Original Article

Preparation of a ^{153}Sm-5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin complex as a possible therapeutic agent

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ABSTRACT

Introduction: Porphyrins are interesting derivatives with low toxicity, tumor avidity and rapid wash-out suggested as potential radiopharmaceuticals in radiolabeled form. In this work we report, synthesis, radiolabeling, quality control, stability, partition coefficient determination and biodistribution studies of 153Sm-5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (^{153}Sm-4-MPP) in wild-type rats.

Methods: [^{153}Sm]-4MPP was prepared using [^{153}Sm]$SmCl_3$ and 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (H_2-4MPP) for 18 h at 80-90°C. Stability of the complex was checked in final formulation and in presence of human serum for 24 h. The biodistribution of the labeled compound in vital organs of wild-type rats was studied. A detailed comparative pharmacokinetic study performed for ^{153}Sm cation and [^{153}Sm]-4-MPP up to 24h.

Results: The radiochemical purity of [^{153}Sm]-4MPP was reported >97±2% and >99±0.5% by ITLC and HPLC, respectively. The specific activity was 220-230 MBq/mmol. The calculated partition coefficient for the compound was (log P=-1.09). The complex is mostly cleared from the circulation through kidneys and liver. The kidney:blood and kidney:muscle ratios 24 h post injection were 14.75 and 42.4, respectively. kidney/liver ratio was almost constant at all time intervals (0.6).

Conclusion: [^{153}Sm]-4MPP was prepared at the optimized conditions and suitable characteristics. Further investigations such as biological studies of this agent on tumor-bearing models are needed.

Key words: Porphyrins; Quality control; Sm-15; Biodistribution

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INTRODUCTION

Metal-based radiopharmaceuticals are widely used in the treatment of cancer and much attention has been focused on designing new coordination compounds with improved pharmacological properties and a broader range of antitumor activity.

Strategies for developing new anticancer agents include the incorporation of carrier groups that can target tumor cells with high specificity. Lanthanide complexes may provide a broader spectrum of antitumor activity [1, 2].

Various metal-porphyrin complexes have shown interesting tumor-avid activity *in vitro* and *in vivo* and have found their ways into clinical studies such as Motexafin gadolinium used in the therapy of brain tumors [3], boronated porphyrin for boron-neutron capture therapy [4] and indium porphyrins for photo/sonotherapy [5].

Radiolabeled therapeutic porphyrins have been developed for the therapeutic purposes such as, ^{109}Pd-protoporphyrins [6], ^{109}Pd-porphyrins [7], ^{109}Pd-derivitized porphyrins [8], ^{188}Re-porphyrins [9] and ^{166}Ho-porphyrins [10].

Samarium-153 has favorable radiation characteristics, medium-energy beta particle emissions (E_{max} = 810 keV) which is desirable for treatment, medium-energy gamma photon (103 keV) which is also 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) [11].

Samarium-153 small labeled molecules including bleomycin and heterocyclic complexes demonstrated significant tumor accumulation in fibrosarcoma-bearing rodents [12, 13].

The effectiveness of Sm-166 chitosan complex therapy for radiosynovectomy is under investigation [14]. Attempts were made to demonstrate the possibility of Sm-153 application in radioimmunotherapy [15, 16].

Due to the interesting pharmacological properties of porphyrins such as solubility in serum, rapid wash-out, tumor avidity and feasible complexation with various bi/tri-valent metals [17], the idea of developing a possible tumor targeting agent by incorporating ^{153}Sm into a suitable porphyrin ligand, *i.e.* $H_2$4-MPP was investigated (Figure 1).

In this work we report, synthesis, radiolabeling, quality control, stability, partition coefficient determination and biodistribution studies of ^{153}Sm-4-MPP in wild-type rats.

The time/activity diagrams for the labeled compound in vital organs have been plotted compared to samarium cation.

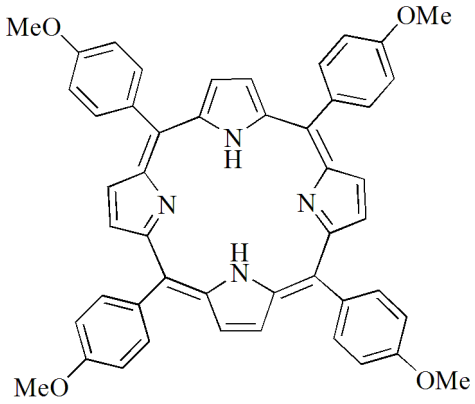

Fig 1. Structure of $H_2$4-MPP.

