tapas Das and Sharmila Banerjee*

Radiopharmaceuticals Chemistry Section, Radiochemistry & Isotope Group, Bhabha Atomic Research Centre, Trombay, Mumbai-400085, India

Sharmila Banerjee

Abstract: Lutetium-177 has been widely discussed as a radioisotope of choice for targeted radionuclide therapy. The simultaneous emission of imageable gamma photons [208 keV (11%) and 113 keV (6.4%)] along with particulate β^- emission [$\beta_{(max)}$ = 497 keV] makes it a theranostically desirable radioisotope. In the present article, the possibility of using two ^{177}Lu-based agents viz. ^{177}Lu-EDTMP and ^{177}Lu-DOTA-TATE for theranostic applications in metastatic bone pain palliation (MBPP) and peptide receptor radionuclide therapy (PRRT), have been explored. In the case of ^{177}Lu-EDTMP, the whole-body images obtained are compared with those recorded using ^{99m}Tc-MDP in the same patient. On the other hand, pre-therapy images acquired with ^{177}Lu-DOTA-TATE are compared with similar images obtained with standard agents, such as ^{99m}Tc-HYNIC-TOC (SPECT) and ^{68}Ga-DOTA-TOC (PET) in the same patient. The advantage of the long physical half-life ($T_{1/2}$) of ^{177}Lu has been utilized in mapping the pharmacokinetics of two additional agents, ^{177}Lu-labeled hydroxyapatite (HA) in radiation synovectomy of knee joints and ^{177}Lu-HA for therapy of hepatocellular carcinoma. Results of these multiple studies conclusively document the potential of ^{177}Lu as a theranostic radioisotope.

Keywords: ^{177}Lu, Theranostic applications, Metastatic bone pain palliation, Peptide receptor radionuclide therapy, Pharmacokinetic mapping.

INTRODUCTION

The term 'Theranostics' was first used by John Funkhouser in 2002 while describing his company's business model in developing diagnostic tests directly linked to the application of specific therapies [1]. 'Theranostic' refers to a combination of two interdependent applications namely therapy and diagnosis, using the same agent [2]. Since both diagnosis and therapy can be effected using this concept of theranosis, the treatment regime can be individualized for planning a specific dose for a specific patient [3]. This possibility forms the basis of 'personalized medicine' wherein the appropriate dose of a drug can be administered with respect to safety and efficacy [3, 4]. Since both diagnostic and therapeutic radionuclides are used in radiopharmaceutical preparation, the concept of, 'theranosis' is particularly relevant in nuclear medicine practices. In actual practice, theranosis can be effected by replacing a diagnostic radioisotope in a radiopharmaceutical with a therapeutic radioisotope usually with same chemical properties (*i.e.* ^{188}Re for ^{99m}Tc), while using the same molecular vector, thereby not compromising the biological avidity of the radiopharmaceuticals [5]. Theranosis helps in augmenting a diagnostic dose to a therapeutic one in order to tailor the therapy in a specific patient. In this modality, necessary pre-therapy information of biopharmacokinetics and dosimetry can be utilized to personalize the therapeutic regime [5].

While making use of theranosis, there are three distinct possibilities. In the first case, a matched radionuclide pair *e.g.* ^{99m}Tc (diagnostic) and ^{188}Re (therapeutic) is used with the same biological vector utilizing the similar complexation chemistries of the two radioisotopes. The second case is exemplified by the classical use of radioiodine where different radioisotopes of the same element such as, ^{123}I and ^{131}I, are used for theranosis. The third and perhaps the most desirable and wide ranging option is the use of a single radionuclide which has imageable gamma photon(s) for pre-therapeutic diagnosis and dosimetry and a particulate emission (β^- or α or Auger electron) for effecting therapy. Due to obvious reasons, theranostic agents based on the individual dual-purpose radionuclides is more attractive as the radiopharmaceutical used for the diagnosis and therapy remains identical in all respects, thereby assuring near-identical biological distribution, pharmacokinetics and an opportunity for very accurate dosimetry planning [4]. ^{177}Lu resides in the third category, since it decays by emission of β^- particle to stable ^{177}Hf with maximum β^- energy of 497 keV, which is suitable for a host of radiotherapeutic applications [6]. This radioisotope also emits several accompanying gamma photons, with the 208 keV (11%) and 113 keV (6.4%) gamma photons being used for diagnostic evaluation [6, 7]. Thus a radiopharmaceutical labeled with ^{177}Lu can be used for diagnosis when injected in lower tracer levels and also for therapy when injected at therapeutic doses [8-10]. This radioisotope ($T_{½}$ = 6.71 d) is additionally advantageous as it can be produced in high activity levels with adequately high specific activity using medium thermal neutron flux research reactors, owing to the high thermal neutron capture cross-section of ^{176}Lu. Recent literature has predicted that ^{177}Lu may play a crucial role in meeting the future global demand of radionuclides required for many targeted radionuclide therapy applications [7] and in this context unraveling the true potential of ^{177}Lu as a

*Address correspondence to this author at the Radiopharmaceuticals Chemistry Section, Radiochemistry & Isotope Group, Bhabha Atomic Research Centre, Mumbai 400 085, India; Tel: 91-22-2559 3910;
Fax: 91-22-2550 5151; E-mail: sharmila@barc.gov.in

1874-4729/16 $58.00+.00

theranostic radioisotope may play a significant role in achieving the anticipated success of personalized medicine.

