

Production, Quality Control and Biological Evaluation of ^{153}Sm -EDTMP in Wild-Type Rodents

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ABSTRACT

Introduction: Nowadays various bone pain palliative therapeutic agents have been developed for bone metastases. Among those, ^{153}Sm -ethylenediamine tetramethylene phosphonic acid (^{153}Sm -EDTMP) is the major therapeutic agent which is widely used in the world. In this study, production, quality control and biodistribution studies of this therapeutic radiopharmaceutical have been presented and followed by imaging studies in a wild-type rabbit for the first time in order to make preparations for this agent to be officially approved in the country.

Methods: ^{153}Sm -EDTMP was produced using ^{153}Sm - SmCl_3 , prepared by neutron activation of an enriched ^{152}Sm sample (purity >98%), and in-house synthesized EDTMP in 4h at 100°C. The analytical data for the structure determination and purity of the ligand was obtained and shown to be identical to an authentic sample from a European vendor. The Radiochemical purity of ^{153}Sm -EDTMP was checked by RTLC and ITLC. The biodistribution of ^{153}Sm -EDTMP in wild-type rodents was checked and SPECT imaging as well as following sacrificing the animal.

Results: The radiolabeled Sm complex was prepared in high radiochemical purity (>99%, RTLC) followed by initial biodistribution data with the significant bone accumulation (>70%) of the tracer in 48h which is comparable with the reported methods.

Conclusion: The produced ^{153}Sm -EDTMP properties suggest good potential for efficient use of this radiopharmaceutical for bone pain palliation and as substitute for other agents, such as $^{89}\text{SrCl}_2$ and ^{32}P , currently used in the country.

Key words: Sm-153, EDTMP, Radiopharmaceutical, Therapy, Biological evaluation

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INTRODUCTION

Bone metastases are a frequent complication in various tumors such as prostate, breast, and lung carcinoma often causing 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 are affected by metastatic bone diseases and nearly half of them experience bone pains (3). In these patients who have progressive diseases despite treatment, a systemic bone-avid radiopharmaceutical has potential benefit for the treatment of widespread bony metastases (4). Radionuclide therapy using ^{32}P , ^{89}Sr , ^{90}Y , ^{186}Re and ^{153}Sm etc. has been proposed as an alternative modality for the management of bone pains (5-7). Samarium-153 has favorable radiation characteristics, such as the medium-energy beta particle emissions ($E_{\text{max}} = 810 \text{ keV}$) which is desirable for treatment, the medium-energy gamma photon (103 keV) which is suitable for imaging, and the short half-life (46.3 h). This radionuclide is the most widely used pain palliation radiopharmaceutical agent in the United States (8). It can be attached to phosphonic acid ligands which have been identified to have *in vivo* localization and clearance properties, these are nearly ideal for radionuclide therapy in patients with metastatic bone cancers (9).

^{153}Sm -ethylenediamine tetramethylene phosphonic acid (Figure 1) is a currently used agent for effective palliative treatment of skeletal metastases (10, 11). This phosphonate complex concentrates in the skeleton, in proportion to osteoblastic activity (12).

In this paper, we reported the preparation, quality control and biodistribution of ^{153}Sm -EDTMP complex and imaging characteristics of this agent in animal model, in order to pave the way for its future clinical application.

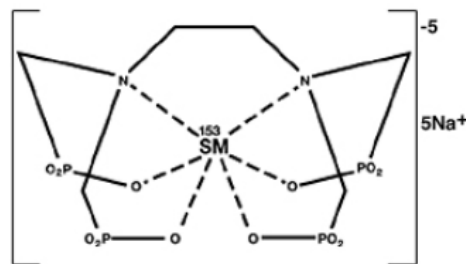


Figure 1. Chemical structure of ^{153}Sm -EDTMP.

METHODS

Production of ^{153}Sm was performed at the Tehran Research Reactor (TRR) using ^{152}Sm (n, γ) ^{153}Sm nuclear reaction. Samarium-152 with purity of >98% was obtained from ISOTEC 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. Gama-spectroscopy on the base of 103 keV peak and beta-spectroscopy were carried out by using the HPGe detector and the Wallac 1220 Quantulus liquid scintillation spectrometer, respectively.