METHODS

Production of ^{153}Sm carried out at the Tehran Research Reactor (TRR) using ^{152}Sm(n, gamma)^{153}Sm nuclear reaction. Natural samarium oxide with purity of >99.99% was obtained from ISOTEC Inc. All chemicals were purchased from Sigma-Aldrich Chemical Co. U.K.. Radio-chromatography was performed by Whatman paper using a thin layer chromatography scanner, Bioscan AR2000, Paris, France. Analytical HPLC to determine the specific activity was performed by a Shimadzu LC-10AT, armed with two detector systems, flow scintillation analyzer (Packard-150 TR) and UV-visible (Shimadzu) using Whatman Partisphere C-18 column 250 × 4.6 mm (Whatman Co. NJ, USA). Calculations were based on the 103 keV peak of ^{153}Sm. All values were expressed as mean ± standard deviation (Mean± SD) and the data were compared using student T-test. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, second edition.

Production and quality control of $^{153}SmCl_3$ solution

Samarium-153 was produced by neutron irradiation of 1 mg of enriched $^{152}Sm_2O_3$ (^{152}Sm, 98.7% from ISOTEC Inc.) according to reported procedures [18] in Tehran Research Reactor at a thermal neutron flux of 5×10^{13} n.cm^{-2}.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 $^{153}SmCl_3$ 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 radiolableing 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 $^{153}SmCl_3$ was checked using 2 solvent systems for ITLC (A: 10mM DTPA pH.4 and B: ammonium acetate 10%: methanol (1:1)) and HPLC.

Preparation of 5,10,15,20-tetrakis(4-methoxyphenyl) porphyrin (H2-4MPP)

This compound was prepared according to the reported method using freshly distilled 4-methoxy benzaldehyde, pyrrole and propionic acid followed by oxidation in darkness [19]. The mixture was purified by column chromatography on alumina (diameter: 2.5 cm, length: 15 cm, mesh 60, eluent: dichloromethane). The fast eluting fraction with purple color was evaporated and recrystalized in CH_2Cl_2: hexane mixture and crystals sent for characterization. Yield; 18%, m.p.> 228-220°C. ^{1}H NMR ($CDCl_3$) δ (ppm) –2.8 (2 H, NH), 3.4-3.7 (12H, 4-OCH3) 7.71–7.82 (12 H), 8.14–8.27 (8 H), 8.85 (8 H). UV (toluene) λ_{max} (ε) = 420 nm (413300), 522 (15560), 549 (8080), 594 (5380), 648 (3870). IR (KBr) 3320, 3055, 3025, 1595. Mass (m/e) 558 (M+3).

Preparation of [^{153}Sm]-4-MPP

The acidic solution (2 ml) of [^{153}Sm]$SmCl_3$ (111-333 MBq, 3-9 mCi) was transferred to a 3 ml-borosilicate vial and heated to dryness using a flow of N_2 gas at 50-60°C. Fifty microlitres of 4-MPP in absolute ethanol (5 mg/ml ≈250 nmoles) was added to the samarium-containing vial followed by the addition of acetate buffer pH 5.5 (450 microliteres). The mixture refluxed at 80°C for 18h. The active solution was checked for radiochemical purity by ITLC and HPLC. The final solution was then passed through a 0.22 µm filter and pH was adjusted to 5.5-7.

Quality control of [^{153}Sm]-4-MPP

Radio thin layer chromatography: A 5 µl sample of the final fraction was spotted on a chromatography Whatman No. 2 paper, and developed in mobile phase mixture, 10% NH_4OAc and methanol 1:1.

High performance liquid chromatography: HPLC was performed with a flow rate of 1 ml/min, pressure: 130 kgF/cm^2 for 20 min. HPLC was performed on the final preparation using a mixture of water:acetonitrile 3:2 (v/v) as the eluent by means of reversed phase column Whatman Partisphere C_{18} 4.6 × 250 mm.

Determination of Partition coefficient

Partition coefficient (log *P*) of [^{153}Sm]-4-MPP was calculated followed by the determination of *P* (*P*= the ratio of specific activities of the organic and aqueous phases). A mixture of 1 ml of 1-octanol and 1 ml of isotonic acetate-buffered saline (pH=7) containing approximately 3.7 MBq of the radiolabeled samarium complex at 37°C was vortexed 1 min and left 5 min. Following centrifugation at >1200*g* for 5 min, the octanol and aqueous phases were sampled and counted in an automatic well-type counter. A 500 µl sample of the octanol phase from this experiment was shaken again two to three times with fresh buffer samples. The reported log *P* values are the average of the second and third extractions from three to four independent measurements.