While using ^{177}Lu as a therapeutic radioisotope, the imageable gamma photons are used to trace the initial *in-vivo* localization of the radiopharmaceutical [11-16]. However, it is of interest to study the possibility of using this radioisotope as a theranostic one by carrying out pre-therapy imaging studies of the ^{177}Lu-labeled radiopharmaceuticals in patients. It is evident that the gamma emission of ^{177}Lu for diagnostic purpose is accompanied with the undesirable, additional radiation burden from beta emission of ^{177}Lu. Therefore, in cases where a therapy is not required, use of ^{177}Lu may deliver an undesirable dose to the patients. However, it is but obvious that the theranostic potential of ^{177}Lu outweighs the above disadvantage, particularly in cancer patients. It is pertinent to mention that although the ability to use the theranostic potential of ^{177}Lu is relatively a recent realization, there are some studies reported in the literature where ^{177}Lu has already been used as a theranostic radioisotope [17, 18]. In the present paper, we report our experience of using two agents, ^{177}Lu-DOTA-TATE (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid coupled Tyr^3-Octreotate) and ^{177}Lu-EDTMP (Ethylenediaminetetramethylene phosphonic acid), towards their potential applicability in theranostic applications. In our studies for exploring the possible applications of the two additional agents, ^{177}Lu-labeled hydroxyapatite particles (HA) [19] for radiation synovectomy (RSV) and ^{177}Lu-HA for hepatocellular carcinoma (HCC) [20]; definite clues to map the post-therapy *in-vivo* pharmacokinetics of the respective ^{177}Lu-labeled agents, utilizing the scintigraphic information have been recorded. This approach is also being reported in the present article. This article attempts to revisit some of our earlier studies using ^{177}Lu-labeled agents, with a view to exploring their utility from the perspective of theranostic applications.

EXPERIMENTAL

Materials and Methods

EDTMP and HA particles (particle size 1-10 μ and 20-60 μ) used for the present study were synthesized and characterized in-house following the procedure reported in the literature [11, 20, 21]. DOTA-TATE was obtained from PiChem (Austria). Gentisic acid (2,5-dihydroxybenzoic acid) and ammonium acetate used for the preparation of patient doses of ^{177}Lu-DOTA-TATE were procured from Aldrich Chemical Company (USA). Lutetium oxide (82% enriched in ^{176}Lu, spectroscopic grade, >99.999% chemically pure) was obtained from Centre for Molecular Research (Russia). High purity supra-pure® water and supra-pure® HCl were obtained from Merck (Germany). All the other chemicals used for the present study were purchased from reputed local manufacturers and were of analytical grade.

^{99m}Tc-labeled MDP (methylene diphosphonate) was prepared by using the freeze-dried MDP kits, obtained from Board of Radiation and Isotope Technology (BRIT, India), following the protocol mentioned in the literature [22]. ^{99m}Tc-labeled HYNIC-TOC (hydroxynicotinamide coupled Tyr^3-Octreotide) and ^{68}Ga-labeled DOTA-TOC (DOTA coupled Tyr^3-Octreotide) were prepared by using the corresponding freeze-dried kits, prepared in-house, following the protocol described in the literature [23].

Paper chromatography (PC) strips were purchased from Whatman (UK). The high performance liquid chromatography (HPLC) system (PU 1580) was obtained from Jasco (Japan). The elution was monitored by detecting the radioactivity signal using a well-type NaI(Tl) detector (Jasco, Japan) coupled with the HPLC system. All the solvents used for HPLC were degassed and filtered prior to use and were of HPLC grade.

The 'theranostic potentials' of ^{177}Lu-EDTMP and ^{177}Lu-DOTA-TATE were ascertained by administering the preparations in patients suffering from metastatic bone pain due to prostate carcinoma and carcinomas of neuroendocrine origins, respectively. In both the cases, the patients were initially subjected to diagnostic evaluation by the administration of the low dose preparation and subsequently the same patient was treated with high dose preparation of the same radiopharmaceutical. In addition, patients having skeletal metastases have also undergone diagnostic imaging with ^{99m}Tc-MDP. Similarly, neuroendocrine cancer patients have undergone additional diagnostic scanning with either ^{99m}Tc-HYNIC-TOC or ^{68}Ga-DOTA-TOC.

The ethical clearances for administration of the agents in human patients were obtained from the competent authorities of the respective nuclear medicine centers and written consents were taken from the patients prior to the administration of the agents.

Production of ^{177}Lu

Lutetium-177 used for the present study, was produced in the BARC (Bhabha Atomic Research Centre) 'DHRUVA' reactor by irradiating isotopically enriched Lu_2O_3 target (82% in ^{176}Lu) at a thermal neutron flux of ~1×10^{14} n.cm^{-2}.s^{-1} for a period of 21 days. The irradiated target was cooled for 24 h and subsequently dissolved in 0.01 M supra-pure HCl by gentle warming. The resulting solution was evaporated to near-dryness and reconstituted with supra-pure water. The evaporation and volume reconstitution steps were repeated two to three times. Finally the radioactive solution was passed through the Millipore® (0.22 μ) filter paper. $^{177}LuCl_3$, thus obtained, was directly used for the formulation of patient doses of ^{177}Lu-EDTMP and ^{177}Lu-DOTA-TATE as well as for the preparation of ^{177}Lu-labeled HA particles.

The total ^{177}Lu radioactivity produced and its radionuclidic purity were determined following the procedure mentioned in the literature [6, 13].

Preparation of ^{177}Lu-EDTMP

Lu-177-labeled EDTMP, suitable for human administration, was prepared aseptically by dissolving 35 mg of EDTMP and 84 mg of $NaHCO_3$ in 1 mL of water for injection and incubating the resulting solution with $^{177}LuCl_3$ (50-100 mCi, 1.85-3.7 GBq) at room temperature for a period of 15 minutes.

For diagnostic imaging, an aliquot (50-100 μL) of the preparation containing 5 mCi (185 MBq) of activity was taken and diluted with normal saline. The preparation was then subsequently subjected to Millipore® filtration before administering to the patients.

On the other hand, for palliative care, the undiluted preparation was directly administered to the patients after rendering it sterile through Millipore® filtration.

Preparation of ^{177}Lu-DOTA-TATE

Lu-177-labeled DOTA-TATE used for the present studies was prepared following the procedure reported by Das *et al*. [24]. According to this methodology, the amount of peptide and radioprotecting agent (gentisic acid) as well as volume of the buffer solution (ammonium acetate) used for a particular preparation were calculated based on the specific activity of ^{177}Lu available at the time of preparation. A typical preparation of 200 mCi (7.4 GBq) of ^{177}Lu-DOTA-TATE was carried out by incubating 260 µg of DOTA-TATE and 55 mg of gentisic acid in 1.4 mL of 0.1 M ammonium acetate buffer (pH ~5) for 45 min at 85-90 °C using ^{177}Lu having a specific activity of 25 mCi.µg^{-1} (925 MBq.µg^{-1}).