All the values were expressed as mean \pm standard deviation (Mean \pm SD) and the data were compared using student T-test. The 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 450-500 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 two solvent systems for ITLC (A: 10mM DTPA pH.4 and B: ammonium acetate 10%: methanol (1:1)).

Synthesis of EDTMP

EDTMP was synthesized using Phosphorous acid (PA), Ethylenediamine and formaldehyde in the presence of HCl by a modified Mannich-type reaction (14). Phosphorous acid (33.66 g) and conc. HCl (33.44 g) stirred in a vessel; then Ethylenediamine dihydrochloric acid (5 g) was added to and it was heated to reflux. Aqueous solution of formaldehyde (37 %) is then added in a drop wise fashion. Reflux (at 100°C) was continued for 4h and the boiling suspension was then filtered under vacuum. It was purified after recrystallization from water/methanol, m.p. 214-215°C. IR (KBr, $\nu \text{ cm}^{-1}$): 3308, 2633, 2311, 1668, 1436, 1356. $^1\text{H-NMR}$ (D_2O , δ ppm): 3.53 (d, $J = 12.3 \text{ Hz}$, 8H, -N-CH₂-P=O), 3.85 (s, 4H, -N-CH₂-). $^{13}\text{C NMR}$ (D_2O , δ ppm): 51.63, 52.73. $^{31}\text{P NMR}$ (D_2O , δ ppm): 10.52.

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

A stock solution of EDTMP was prepared by dissolving it in 1 N NaOH and diluting the solution to the appropriate volume with

ultra pure water, to produce a solution of 50 mg/ml. For Labeling, an appropriate amount of the $^{153}\text{SmCl}_3$ solution containing the 30-40 mCi of activity was added to the 400 μl of EDTMP solution. The complex solution was kept at room temperature for 45 min. The final solution was passed through a 0.22 μm membrane filter and pH was adjusted to 7-8.5 with 0.05 M phosphate buffer. Sterility, apyrogenicity and toxicity were ascertained by routine methods. The complex yield and the radiochemical purity were determined using both Whatman No. 2 chromatography paper and ITLC-SG eluted with $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$ (0.2:2:4). Stability tests were carried out for one week by chromatography on ITLC-SG using mentioned solvent.

Biodistribution of ^{153}Sm -EDTMP in wild-type rats

To determine its biodistribution, ^{153}Sm -EDTMP was administered to normal rats. After diluting of final complex solution, a volume of 50-100 μl containing $180 \pm 5 \mu\text{Ci}$ of radioactivity was injected intravenously to rats through their tail veins. The animals were sacrificed at the exact intervals of 4, 24 and 48 h post injection, and the specific activity of different organs was calculated as percentage of the injected dose per gram tissues using the NaI detector.

SPECT imaging of ^{153}Sm -EDTMP in wild-type rabbit

In addition, an activity (1 mCi/kg) of diluted ^{153}Sm -EDTMP solution was injected intravenously to a male rabbit through its ear vein. The Image was taken at 48 hours after administration of the radiopharmaceutical by a dual-head SPECT system. The mouse-to-high energy septa distance was 12 cm. The useful field of view (UFOV) was 540 mm \times 400 mm. The spatial resolution was 10 mm FWHM at the CFOV. Sixty four projections were acquired for 30 seconds per view with a 64 \times 64 matrix.

RESULTS

The radionuclide was prepared in a research reactor according to regular methods with a specific activity of 450-500 mCi/mg for radiolabeling use. The radioisotope was dissolved in acidic media as a starting sample. Then the solution 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 2). Beta spectroscopy depicted a continual spectrum (Figure 3).

