

Production, quality control and biological evaluation of ^{153}Sm -TTHMP as a possible bone palliation agent

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ABSTRACT

Introduction: Various bone palliative therapeutic agents have been developed and widely used for bone metastasis such as ^{153}Sm -EDTMP. In this study, production, quality control and biodistribution studies of a newly developed therapeutic compound have been presented followed by imaging studies in wild-type rodents.

Methods: ^{153}Sm -TTHMP was prepared starting from ^{153}Sm - SmCl_3 , prepared by neutron activation of an enriched ^{152}Sm sample (purity >98%), and in-house synthesized TTHMP in 1h at 25°C followed by stability tests, partition coefficient determination and biodistribution studies of in wild-type rodents using scarification and SPECT imaging.

Results: The radiolabeled Sm complex was prepared in high radiochemical purity (>99%, ITLC) and specific activity of 278 GBq/mmol and demonstrated significant stability at 4, 25 and 37°C (in presence of human serum). Initial biodistribution data showed significant bone accumulation of the tracer in 48h.

Conclusion: ^{153}Sm -TTHMP can be a potential candidate for bone pain palliation therapy in skeletal metastases, although further biological studies in other mammals is still needed.

Keywords: Sm-153, TTHMP, Radiopharmaceutical, Therapy, Biodistribution, Imaging

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INTRODUCTION

Bone metastases are common in the course of various tumors such as prostate, breast, and lung carcinoma and they often entail an occurrence of progressive pain (1). Bone metastases occur in many patients with solid malignant tumors (2). Approximately 50% of patients with breast carcinoma and 80% of patients with prostate carcinoma develop metastatic bone disease and nearly half of them experience bone pain (3). In these patients who have progressive disease despite treatment, a systemic bone-avid radiopharmaceutical for treatment of widespread bony metastases has potential benefit (4). Radionuclide therapy using ^{32}P , ^{89}Sr , ^{90}Y , ^{153}Sm and ^{186}Re has been proposed as an alternative modality for management of bone pain (5).

Samarium-153 has favorable radiation characteristics, medium-energy beta particle emissions ($E_{\text{max}} = 810 \text{ keV}$) which is desirable for treatment, medium-energy gamma photon (103 keV) which is suitable for imaging, and short half-life (46.3 h). This radionuclide is the most widely used pain palliation radiopharmaceutical in the United States in form of EDTMP complex (Lexidronam) (6).

However, the search for the development of new ligands with higher stability, better pharmacokinetics and lower unwanted tissue uptakes (liver and GI) is still ongoing. Various complexes including new cyclic mixed phosphonate/carbonate ligands (7), alkyl phosphonates (PDTMP) (8) and hydroxyl-containing phosphonates (APDDMP) (9) have been developed and evaluated while none of these ^{153}Sm -complexes demonstrated better performance compared to Lexidronam (Figure 1).

In continuation of developing bone pain palliation agents for the use in the country as well as developing better quality compounds (10, 11), in this work, we report the preparation, quality control and

biodistribution of a new Sm-153 complex of recently synthesized ligand (12), Triethylene Tetramine Hexa (Methylene Phosphonic acid) (^{153}Sm -TTHMP) developed for ultimate bone pain palliation therapy (Figure 1).

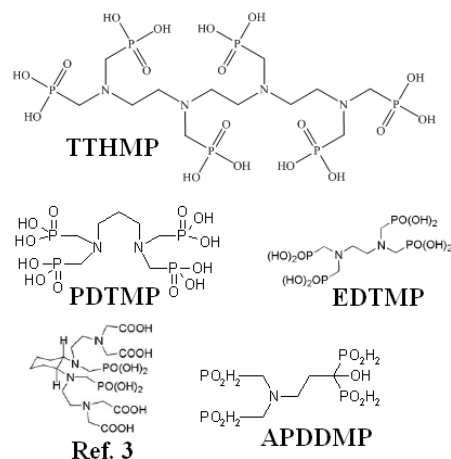


Figure 1. Structures for some phosphonates ligands used in ^{153}Sm labeling.

METHODS

Production of ^{153}Sm was performed at the Tehran Research Reactor (TRR) using ^{152}Sm (n, gamma) ^{153}Sm nuclear reaction. Samarium-152 with purity of >98% was obtained from ISOTECH Inc., USA. All chemicals were purchased from Sigma-Aldrich Chemical Co. U.K. Radiochromatography was performed by counting of Whatman No.2 using a thin layer chromatography scanner, Bioscan AR2000, Paris, France. Calculations were based on the 103 keV peak for ^{153}Sm . All values were expressed as mean \pm standard deviation (Mean \pm SD) and the data were compared using student T-test. Statistical significance was defined as $P < 0.05$. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd edn.