An aliquot of the preparation containing 10 mCi (370 MBq) of activity was withdrawn and diluted with saline. The ^{177}Lu-DOTA-TATE preparation, thus obtained, was administered to the patients after Millipore® filtration for diagnostic imaging.

For providing radiotherapeutic treatment, the undiluted ^{177}Lu-DOTA-TATE preparation was administered to the patients after Millipore® filtration following the standard protocol reported in the literature [10, 25].

Preparation of ^{177}Lu-HA

Lu-177-labeled HA particles, for both RSV and HCC application, were prepared following the protocol mentioned in the literature [19, 20]. 5 mg of HA particles were suspended in 800 µL of saline and 100 µL 0.5 M $NaHCO_3$ solution and thoroughly mixed after the addition of 100 µL of $^{177}LuCl_3$ (5 mCi, 185 MBq). The pH of the resulting suspension was adjusted between 7 and 8 using dilute NaOH solution prior to vortex mixing which was continued at room temperature for 30 minutes. Subsequently, the reaction mixture was centrifuged at 3000 rpm for 5 min. The supernatant was carefully separated from the precipitate. The radiolabeled HA particles, thus obtained as a precipitate, were subjected to further washing using 1 mL of sterile, pyrogen free normal saline to ensure the removal of free ^{177}Lu activity, if any. The last step was repeated twice. Finally, the radiolabeled particulates were suspended in sterile saline, autoclaved and used for the subsequent studies.

Quality Control of ^{177}Lu-EDTMP, ^{177}Lu-DOTA-TATE and ^{177}Lu-HA

Radiochemical purities of ^{177}Lu-EDTMP, ^{177}Lu-DOTA-TATE and ^{177}Lu-HA complexes were carried out following the procedures reported in the literatures [11,19,20,24]. In brief, radiochemical purity of ^{177}Lu-EDTMP was determined by PC using normal saline as the eluting solvent. Radiochemical purity of ^{177}Lu-DOTA-TATE was ascertained by both PC and HPLC studies. PC was carried out using acetonitrile in water (1:1, v/v) as the eluting solvent while HPLC was performed using water (A) and acetonitrile (B) mixed with 0.1 % trifluoroacetic acid (TFA) as the eluting solvent employing gradient elution technique (0-4 min 95% A, 4-15 min 95% A to 5% A, 15-20 min 5% A, 20-25 min 5% A to 95% A, 25-30 min 95% A).

Radiochemical purity of ^{177}Lu-labeled HA particles was determined by measuring the activity associated with HA particles and the solution of the reaction mixture. The reaction mixture was vortexed thoroughly and centrifuged after the completion of the reaction. Subsequently, half the volume of the supernatant solution was carefully pipetted out and ^{177}Lu activity associated with this solution was measured. Similarly, ^{177}Lu activity associated with HA particles along with the remaining half of the supernatant solution was also measured. From these data the percentage Radiochemical purity of ^{177}Lu-HA was calculated by using the following equation,

$$\% \text{ Radiochemical purity} = [(Y-X)/(Y+X)]\times 100$$

where, X represents background-corrected activity associated with half the volume of the supernatant solution and Y is that of the precipitated HA particles along with the remaining half of the supernatant.

RESULTS AND DISCUSSION

Production of ^{177}Lu

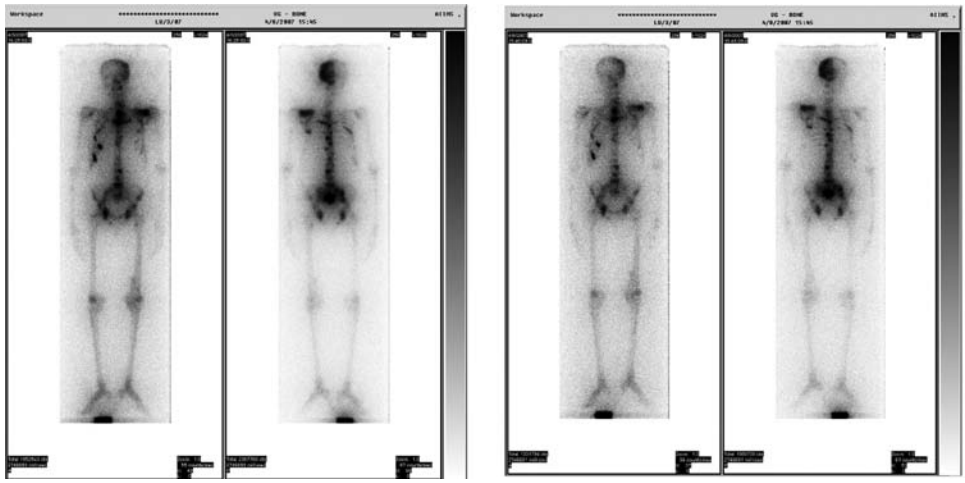
Lutetium-177 was produced with a specific activity of 20-25 mCi.µg^{-1} (740-925 MBq.µg^{-1}) when enriched (82% in ^{176}Lu) Lu_2O_3 target was irradiated at a thermal neutron flux of ~1×10^{14} n.cm^{-2}.s^{-1} for a period of 21 d in our Institute's reactor. Lu-177m was the only radionuclidic impurity present in the processed $^{177}LuCl_3$ and it was found that on an average 0.015 µCi (5.55 kBq) of ^{177m}Lu was present in 1 mCi (37 mCi) of $^{177}LuCl_3$ at the end-of bombardment (EOB). This implies that ^{177}Lu was obtained with ~99.98% radionuclidic purity at EOB [6, 13]. $^{177}LuCl_3$, obtained after radiochemical processing, was subjected to Millipore filtration and subsequently used for the preparation of radiopharmaceuticals for human/animal administration.