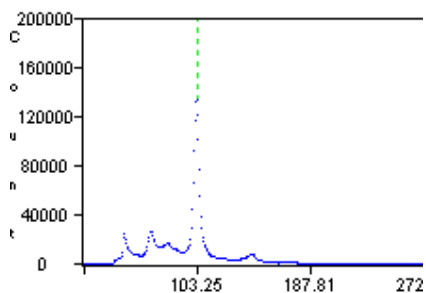


Figure 2. Gamma spectrum for Sm-153 prepared by neutron irradiation of Sm-152 sample using an HPGe detector.

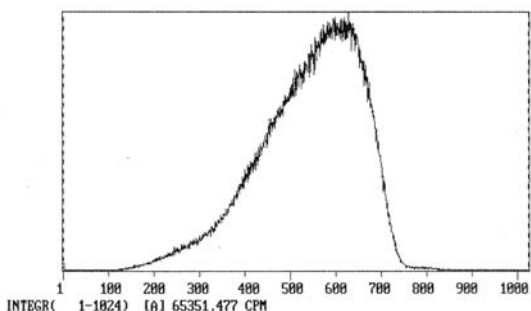


Figure 3. Beta spectrum for Sm-153 prepared by neutron irradiation of Sm-152 sample using a liquid scintillation counter.

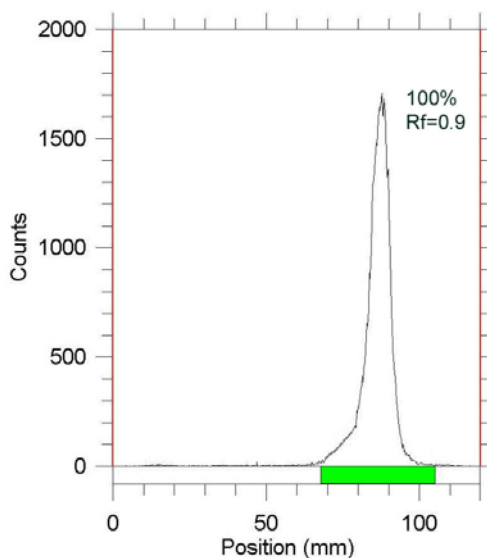
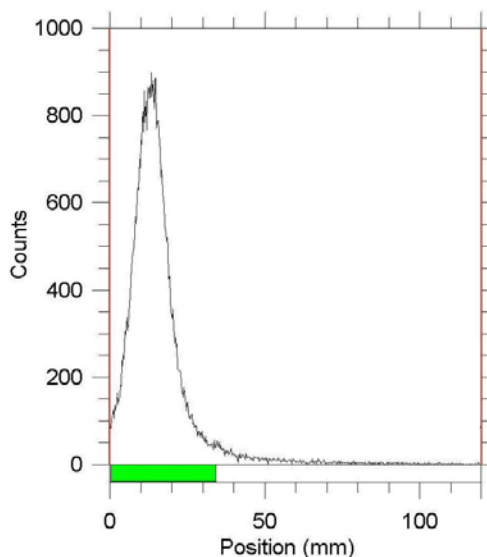


Figure 4. ITLC chromatograms of ^{153}Sm - SmCl_3 (above) and ^{153}Sm -EDTMP (below) solutions on Whatman no. 2 paper using $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$ (0.2:2:4)

Radiochemical impurities in the ^{153}Sm sample used in the radiolabeling step were checked by two systems. A mixture of $\text{NH}_4\text{OH}:\text{MeOH}:\text{H}_2\text{O}$ (0.2:2:4) solution was used as mobile phase for both systems. As stationary phase, Whatman No. 2 paper was used for paper chromatography system, and a silica-gel sheet (10×1.5 cm) was used for ITLC system. In both systems, the free

samarium cation in $^{153}\text{Sm}^{3+}$ form remains at the origin ($R_f = 0.0$) and the ^{153}Sm -EDTMP complex migrates to higher R_f s ($R_f = 0.8$ - 0.9) (Figure 4). Another eluent for the Sm^{3+}

detection was 10 mM DTPA aqueous solution at pH.3 ($R_f = 0.8$) (Figure 5).