Production and quality control of $^{153}\text{SmCl}_3$ solution

Samarium-153 was produced by neutron irradiation of 1 mg of enriched $^{152}\text{Sm}_2\text{O}_3$ (^{152}Sm , 98.7% from ISOTECH Inc.) according to reported procedures (13) in the Tehran Research Reactor at a thermal neutron flux of $5 \times 10^{13} \text{ n.cm}^{-2}.\text{s}^{-1}$ for 2 days. Specific activity of the produced ^{153}Sm was 345 mCi/mg. The irradiated target was dissolved in 100 μl of 1.0 M HCl, to prepare $^{153}\text{SmCl}_3$ and diluted to the appropriate volume with ultra pure water, to produce a stock solution. The mixture was filtered through a 0.22 μm biological filter and sent for use in the radiolabeling step. The radionuclidic purity of the solution was tested for the presence of other radionuclides using beta spectroscopy as well as HPGe spectroscopy for the detection of various interfering beta and gamma emitting radionuclides. The radiochemical purity of the $^{153}\text{SmCl}_3$ was checked using 2 solvent systems for ITLC (A: 10mM DTPA pH.4 and B: ammonium acetate 10%: methanol (1:1)) and HPLC.

Synthesis of triethylene tetramine hexa(methylenephosphonic Acid) (TTHMP)

The experimental procedure for the synthesis of TTHMP ligand was according to other bis-phosphonates as reported (14). Briefly, a quantity of 0.48 g (3.3 mmoles) of triethylenetetramine was dissolved in 0.75 ml of concentrated HCl and a concentrated aqueous solution of 1.62 g (20 mmoles) of phosphorous acid. The resulting solution was heated to reflux temperature and 3.2 ml of 37% aqueous formaldehyde solution (40 mmoles) was added dropwise in the course of 1 h to the refluxing solution and refluxing was continued for another 1h. The result of reaction is an ethanol precipitated of a slightly yellow product from the concentrated reaction solution [m.p. 90-92°C, $^1\text{H-NMR}$ (D_2O , δ ppm): 3.02-3.25(m,

12 H, $>\text{N-CH}_2\text{CH}_2\text{-N}<$), 3.37-3.47(m, 12 H, $-\text{NCH}_2\text{-PO}_3\text{H}_2$)].

Radiolabeling of TTHMP with $^{153}\text{SmCl}_3$

A stock solution of TTHMP was prepared by dissolution in 1 N NaOH and diluted to the appropriate volume with ultra pure water by dissolving 250 mg of TTHMP in 1.5 ml NaOH (2N) and 3.5 ml distilled H_2O , pH. 12. Then 0.3 ml of this solution was added to 200 μl $^{153}\text{SmCl}_3$ (5.7 mCi) (S.A. 345 mci/mg) and pH adjusted to 7 using phosphate buffer. The reaction mixtures were incubated with stirring at room temperature for 1h. Various parameters such as ligand concentration, pH of the reaction mixture, incubation time, reaction temperature were optimized to achieve maximum complexation yield. Sterility, apyrogenicity and toxicity were ascertained by routine methods. The radiolabeling yield of the ligand was determined with paper chromatography using Whatman No. 2 paper by sampling 5 μl of the reaction mixture on the paper strip followed by developing in $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$ (2:20:40) mixture.

Stability studies

The stability of the complex stored at room temperature (22°C ambient), fridge (4°C) and presence of freshly-prepared human serum (at 37°C) was studied at different intervals of time by determining the radiochemical purity of the complex by paper chromatography in $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$ (2:20:40) system.

Determination of partition coefficient

Partition coefficient ($\log P$) of ^{153}Sm -TTHMP complex was calculated followed by the determination of P (P= the ratio of specific activity of the aqueous to organic phase). A mixture of 100 μl of 2-octanol and 100 μl of radiolabeled Samarium complex at 37°C was vortexed for 2 hours and then left for 30 minutes in room temperature. Then 5 μl of the octanol and aqueous phases were

sampled and counted in HPGe detector for 20 seconds.