Quality Control of ^{177}Lu-based Radiopharmaceuticals

PC was carried out with normal saline as the eluting solvent where ^{177}Lu-EDTMP moved towards the solvent front (R_f = 0.9-1), while uncomplexed ^{177}Lu remained at the point of spotting (R_f = 0). ^{177}Lu-EDTMP was obtained with 99.0±0.5 % radiochemical purity under the optimized reaction conditions. On the other hand, in PC, carried out with 50% acetonitrile in water (1:1, v/v) as the eluting solvent, ^{177}Lu-DOTA-TATE showed a R_f value of 0.7-0.9, while uncomplexed ^{177}Lu remained at the point of application (R_f = 0) under identical conditions. In HPLC studies, ^{177}Lu-DOTA-TATE exhibited a retention times of ~18 min while uncomplexed ^{177}Lu was eluted from the column at ~4 min. Radiochemical purity of ^{177}Lu-DOTA-TATE was determined by employing the above two techniques and was found to be 98.8±0.6%. Radiochemical purity of ^{177}Lu-HA, prepared for both the applications using two different particle sizes, were determined using the procedure mentioned in the 'Experimental' section and was found to be 99.1±0.2% under optimized reaction conditions.

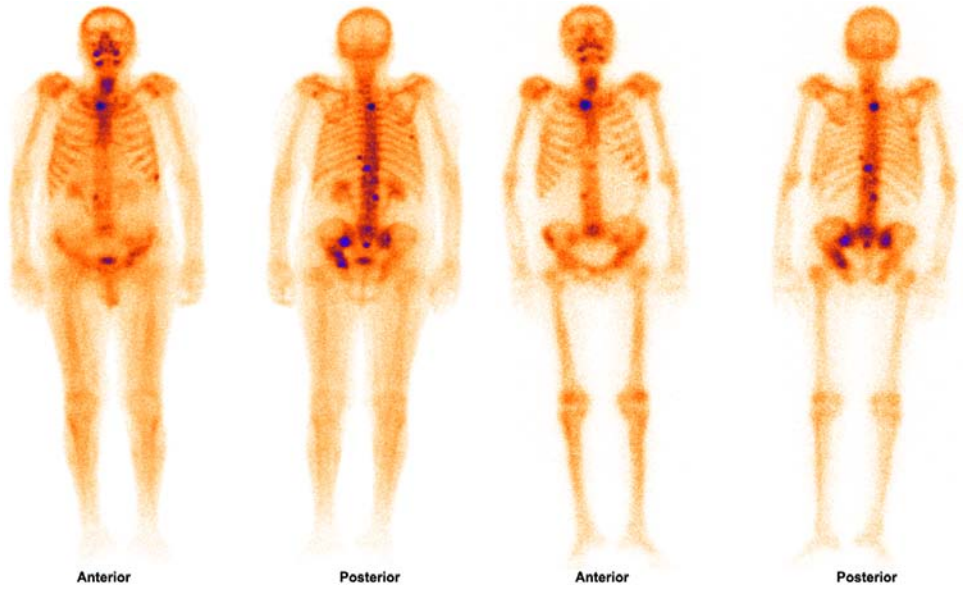
Theranostic Applications of ^{177}Lu-EDTMP

^{177}Lu-EDTMP scores over other radiopharmaceuticals used in bone pain palliation such as $Na_3{}^{32}PO_4$, $^{89}SrCl_2$ (Me-



(A) **(B)**

Fig. (1). Whole-body scintigraphic images of a patient recorded (**A**) 30 min and (**B**) 6 d post-administration of 5 mCi (185 MBq) of ^{177}Lu-EDTMP.



(A) **(B)**

Fig. (2). Whole-body scintigraphic images of a patient recorded after administration of (**A**) ^{99m}Tc-MDP and (**B**) therapeutic doses of ^{177}Lu-EDTMP at 4 weeks post-injection.

tastron®), ^{188}Re-HEDP (Hydroxyethylenediphosphonate) and ^{153}Sm-EDTMP with respect to its suitable nuclear decay characteristics, ease of production and logistic advantages for distribution [14]. Its clinical efficacy is recently documented [8, 26]. To study the possibility of using ^{177}Lu-EDTMP as a diagnostic agent, patients suffering from metastatic skeletal carcinoma arising from prostate or breast cancer, were subjected to pre-therapy scanning using the agent. Towards this, 5 mCi (185 MBq) of the preparation was administered to the patients and sequential whole-body scintigraphic images were recorded at different post-administration time points. Figs. (**1A**) and (**1B**) represents the whole-body scintigraphic images of a patient recorded 30 min and 6 d post-administration of the agent, respectively [27]. The images clearly demonstrate the excellent bio-localization of the radiotracer in the cancerous lesions with minimal soft-tissue uptake which is the prelude to initiate the therapeutic regimen. The images recorded were found to be similar to those recorded with ^{99m}Tc-MDP in the same patient. Fig. (**2A**) and (**2B**) depicts the whole-body images of a patient recorded after administration of ^{99m}Tc-MDP and therapeutic doses of ^{177}Lu-EDTMP at 4 weeks post-injection. The ^{177}Lu-EDTMP image, although recorded after the administration of therapeutic doses of the agent and after 4 weeks post-administration, bears the testimony that ^{177}Lu-EDTMP has the potential to be used as a diagnostic marker and thus can be considered for theranostic application. Moreover, the image proves that the agent can suitably be used for the post-therapy monitoring of the patients.