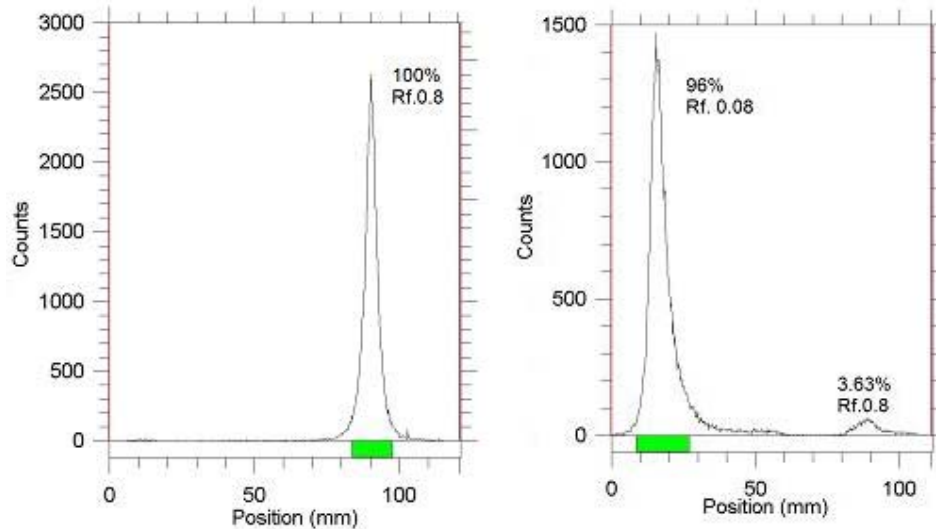


Figure 5. ITLC chromatograms of ^{153}Sm - SmCl_3 solution on Whatman no. 2 paper using 10 mM DTPA solution (pH.3) (Left) and 10% ammonium acetate:methanol (1:1) (Right).

Table 1. Percentage of injected dose per gram of tissues (ID/g %) at 4, 24 and 48hrs post injection of ^{153}Sm -EDTMP in wild type rats.

| Tissue | 4h | 24h | 48h |
|-----------|---------------|---------------|---------------|
| Blood | 0.006 (0.003) | 0.000 (0.000) | 0.000 (0.000) |
| Liver | 0.088 (0.009) | 0.097 (0.020) | 0.139 (0.055) |
| Spleen | 0.024 (0.018) | 0.010 (0.006) | 0.002 (0.003) |
| Stomach | 0.026 (0.008) | 0.044 (0.022) | 0.023 (0.009) |
| Intestine | 0.052 (0.020) | 0.045 (0.013) | 0.023 (0.014) |
| Kidney | 0.161 (0.039) | 0.098 (0.046) | 0.147 (0.080) |
| Bone | 1.774 (0.290) | 1.596 (0.060) | 2.463 (0.943) |

- . Three rats were used at each interval
- . All the values in the table have been expressed as average
- . The figures in the parentheses represent standard deviation

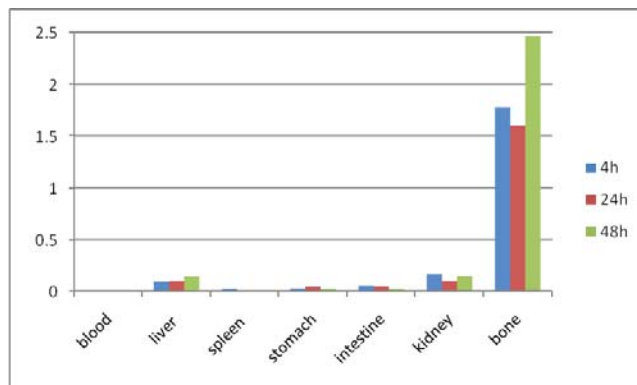


Figure 6. Percentage of injected dose per gram of tissues (ID/g %) at 4, 24 and 48hrs post injection of ^{153}Sm -EDTMP in wild type rats.