Stability tests

The stability of the complex was checked according to the conventional ITLC method [20]. A sample of [^{153}Sm]-4-MPP (37 MBq) was kept at room temperature for 24 h while being checked by ITLC at time intervals in order to check stability in final product using above chromatography system. For serum stability studies, 500µl of freshly collected human serum was added to 36.1 MBq (976 µCi) of [^{153}Sm]-4-MPP, and the resulting mixture was incubated at 37°C for 24 h, aliquots (5 µl) were analyzed by ITLC.

Biodistribution of ^{153}Sm cation and ^{153}Sm-4-MPP in wild-type rats

To determine its biodistribution, ^{153}Sm-4-MPP was administered to normal rats. For comparison, free Sm^{3+} cation buffer solution was also administered. Briefly, 200µl of final ^{153}Sm-4-MPP solution with 100-150 µCi radioactivity was injected through the tail vein. The animals were sacrificed at the exact time intervals (2, 4 and 24 hours), and specific activity of different organs was calculated as percentage of injected dose per gram using HPGe detector (%ID/g).

RESULTS AND DISCUSSION

Ligand synthesis

4-MPP ligand was synthesized and the structure was determined using ^{1}H NMR, UV, MASS and IR methods which was equivalent to other commercial authentic samples of porphyrins according to the conventional method (Figure 2).

Radionuclide production

The radionuclide was prepared in Tehran Research Reactor according to conventional methods with a specific activity of 350 mCi/mg.

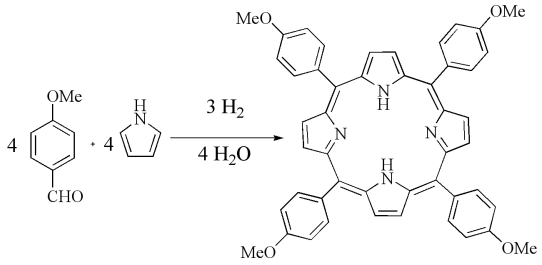

Fig 2. Synthetic scheme of H_2-4MPP.

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 (<4.7×10^{-5}% of ^{153}Sm) and ^{155}Eu (<2.4×10^{-5}% of ^{153}Sm) at the end of irradiation (Figure 3).

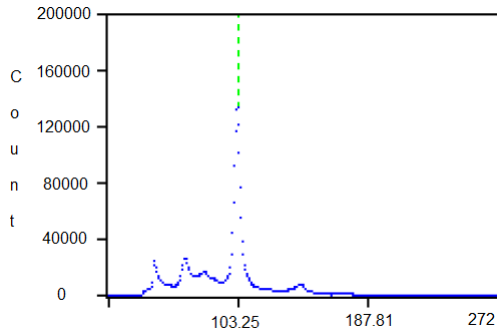

Fig 3. Gamma Spectrum for Sm-153 prepared by neutron irradiation of Sm-152 sample using HPGe detector.

Radiochemical impurities were checked by two systems. Whatman 2 MM paper was used -as stationary phase- for paper chromatography system. In %10 ammonium acetate:methanol, the free samarium cation in $^{153}Sm^{3+}$ form remains at the origin (R_f = 0.0) while other Sm-153 species migrate to higher R_fs (0.8).

The other eluent for Sm^{3+} detection was 10 mM DTPA aquoes solution at pH.3 (R_f = 0.8) (Figure 4).

Labeling optimization studies

Because of the engagement of NH polar functional groups in its structure, labeling of $H_2$4-MPP with samarium cation affects its chromatographic properties and the final complex is more lipophilic. Both HPLC and ITLC data confirmed the formation of the complex.

The water solubility of the radiocomplex leads to less soft tissue unwanted uptakes including liver and adipose tissue and faster renal clearance.

ITLC studies confirmed the production of a single radiolabeled compound (Figure 5), HPLC studies also demonstrated the existence of only one radiolabeled species using both UV and scintillation detectors. A slow-eluting compound at 5 min (scintillation detector) demonstrated a more lipophilic compound compared to Sm cation and unlabeled compound. Free Sm-153 cation eluted at 1.1 minutes (not shown) (Figure 6).