Theranostic Applications of ^{177}Lu-DOTA-TATE

Theranostic application of ^{177}Lu in peptide receptor radionuclide therapy (PRRT) has been studied through admini-

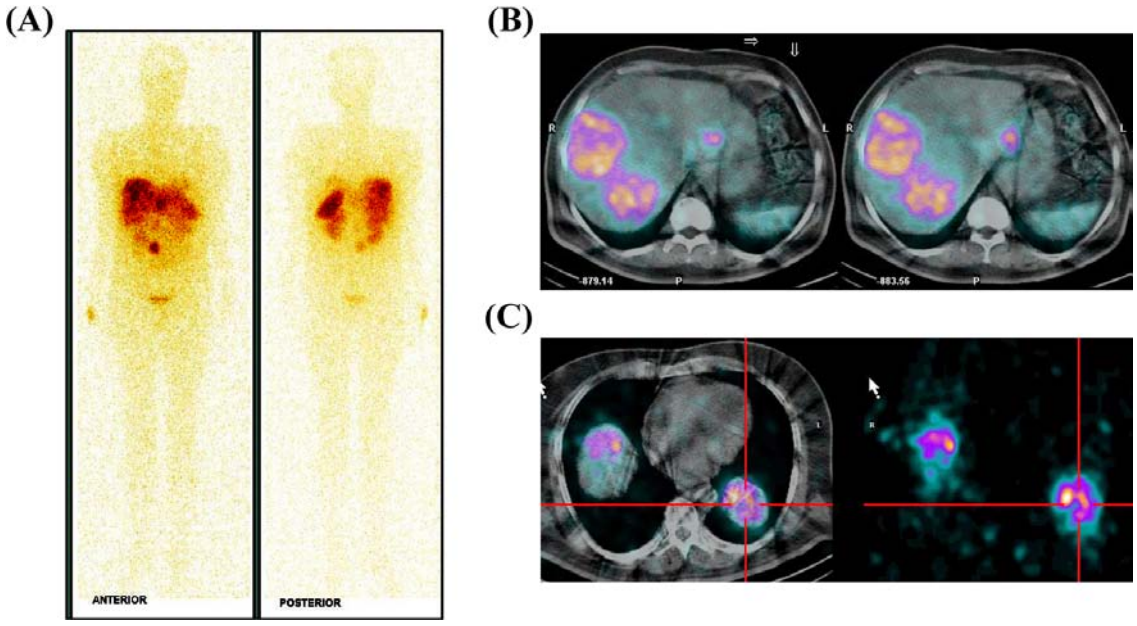


Fig. (3). Whole-body scintigraphic images of a patient recorded after administration of 10 mCi (370 MBq) of ^{177}Lu-DOTA-TATE. SPECT-CT images of (B) liver and (C) lung lesions of the same patient.

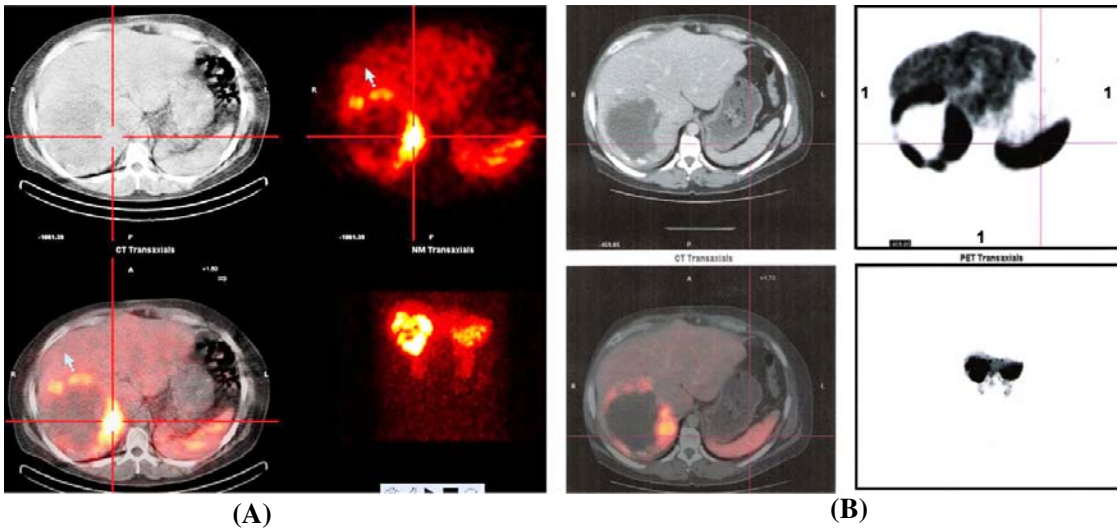


Fig. (4). (**A**) SPECT-CT scan of a patient recorded after administration of 10 mCi (370 MBq) of ^{177}Lu-DOTA-TATE. (**B**) PET-CT scan of the same patient recorded after administration of ^{68}Ga-DOTA-TOC.

stration of ^{177}Lu-DOTA-TATE in 39 patients suffering from metastatic neuroendocrine cancers [27]. The patients were administered 10 mCi (370 MBq) of the preparation and planar images of the whole-body as well as SPECT-CT (SPECT: Single Photon Emission Computed Tomography, CT: Computed Tomography) images were recorded at various post-administration time points. The whole-body scintigraphic images of a patient recorded after the administration of diagnostic doses of ^{177}Lu-DOTA-TATE are shown in Fig. (**3A**) while the SPECT-CT images of liver and lung lesions are shown in Fig. (**3B**) and (**3C**). These images showed excellent normal physiological distribution and abnormal increased uptakes in all the lesions as observed in the contrast CT scan of the patient. In addition, the SPECT-CT images not only demonstrated the heterogeneous versus homogeneous distribution of receptor uptake, but also aided in estimation of the tumor volume [28].

To study and compare the SPECT-CT images recorded with ^{177}Lu DOTA-TATE with those obtained with commonly used radiopharmaceuticals employed to diagnose neuroendocrine cancers, ^{68}Ga-DOTA-TOC was administered in the same patient and PET-CT images were recorded. Fig. (**4A**) and (**4B**) depicts the PET-CT images which were found to be comparable in terms of sensitivity and lesion characteristics and showed all the metastatic lesions seen on the contrast CT. The pre-treatment dosimetry of ^{177}Lu-DOTA-TATE based on the whole-body diagnostic scans could therefore be utilized to tailor therapies with more accuracy [28].