Biodistribution of ^{153}Sm cation and ^{153}Sm -TTHMP in wild-type rats

To determine its biodistribution, ^{153}Sm -TTHMP was administered to normal rats. For comparison, free Sm^{3+} cation buffer solution was also administered. Briefly, 200 μl of final ^{153}Sm -TTHMP solution with 0.7 mCi radioactivity was injected intravenously to rats through their tail vein. The animals were sacrificed at the exact time intervals (2, 4, 24, 48 hours), and specific activity of different organs was calculated as percentage of injected dose per gram using HPGe detector (%ID/g).

Single photon emission computed tomography (SPECT) imaging of ^{153}Sm -TTHMP in wild-type rats

For imaging studies, ^{153}Sm -TTHMP solution (7.4 MBq, 200 μL) was injected intravenously to rats through their tail veins followed by propofol-xylazine mixture injection for anaesthetization. The images were acquired after administration of the radiopharmaceutical by a single-head SPECT system (Siemens, Germany) based on 103 keV peak (15% energy window). The rat-to-septa distance was 12 cm.

RESULTS AND DISCUSSION

Ligand synthesis

TTHMP ligand was synthesized and the structure was determined using H NMR, C NMR, P NMR and IR methods which was equivalent to other commercial authentic samples of bis-phosphonates used in radiopharmacy, according to the conventional method (Figure 2).

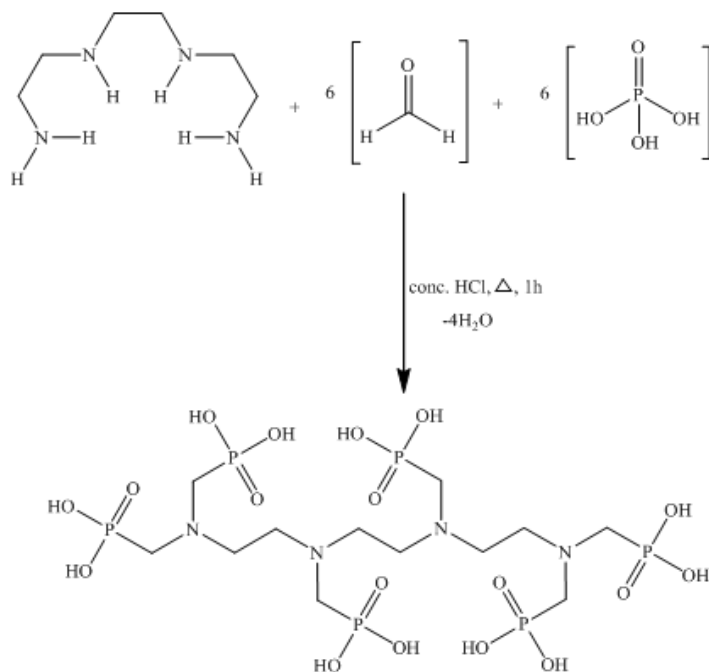


Figure 2. Synthetic scheme of TTHMP.

Radionuclide production

The radionuclide was prepared in a research reactor according to regular methods with a specific activity of 350 mCi/mg for radiolabeling use. The radioisotope was dissolved in acidic media as a starting sample and was further diluted and evaporated for obtaining the desired pH and volume followed by sterile filtering. Gamma-ray spectrum revealed the presence of ^{154}Eu ($<4.7 \times 10^{-5}\%$ of ^{153}Sm) and ^{155}Eu ($<2.4 \times 10^{-5}\%$ of ^{153}Sm) at the end of irradiation.

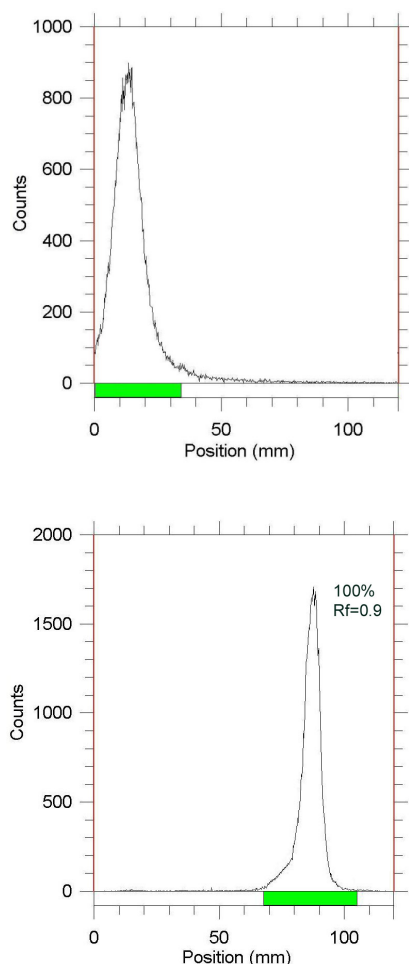


Figure 3. ITLC chromatograms of ^{153}Sm - SmCl_3 (above) and ^{153}Sm -TTHMP (below) solutions on Whatman 2 MM paper using NH_4OH : MeOH : H_2O (0.2:2:4).