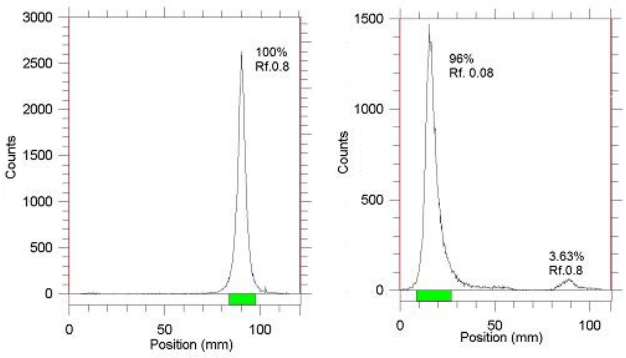

Fig 4. ITLC chromatograms of ^{153}Sm-$SmCl_3$ solution on Whatman no. 1 paper using 10 mM DTPA solution (pH.3) (Left) and 10% ammonium acetate:methanol (1:1) (Right).

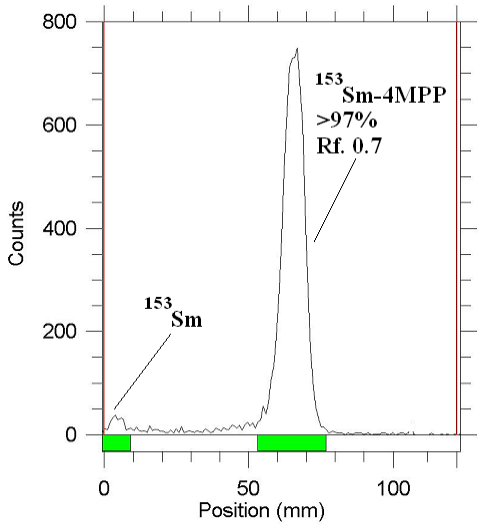

Fig 5. ITLC chromatograms of ([^{153}Sm]-4MPP) solution on Whatman no. 1 paper using 10% ammonium acetate:methanol (1:1).

Partition coefficient of [^{153}Sm]-4-MPP

As expected from the chemical formula, the lipophilicity of the [^{153}Sm]-4-MPP compound is not that high due to the ionic nature of the radiocomplex. The measured octanol/water partition coefficient, *P*, for the complex was found to depend on the pH of the solution. At the pH.7 the logP was -1.09.

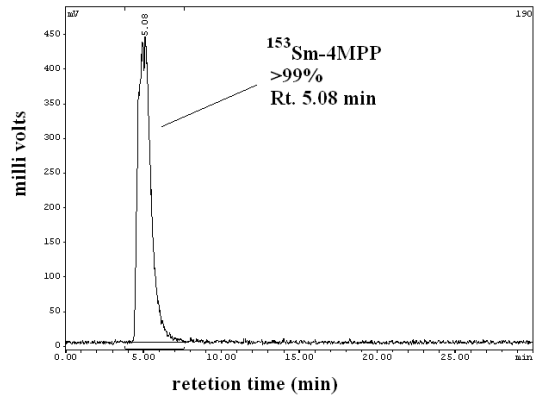

Fig 6. HPLC chromatograms of [^{153}Sm]-4-MPP on a reversed phase column using acetonitrile:water 40:60.

Stability

The chemical stability of [^{153}Sm]-4-MPP was high enough to perform further studies. Incubation of [^{153}Sm]-4-MPP in freshly prepared human serum for 24 h at 37°C showed no loss of ^{153}Sm from the complex. The radiochemical purity of complex remained >90% for 24 h under physiologic conditions (Figure 7).

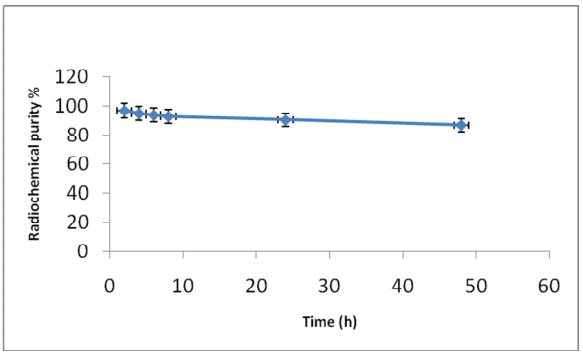

Fig 7. The stability of ^{153}Sm-4-MPP in 48 h.

Biodistribution studies

In order to investigate biodistribution of ^{153}Sm-4-MPP in wild-type animals we had to study the biodistribution data for free samarium, thus after injection of 6.7 MBq of the $^{153}SmCl_3$ pre-formulated by the normal saline (pH. 6.5-8) through the tail vein of adult wild-type rats the biodistribution of the cation was checked in various vital organs.