Recently an attempt has also been made to compare the diagnostic potential of ^{177}Lu-DOTA-TATE with that ^{99m}Tc-HYNIC-TOC, which is regularly used for the diagnostic evaluation of the cancer patients of neuroendocrine origin [23]. The authors have compared ^{99m}Tc-HYNIC-TOC scinti-

graphic images with the PET-CT images obtained with ^{68}Ga-DOTA-TATE and post-therapy SPECT images acquired with ^{177}Lu-DOTA-TATE. The perfect correlation between the images for the detection of particular lesions in patients with neuroendocrine cancers provides conclusive evidence towards the theranostic potential of ^{177}Lu.

It is worthwhile to mention that DOTA-TATE is the somatostatin analogue which has high affinity for somatostatin receptor subtype 2 (SSTR-2) and is used as the peptide vector in PRRT application. On the other hand, DOTA-TOC and DOTA-NOC (DOTA coupled Nal^3-octreotide), have affinity for SSTR-3 and SSTR-5 also. It is therefore evident that the prediction of therapeutic response would be more accurate when ^{177}Lu-DOTA-TATE diagnostic scans are used for determining the localization of the radiopharmaceutical. Since ^{68}Ga-DOTA-TOC / DOTA-NOC, which are commercially used diagnostic agents for neuroendocrine cancers, could be indicative of the presence of SSTR-3 and 5, besides SSTR-2; therapy with ^{177}Lu-DOTA-TATE using ^{68}Ga-DOTA-NOC scans could be misleading and may not yield any therapeutic benefit. It is worthwhile to mention that to avoid this problem, use of ^{68}Ga-DOTA-TATE for diagnostic scanning of the neuroendocrine cancer patients have been advocated [29, 30]. However, it is also reported that even the simple change of radioisotope from ^{68}Ga to ^{177}Lu changes the pharmacokinetics and binding affinity of the agent thereby making the scanning with diagnostic dose of ^{177}Lu-DOTA-TATE more relevant [31]. This makes ^{177}Lu DOTA-TATE an ideal theranostic agent for PRRT applications.

^{177}Lu-labeled HA for Mapping of Pharmacokinetics in Radaiation Synovectomy

In our studies using ^{177}Lu-HA (particle size 1-10 μ) for RSV, the theranostic potential of ^{177}Lu has been utilized to map the pharmacokinetics of the radiopharmaceutical *in-vivo* [17]. Scintigraphic images were obtained at various time intervals in patients bearing arthritis in one of the knee joints, where the ^{177}Lu-HA preparation was injected. The images clearly revealed that the injected activity remained localized in the synovium even at 1 month post-injection (Fig. **5**). The whole-body images recorded did not show any detectable activity in any other organs thereby confirming that the radiolabeled particulates remained intact in the point of injection and no leaching out was observable. This observation conclusively indicates that the long $T_{1/2}$ of ^{177}Lu can be utilized as an ideal parameter to visualize the localization of the radiopharmaceutical in the body and thereby ascertaining the *in-vivo* pharmacological stability of the agent. The results obtained by gamma scintigraphy of the radiopharmaceutical has been further corroborated by observing the activity in the blood samples at different time points which showed no radioactivity over the background activity post-injection at any of the time points under study.

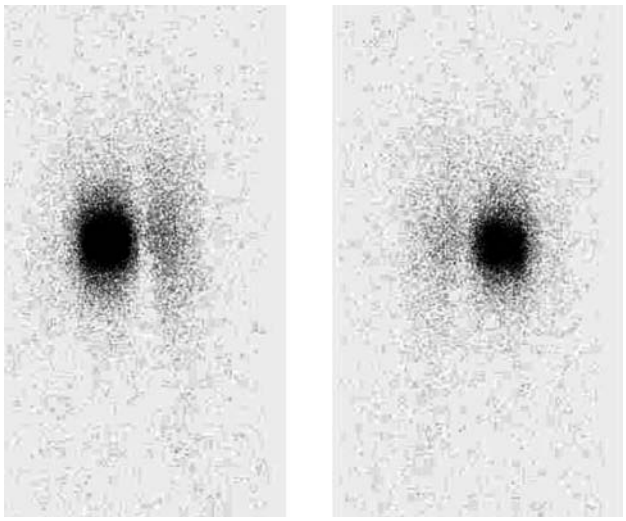


Fig. (5). Whole-body scintigraphic images of a patient injected with 5 mCi (185 MBq) of ^{177}Lu-HA (particle size: 1-10 μ) in the knee joint 1 month post-administration.

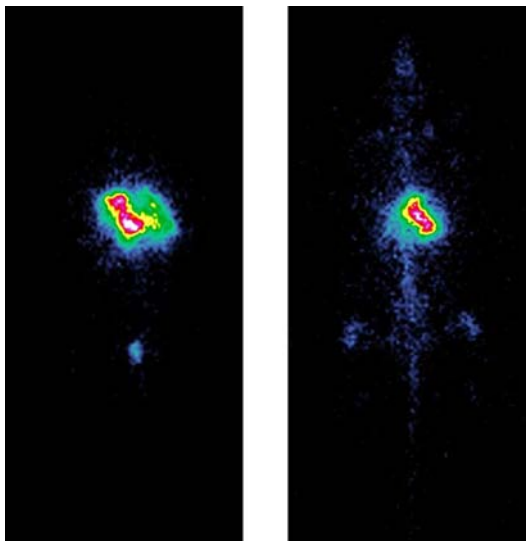


Fig. (6). Whole-body scintigraphic images of normal Wistar rat injected with ^{177}Lu-HA (particle size: 20-60 μ) through hepatic artery at (**A**) 2 h and (**B**) 7 d post-administration.

^{177}Lu-labeled HA for Mapping of Pharmacokinetics in Hepatocellular Carcinoma

In a similar study, using ^{177}Lu-HA (particle size 20-60 μ) for possible application in therapy of HCC, the potential applicability of the agent could be assessed using the scintigraphic images obtained by administering the agent in animal models [18]. One of the key issues concerning the use of radiolabeled particulates for *in-vivo* therapy of HCC is the radiation dose burden to the non-target organs due to the leakage of the particulates from liver. The primary criterion of an ideal agent for this application is that it should be retained in liver and deliver adequate therapeutic dose to the proliferating tissue. Vital clues could be obtained with respect to the pharmacological stability of the agent through serial scintigraphic imaging (Fig. **6**) wherein it was observed that, the activity was not completely retained in the liver. The images showed that though no appreciable uptake was observed in any other non-target organs, the skeletal uptake increased with passage of time, thereby indicating the gradual leakage of the injected agent from the liver due to *in vivo* instability. This significantly high skeletal uptake being an undesirable feature limits the potential of the agent. In this case again, the initial information obtained from imaging studies utilizing the long half-life advantage of ^{177}Lu indicated the limitation of the agent as a radiotherapeutic in treatment of HCC.