Radiochemical impurities in the ^{153}Sm sample used in the radiolabeling step were checked by two systems. As stationary phase for paper chromatography system, Whatman 2 MM paper was used. In %10 ammonium acetate:methanol, the free samarium cation in $^{153}\text{Sm}^{3+}$ form remains at the origin ($R_f = 0.0$) and while other Sm-153 species migrate to higher R_f s (0.8). Another eluent for Sm^{3+} detection was 10 mM DTPA aqueous solution at pH.3 ($R_f = 0.8$).

Labeling optimization studies

In order to obtain maximum complexation yields, several experiments were carried out by varying different reaction parameters such as ligand concentration, pH, reaction time and temperature. Ligand concentration was varied between a wide ranges starting from 10 to 50mg/ml for TTHMP. It was observed that at room temperature 99% complexation was achieved with 15mg/ml of TTHMP. The best ITLC mobile phase was considered Whatman 2 MM paper using NH_4OH : MeOH : H_2O (0.2:2:4) as shown in Figure 3.

Variation of complexation yields with respect to TTHMP concentration is shown in Figure 4. The effect of variation of pH on complexation yield at room temperature was also studied by varying the pH of the reaction mixture from 2 to 12 using 1M HCl or 2M NaOH solution. Maximum yield of 100% was observed at pH 7-8 for complex. The effect of pH on the complexation yield for ^{153}Sm -TTHMP complex is shown in Figure 5.

The effect of reaction temperature on complexation yield was not studied for this complex, as sufficiently high complexation yield was achieved at room temperature. The reaction mixture was incubated at room temperature for different time periods and 60 min incubation was found to be adequate to yield maximum complexation.

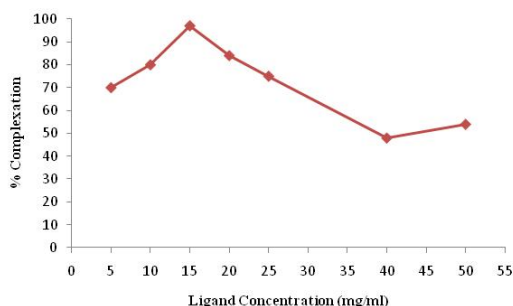


Figure 4. Effect of ligand concentration on complexation yield of ^{153}Sm -TTHMP.

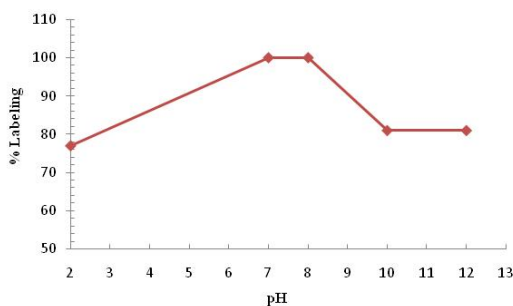


Figure 5. Effect of variation of pH on complexation yield of ^{153}Sm -TTHMP at room temperature.

Stability

The stability of the ^{153}Sm -TTHMP complex prepared under optimized reaction conditions was studied and observed that the complex showed excellent stability even when stored at room temperature. The complex remained stable to the extent of 96% up to 72h, whereas stability this compound was shown 90% for 48h in refrigerator.

In vitro stability against human serum

This was carried out by incubating 0.1 ml of the radioactive complex and 0.3 ml human serum at 37°C and at $\text{pH}=7$ for 4 h. After 4

h, activity was measured by RTLC in $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$ (2:20:40) system. The result of RTLC was shown that this complex is stable (95%) in this condition.

Determination of water/lipid solubility

A mixture of 100 μl of 2-Octanol and 100 μl of radiolabeled Samarium complex at 37°C was vortexed for 2 hours and left for 30 minutes in room temperature. Then 5 μl of the octanol and aqueous phases were sampled and counted in HPGe detector for 20 seconds. P was calculated from the ratio showing very high water solubility of the complex leading to very low unwanted liver and gastrointestinal tract uptake of the complex and also high excretion through kidneys.