The animals were sacrificed by CO_2 asphyxiation at selected times after injection. Dissection began by drawing blood from the aorta, followed by collecting heart, spleen, muscle, brain, bone, kidneys, liver, intestine, stomach, lung and skin samples. The tissue uptakes were calculated as the percent of area under the curve of 103 keV peak per gram of tissue (% ID/g) (Figure 8).

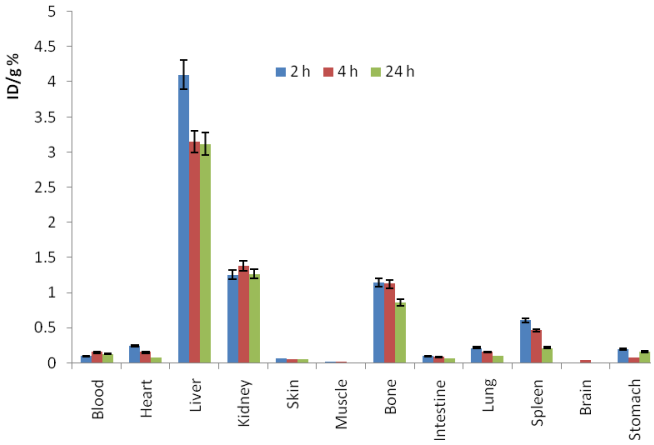

Fig 8. Biodistribution of ^{153}Sm-free in different organs of normal rats 2, 24 and 48 h post-injection (n=3).

The blood count was low at all time intervals and this shows the rapid removal of activity in the circulation. The lung, muscle and also skin did not demonstrate significant uptake which is in accordance with other cations biodistribution.

A %1 bone uptake was observed for the cation which remained almost constant after 96 h (data not shown).

The liver uptake of the cation was comparable with many other radio-lanthanides mimicking calcium cation accumulation. The cation is taken up in plasma as bonded to transferrin as well as other proteins which finally accumulate in the liver [21].

The spleen also showed significant activity possibly related to reticuloendothelial uptake. The kidney plays an important role in ^{153}Sm cation excretion especially after 24 h.

Due to ionic nature of the porphyrin complex, the majority of activity in 2 hours post injection remains in blood, lung, kidney and spleen thus the major route of excretion for the labeled compound is billiary tract after 24h. High intestinal activity demonstrates the extensive hepatobiliary excretion route (Figure 9). Considering kidneys as the second major excretion route the kidney:tissue uptake ratios are considered as excretion criterion especially at 24h post injection (Table 1).

For better comparison of the ^{153}Sm-4-MPP and $^{153}Sm^{3+}$ species behavior, Figure 10 demonstrates the cardial accumulation from 2 to 24h.

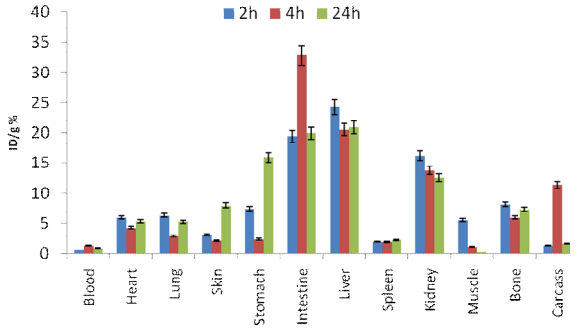

Fig 9. Biodistribution of [^{153}Sm]-4-MPP (1.85 MBq, 50µCi) in wild type rats 2, 24 and 48 h after iv injection via tail vein (ID/g%: percentage of injected dose per gram of tissue calculated based on the area under curve of 103 keV peak in gamma spectrum) (n=3).

Table 1. Kidney:tissue activity ratios of ^{153}Sm-4-MPP in rats 2-24h post injection.

Ratios/Time	2h	4h	24h
Kidney:blood	23.67	10.51	14.74
Kidney:liver	0.67	0.67	0.60
Kidney:muscle	2.97	13.26	42.41

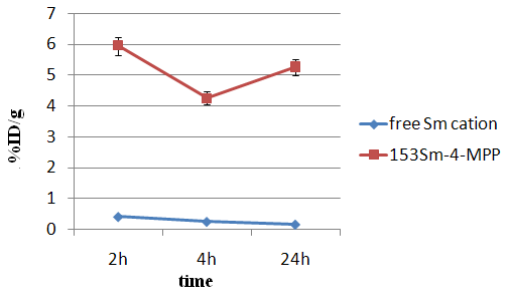

Fig 10. The comparative study of heart uptake for ^{153}Sm-4-MPP and $^{153}SmCl_3$ in 2-24h.