CONCLUSION

In all the studies which aim at utilizing the potential of ^{177}Lu as a theranostic isotope, the long half-life of the radionuclide besides the presence of the imagable gamma photons is a distinct advantage. This nuclear property aids clinical tracking of the radiopharmaceutical *in-vivo*, long after its administration. From this observation, clues about the *in-vivo* localization as well as pharmacokinetic stability can be assessed. The studies described in the present paper demonstrate the various options while utilizing the theranostic potential of ^{177}Lu. While on one hand, pre-therapy information can be derived towards deciding the therapeutic regime, estimating the therapeutic efficacy from the post-therapy scintigraphic images is also possible. The third option is the possibility to be able to carry out mapping of the pharmacokinetic pattern of the injected radiopharmaceutical. Therefore, ^{177}Lu will definitely be the radionuclide of choice in nuclear medicine 'theranosis' and focusing on the utilization of its full potential in a variety of other applications will constitute a relevant area of research in radiopharmaceutical applications in nuclear medicine.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the collaborative support received from the clinical partners viz. Prof. C.S. Bal, Head, Department of Nuclear Medicine, All India Institute of Medical Sciences, New Delhi; Dr. Ajit Shinto, Head, Department of Nuclear Medicine and PET, Kovai Medical Centre and Hospital, Coimbatore and Dr. Natasha Singh, Consultant, Nuclear Medicine, P.D. Hinduja National Hospital, Mumbai. The authors express their sincere thanks to all the coauthors for providing the images of human patients involved in the respective clinical studies (referenced in appropriate places in the manuscript).

REFERENCES

[1] Kelkar, S.S.; Reineke, T.M. Theranostics: Combining imaging and therapy. *Bioconj. Chem.,* **2011**, *22*, 1879-1903.

[2] Wang, P.; Moore, A. Theranostic magnetic resonance imaging of type 1 diabetes and pancreatic islet transplantation. *Quant. Imaging Med. Surg.,* **2012**, *2*, 151-162.

[3] DeNardo, G.L; Denardo, S.J. Concept, consequences, and implications of theranosis. *Semin. Nucl. Med.,* **2012**, *42*, 147-150.

[4] Srivastava, S.C. Paving the way to personalized medicine: Production of some promising theragnostic radionuclides at Brookhaven National Laboratory. *Semin. Nucl. Med.,* **2012**, *42*, 151-163.

[5] Baum, R.P.; Kulkarni, H.R. Theranostics: From molecular imaging using Ga-68 labeled tracers and PET/CT to personalized radionuclide therapy - the Bad Berka experience. *Theranostics,* **2012**, *2*, 437-447.

[6] Pillai, M.R.A.; Chakraborty, S.; Das, T.; Venkatesh, M.; Ramamoorthy, N. Production logistics of ^{177}Lu for radionuclide therapy. *Appl. Radiat. Isot.,* **2003**, *59*, 109-118.

[7] Das, T.; Pillai, M.R.A. Options to meet the future global demand of radionuclides for radionuclide therapy. *Nucl. Med. Biol.*, **2013**, *40*, 23-32.

[8] Shinto, A.S.; Shibu, D.; Kamaleshwaran, K.K.; Das, T.; Chakraborty, S.; Banerjee, S.; Thirumalaisamy, P.; Das, P.; Veersekar, G. ^{177}Lu-EDTMP for treatment of bone pain in patients with disseminated skeletal metastases. *J. Nucl. Med. Technol.*, **2014**, *42*, 55-61.

[9] Solá, G.A.R.; Argüelles, M.G.; Bottazzini, D.L.; Furnari, J.C.; Parada, I.G.; Rojo, A.; Ruiz, H.V. Lutetium-177-EDTMP for bone pain palliation. Preparation, biodistribution and pre-clinical studies. *Radiochim. Acta*, **2000**, *88*, 157-162.

[10] Das, T.; Bhadwal, M.; Banerjee, S.; Sarma, H.D.; Shinto, A.; Kamaleshwaran, K.K. Preparation of DOTA-TATE and DOTA-NOC freeze-dried kits for formulation of patient doses of ^{177}Lu-labeled agents and their comparison for peptide receptor radionuclide therapy application. *J. Radioanal. Nucl. Chem.,* **2014**, *299*, 1389-1398.

[11] Chakraborty, S.; Das, T.; Banerjee, S.; Balogh, L.; Chaudhari, P.R.; Sarma, H.D.; Polyák, A.; Máthé, D.; Venkatesh, M.; Janoki, G.A.; Pillai, M.R.A. ^{177}Lu-EDTMP : A viable bone pain palliative in skeletal metastasis. *Cancer Biother. Radiopharm.*, **2008**, *23*, 202-213.

[12] Das, T.; Chakraborty, S.; Sarma, H.D.; Banerjee, S.; Venkatesh, M. A novel ^{177}Lu-labeled porphyrin for possible use in targeted tumor therapy. *Nucl. Med. Biol.*, **2010**, *37*, 655-663.

[13] Banerjee, S.; Das, T.; Chakraborty, S.; Venkatesh, M. Emergence and present status of Lu-177 in targeted radiotherapy: the Indian scenario. *Radiochim. Acta*, **2012**, *100*, 115-126.

[14] Máthé, D.; Balogh, L,; Polyák, A.; Király, R.; Márián, T.; Pawlak, D.; Zaknun, J.J.; Pillai, M.R.A.; Jánoki, G. Multispecies animal investigation on biodistribution, pharmacokinetics and toxicity of ^{177}Lu-EDTMP, a potential bone pain palliation agent. *Nucl. Med. Biol.*, **2010**, *37*, 215-226.