Biodistribution of ^{153}Sm cation and ^{153}Sm -TTHMP in wild-type rats

The animals were sacrificed by CO_2 asphyxiation at selected times after injection (2, 4, 24 and 48h). Dissection began by drawing blood from the aorta followed by removing the heart, spleen, muscle, bone, kidneys, liver, intestine, stomach, lungs and skin samples. The tissue uptakes were calculated as the percent of area under the curve of the related photo peak per gram of tissue (% ID/g) (Figure 6). The liver uptake of the cation is comparable with many other radio-lanthanides mimicking calcium cation accumulation; about %3 of the activity accumulates in the liver after 48 h. The blood content is low at all time intervals and this shows the rapid removal of activity in the circulation. The lung, muscle and also skin do not demonstrate significant uptake which is in accordance with other cations accumulation. A %4 bone uptake is observed for the cation which remains almost constant after 96 h (data not shown). The spleen also has significant uptake possibly related to reticuloendothelial uptake.

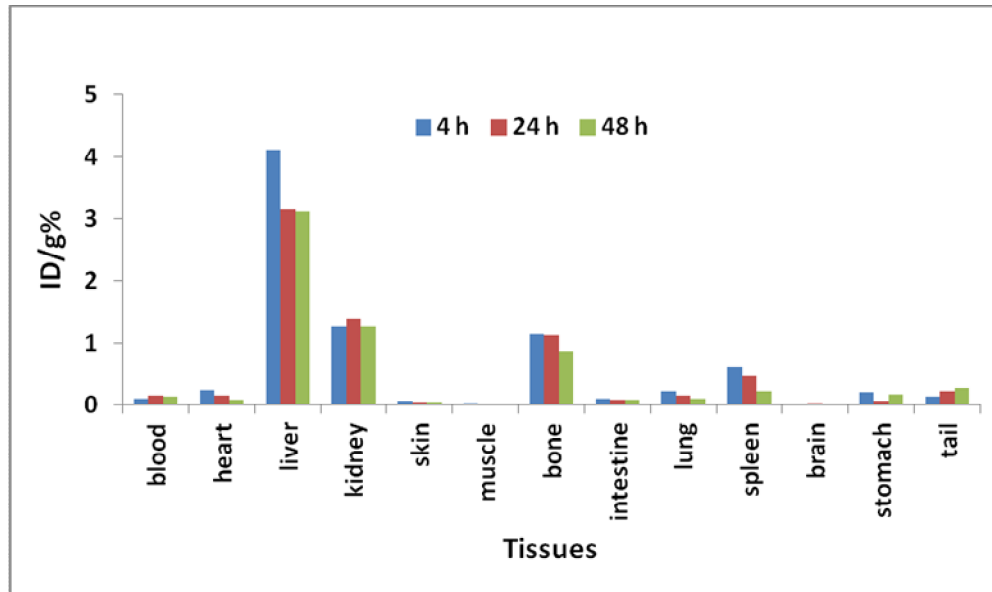


Figure 6. Biodistribution of ^{153}Sm -free in different organs of normal rat.