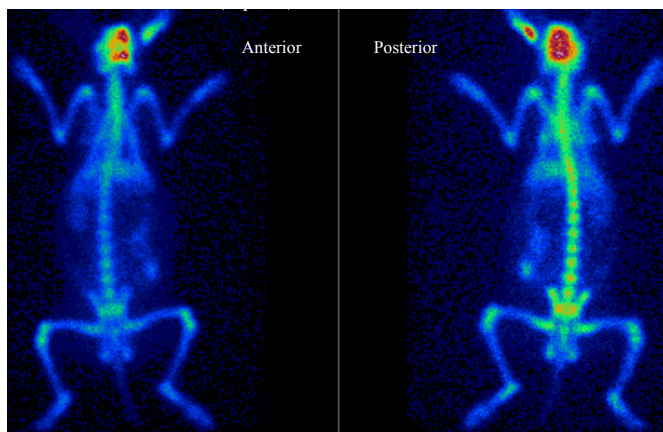


Figure 7. SPECT images of ^{153}Sm -EDTMP in a wild-type rabbit 48 hours post injection

For optimization of the labeling conditions, at room temperature, the best range of pH was 7.5-8. In addition, ^{153}Sm -EDTMP was labeled at different molar ratio of ^{153}Sm :EDTMP in order to determine the best molar ratio which was obtained 1:50. The Radiochemical purity of ^{153}Sm -EDTMP was more than 99%.

As shown in table 1 and figure 6, the complex is mainly washed out from the circulation in first few hours and trapped in bone tissues as the major part; the only significant activities are accumulated in liver and kidney which are the important target tissues for the free Sm.

This minor content of Sm cation is possibly a product of biodegradation and/or a part of natural complex accumulation. This trend is continuing for 48 hours for all tissues and more than 2% of the injected dose per gram is shown to be in target organs. A small portion of the radiopharmaceutical is also excreted via kidneys and is diminished as a function of time in 48h. For the imaging studies of the radiopharmaceutical a wild type rabbit was used showing a distinct skeletal uptake in all time intervals (Figure 7), this is in accordance with other reported studies of this complex in animals and human (15, 16).

DISCUSSION

EDTMP ligand was synthesized in house and the structure was determined using H NMR, C NMR, P NMR and IR methods which was better or equivalent to commercial authentic samples used in radiopharmacy. For ^{153}Sm -EDTMP, the radiochemical purity was higher than 99% and the labeling and quality control took one hour. The final preparation was administered to normal rats and biodistribution of the radiopharmaceutical was checked 4-48 hours post injection showing at least 70% accumulation of the drug in the bone tissues. ^{153}Sm -EDTMP was finally administered to a rabbit in order to access the SPECT imaging model for skeletal studies and the biodistribution was shown to be consistent with the other reported methods for this radiopharmaceutical.

Although there are various agents used in bone pain palliation, such as ^{32}P , $^{89}\text{SrCl}_2$, ^{186}Re -HEDP and ^{177}Lu -EDTMP, applying each of them imposes some limitations and disadvantage. For example, some of these agents are not routinely used, like ^{32}P and $^{89}\text{SrCl}_2$, because of their destructive side effects originated from their high beta-energy particles. However ^{186}Re -HEDP is used in some countries at present, the function of radiolabeling for preparation of this radiopharmaceutical is not easily practicable which burdens a kind of limitation to its use. ^{177}Lu -EDTMP is the other agent which is already under consideration; however, high cost of preparation limits its widespread uses.

^{153}Sm -EDTMP is a promising agent for bone pain palliation in skeletal metastases which has been used for more than a decade around the world. Our preliminary study for preparation of ^{153}Sm -EDTMP has been satisfactory and research groups in the Research Institute for Nuclear Medicine at Tehran University of Medical Sciences are

prepared to start its clinical trials in the country.

CONCLUSION

The produced ^{153}Sm -EDTMP properties suggest good potential for efficient use of this radiopharmaceutical for bone pain palliation and as substitute for other agents, such as $^{89}\text{SrCl}_2$ and ^{32}P , currently used in the country.

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