[15] Sola, G.A.R.; Arguelles, M.G.; Bottazzini, D.L.; Furnari, J.C.; Parada, I.G.; Rojo, A.; Vera Ruiz, H. Lutetium-177-EDTMP for bone pain palliation. Preparation, biodistribution and preclinical studies. *Radiochim. Acta*, **2000**, *88*, 157-161.

[16] Ando, A.; Ando, I.; Tonami, N.; Kinuya, S.; Kazuma, K.; Kataiwa, A.; Nakagawa, M.; Fujita, N. ^{177}Lu-EDTMP: A potential therapeutic bone agent. *Nucl. Med. Commun.,* **1998**, *19*, 587-591.

[17] Fellner, M.; Baum, R.; Kubicek, V.; Hermann, P; Roesch, F. ^{177}Lu-BPAMD - From bone imaging to therapy with a macrocycle-bisphosphonate ligand. *J. Nucl. Med.*, **2010**, *51(Suppl. 2)*, 1164.

[18] Shultz, M.D.; Wilson, J.D.; Fuller, C.E.; Zhang, J.; Dorn, H.C.; Fatouros, P.P. Metallofullerene-based nanoplatform for brain tumor brachytherapy and longitudinal imaging in a murine orthotopic xenograft model. *Radiology,* **2011**, *261*, 136-143.

[19] Chakraborty, S.; Das, T.; Banerjee, S.; Sarma, H.D.; Venkatesh, M. Preparation and preliminary biological evaluation of ^{177}Lu labeled hydroxyapatite as a promising agent for radiation synovectomy of small joints. *Nucl. Med. Commun.*, **2006**, *27*, 661-668.

[20] Chakraborty, S.; Das, T.; Sarma, H.D.; Venkatesh, M.; Banerjee, S. Preparation and preliminary studies on ^{177}Lu-labeled hydroxyapatite particles for possible use in therapy of liver cancer. *Nucl. Med. Biol.*, **2008**, *35*, 589-597.

[21] Unni, P.R.; Chaudhari, P.R.; Venkatesh, M.; Ramamoorthy, N.; Pillai, M.R.A. Preparation and bioevaluation of ^{166}Ho labeled hydroxyapatite (HA) particles for radiosynovectomy. *Nucl. Med. Biol.*, **2002**, *29*, 199-209.

[22] Available from: http://www.britatom.gov.in/docs/rph_ria_cat/RPH_COLD/RPH _ TCK30.pdf [Accessed Oct 20, 2014].

[23] Basu, S.; Kand, P.; Mallia, M.; Korde, A.; Shimpi, H. Gratifying clinical experience with an indigenously formulated single-vial lyophilized HYNIC-TOC kit at the Radiopharmaceutical Division of BARC: A pivotal boost for building up a peptide receptor radionuclide therapy programme in an Indian setting. *Eur. J. Nucl. Med. Mol. Imaging*, **2013**, *40*, 1622-1624.

[24] Das, T.; Chakraborty, S.; Kallur, K.G.; Venkatesh, M.; Banerjee, S. Preparation of patient doses of ^{177}Lu-DOTA-TATE using indigenously produced ^{177}Lu: The Indian experience. *Cancer Biother. Radiopharm.,* **2011**, *26*, 395-400.

[25] Danthala, M.; Kallur, K.G.; Prashant, G.R.; Rajkumar, K.; Rao, M.R. ^{177}Lu-DOTATATE therapy in patients with neuroendocrine tumours: 5 years' experience from a tertiary cancer care centre in India. *Eur. J. Nucl. Med. Mol. Imaging*, **2014**, (In-Press) DOI: 10.1007/s00259-014-2710-1.

[26] Yuan J.; Liu, C.; Liu, X.; Wang, Y.; Kuai, D.; Zhang, G.; Zaknun. J. J. Efficacy and Safety of ^{177}Lu-EDTMP in Bone Metastatic Pain Palliation in Breast Cancer and Hormone Refractory Prostate Cancer: A Phase II Study. *Clin. Nucl. Med.,* **2013**; *38*, 88-92.

[27] Das, T.; Chakraborty, S.; Banerjee, S.; Nair, K.V.V.; Chirayil, V.; Venkatesh, M.; Sarma, H.D.; Bal, C.S.; Arora, G. ^{177}Lu-EDTMP: A new radiopharmaceutical for palliation of bone pain in cancer patients with skeletal metastases. *BARC Newsletter*, **2009**, *305*, 2-11.

[28] Singh, N.; Krishna, B.A.; Vyas, M.; Venkatesh, M.; Banerjee, S.; Das, T.; Nair, K.V.V.; Chakraborty, S. Lutetium DOTATATE whole body scans - A novel approach for evaluation of neuroendocrine tumors. *Ind. J. Nucl. Med.*, **2011**, *26*, 135-138.

[29] Virgolini, I., Ambrosini, V., Bomanji, J.B., Baum, R.P., Fanti, S., Gabriel, M., Papathanasiou, N.D., Pepe, G., Oyen, W., De Cristoforo, C., Chiti, A. Procedure guidelines for PET/CT tumour imaging with ^{68}Ga-DOTA- conjugated peptides: ^{68}Ga-DOTA-TOC, ^{68}Ga-DOTA-NOC, ^{68}Ga-DOTA-TATE. *Eur. J. Nucl. Med. Mol. Imaging*, **2010**, *37*, 2004-2010.

[30] Ambrosini, V., Campana, D., Tomassetti, P., Fanti, S. ^{68}Ga-labelled peptides for diagnosis of gastroenteropancreatic NET. *Eur. J. Nucl. Med. Mol. Imaging*, **2012**, *39 (Suppl. 1)*, S52-S-60.

[31] Maecke, H.R., Hofmann, M., Haberkorn, U. ^{68}Ga-labeled peptides in tumor imaging. *J. Nucl. Med.,* **2005**, *46(Suppl. 1)*, 172S-178S.

Received: May 01, 2014 Revised: June 08, 2014 Accepted: March 09, 2015