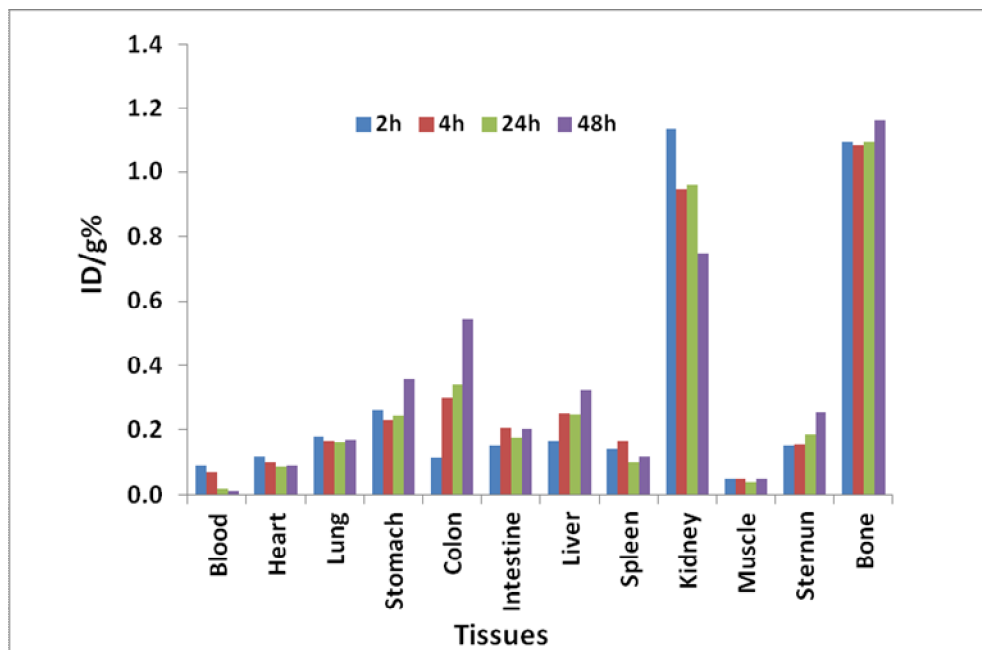


Figure 7. Biodistribution of ^{153}Sm -TTHMP in different organs of wild-type rats.

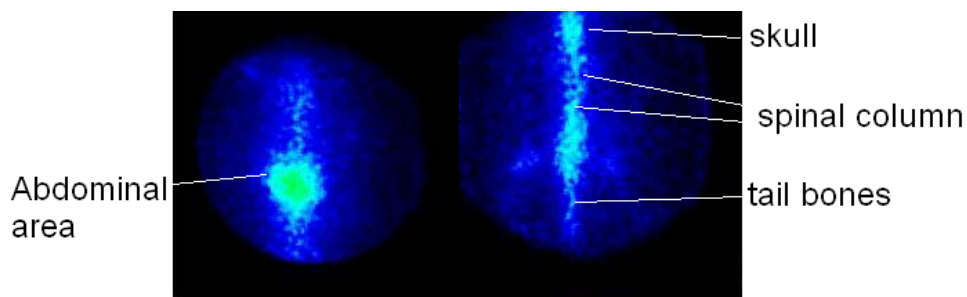


Figure 8. SPECT images of ^{153}Sm -TTHMP (right) and ^{153}Sm (left) 24 h post injection in wild-type rats.

The kidney plays an important role in ^{153}Sm cation excretion especially after 24 h.

A volume (200 μl) of final ^{153}Sm -TTHMP solution with 0.7 mCi radioactivity was injected intravenously to rats through their tail vein. The animals were sacrificed at the exact time intervals (2, 4, 24, 48 hours), and specific activity of different organs was calculated as percentage of injected dose per gram using HPGe detector (Figure 7).

As shown in Fig 7. The major radioactivity is accumulated in bones as expected for bone-avid radiopharmaceuticals, also due to the presence of anionic properties of the complex and relatively small size of the molecules, the complex is also excreted through the kidneys. Due to liver uptake a significant GI uptake is observed.

The cation radioactivity is almost reaching a maximum after 48 h while the complex is rapidly removed from the circulation into the bones as expected for a bone pain palliation such as ^{153}Sm -EDTMP (3-4%). Also a significant difference is observed for the liver accumulation among two species, free cation, like many other lanthanides, accumulates 1-4% in the liver while the complex is less than %0.5 at all time intervals as expected for a suitable bone agent. The spleen uptake is low for ^{153}Sm -TTHMP while a minor cation uptake is observed in this tissue as observe in other radio metals.

The bone uptake among the two species is not significantly different in 24h while after 48 hours the bone uptake increases in case of ^{153}Sm -TTHMP and the cation uptake.

As shown in other works (12), the Lu-177 uptake of the complex. Compared to that work, the liver uptake of the Sm-complex is less that than the Lu-complex, leading to more safety aspects of the complex due to unwanted irradiation to the surrounding tissues. On the other hand comparison of the complex with the well known Sm-153 EDTMP complex demonstrates the high specific activity of Sm-153 EDTMP in bones and less unwanted uptake in the other organs. That makes the Sm complexes of the discussion superior to the Lu-177 analogs.

For the imaging studies of the radiopharmaceutical a wild type rats was used showing a distinct skeletal uptake in all time intervals (Figure 8).

CONCLUSION

For ^{153}Sm -TTHMP the radiochemical purity was higher than 99% and the labeling and quality control took one hour. The radiolabeled Sm complex was prepared in high radiochemical purity (>99%, ITLC) and specific activity of 278 GBq/mmol and demonstrated significant stability at 4, 25

and 37°C (in presence of human serum). The final preparation was administered to wild-type rats and biodistribution of the radiopharmaceutical was checked 4-48 hours later showing major accumulation of the drug in the bone tissues. Preliminary imaging studies also demonstrate bone uptake in rats after 24h. ^{153}Sm -TTHMP can be a potential candidate for bone pain palliation therapy in skeletal metastases, although further biological studies in other mammals is still needed.

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