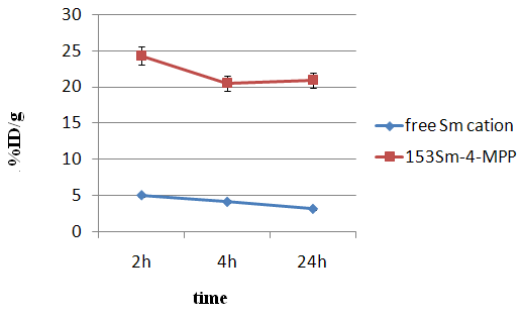

Fig 11. The comparative study of liver uptake for ^{153}Sm-4-MPP and $^{153}SmCl_3$ in 2-24h.

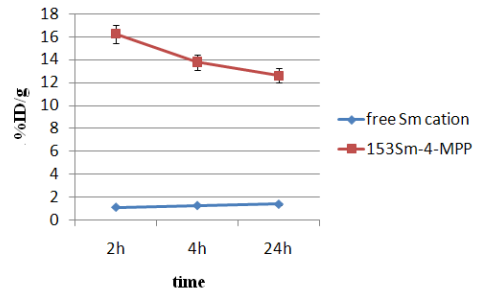

Fig 12. The comparative study of kidney uptake for ^{153}Sm-4-MPP and $^{153}SmCl_3$ in 2-24h.

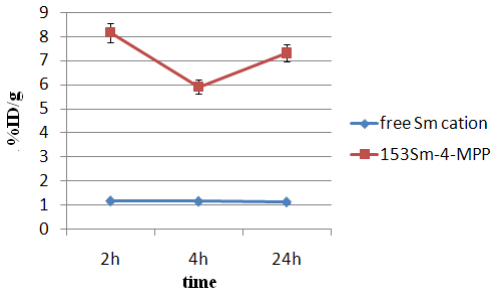

Fig 13. The comparative study of bone uptake for ^{153}Sm-4-MPP and $^{153}SmCl_3$ in 2-24h.

The expression of proteins that possibly affect porphyrin accumulation including ferrochelatase and ATP-binding cassette transporter G2 (ABCG2), in several tumor cell lines as well as normal tissues has been reported.

The complex is distributed not only in the plasma membrane but also intracellular organelles, including mitochondria. Thus mitochondria-rich organs such as liver and myocardial cells may demonstrate radiolabeled porphyrins uptake followed by the release of the cation in mitochondrion matrix [22]. Thus the various uptakes of the labeled porphyrins compounds in heart, liver and even lungs can be an outcome of above mentioned mechanism as already reported for other radiolabeled TDMPP analogs [13, 23].

Several liver accumulation mechanisms are suggested other than the above-mentioned mechanism, such as specific lipoprotein binding in serum leading to the final entrance in liver. As shown in Figure 11, the liver uptake is also detected for free samarium which is a result of lanthanide similarity to calcium cation in vivo.

Although the liver uptake for the complex is the major site, a significant portion of the radioactivity is also detected in the kidneys 2-24 h (ranging 12-16%),

this can be explained by the possible ionic properties of radiocomplex which could be predicted by partition coefficient results (log P. -1.08) (Figure 12).

Bone and teeth uptake is also significant which has been already reported in some cases of porphyrins [24] in humans and animals, while free cation uptake is also constant (1%) with regards to calcium/lanthanide similarities (Figure 13).

CONCLUSION

Total labeling and formulation of [^{153}Sm]-4-MPP took about 18 h at 80°C (radiochemical purity: >97±2% ITLC, >99±0.5% HPLC, specific activity specific activity: 220-230 MBq/mmol). The complex was stable in final formulation and in presence of human serum for 24 h. The partition coefficient was calculated for the compound (log P=-1.09). A detailed comparative pharmacokinetic study performed for ^{153}Sm cation and [^{153}Sm]-4-MPP performed up to 24h.

The complex is mostly cleared from the circulation through kidneys and liver. The kidney:blood and kidney:muscle ratios 24 h post injection were 14.75 and 42.4 respectively. kidney/liver ratio was almost constant at all time intervals (0.6). biological studies on tumor-bearing models are underway.

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