Preparation and preliminary biological evaluation of [^{153}Sm] samarium AMD3100; towards a possible therapeutic chemokine receptor CXCR4 targeting complex

Original Article

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ABSTRACT

Introduction: In continuation of recent development of possible C-X-C chemokine receptor type 4 (CXCR4) imaging agents, we report the development of a possible CXCR4 targeted therapy agent.

Methods: [^{153}Sm]labeled 1,1′-[1,4-phenylenebis(methylene)] bis-1,4,8,11-tetraazacyclo- tetradecane ([^{153}Sm]-AMD3100) was prepared using [^{153}Sm]$SmCl_3$ and AMD-3100 for 24h at 50°C in acetate buffer. Stability tests, partition coefficient determination, toxicity tests and biodistribution studies of the complex in wild-type rats were determined.

Results: The radiolabeled complex was prepared in high radiochemical purity (>95%; RTLC and >99% HPLC) and specific activity of 278 GBq/mmol and demonstrated significant stability up to 48h at 37 °C (in presence of human serum). Partition coefficient determination was calculated Log P= -1.09. Hepatotoxicity experiments demonstrated no distinguishable effect on hepatic enzymes in 10 days post injection. Initial complex biodistribution data showed significant liver and kidney accumulation in wild-type rats.

Conclusion: Since lung and spleen are considered as CXCR4 rich organs, the best lung/blood and spleen/blood ratios were achieved 12 and 7 at 24 h post injection. Further investigations are needed especially on therapeutic properties of this agent.

Key words: AMD3100; Targeted radiotherapy; Radiolabeling; Biodistribution; Sm-153

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INTRODUCTION

The chemokine receptor subtype CXCR4 is an attractive possible target for cancer treatment as it is overexpressed in more than 70% of human solid tumors, including mammary cancer, prostate cancer, B-cell lymphoma, neuroblastoma, melanoma, cervical adenocarcinoma and glioma [1], involving in three fundamental aspects of cancer: primary tumor growth, cancer cell migration, and establishment of metastatic sites. Many peptidic and nonpeptidic ligands with different modes of antagonistic activity have been developed against this receptor [2].

Recent studies confirmed the necessity of CXCR4 in breast cancer metastasis [3] and imaging studies demonstrated that the CXCR4 is required to initiate proliferation and/or promote survival of breast cancer cells *in vivo* and suggest that CXCR4 inhibitors, such as; 1,1′-[1,4-Phenylenebis(methylene)]bis-1,4,8,11-tetraazacyclotetradecane (AMD3100; Plerixafor), can improve treatment of patients with primary and metastatic breast cancers [4].

Previous studies have demonstrated that metals bound to the cyclam core increases the affinity of AMD3100 to the CXCR4 receptor, for instance, copper complex affinity is increased by 6-fold [5]. Also the binuclear ZnII, CuII and NiII complexes of AMD3100 have shown to enhance the binding properties of AMD3100.

Interestingly, the Zn(II)-AMD3100 complex (carrying overall +4 charge), revealed marginally higher specificity and reduced toxicity *in vitro* compared to the free ligand [6].

Various CXCR4 imaging compounds based on AMD-3100, using positron emission tomography (PET) and single photon emission tomography (SPECT) including Tc-99m [7], Cu-64 [8, 9], Zn-62/Cu-62 [10] and Ga-67 [11] have been reported, however the attempt to develop therapeutic radionuclide based AMD-3100 complexes has not been documented according to the authors' knowledge.

The complex formation potentials of various tetraaza macrocycles have been studied with lanthanides including ^{153}Sm and ^{166}Ho with medium to high stability in physiological solutions and in human serum [12] (Figure 1).

Radioisotopes with medium-energy beta emissions and half-life of a few days are attractive candidates for systemic delivery of targeted irradiation. Samarium-153 ($T_{1/2}$ = 46.7 h) has favorable radiation characteristics, medium-energy beta particle emissions (E_{max}=810 keV) which is desirable for treatment, and range of about 3.0 mm in tissue has potential for targeted tumor radiotherapy.

It emits medium-energy gamma photon (103 keV) in addition to particle emissions which make it suitable for monitoring the therapy with imaging, and for continuous follow-up of the absorbed dose distribution. This radionuclide is the most widely used pain palliation radiopharmaceutical in the form of EDTMP complex in the world as well as in our country [13, 14] which can also be prepared by neutron activation of natural and/or enriched $^{152}Sm_2O_3$ [15].

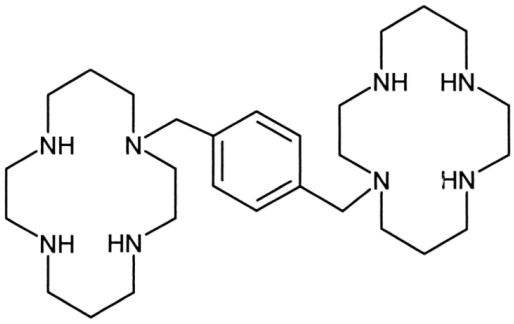

Fig 1. Chemical structure of AMD3100.

In this work, we were interested in developing a ^{153}Sm-AMD3100 complex followed by quality control and biological studies in wild type rats for possible therapeutic application.

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.

Radio-chromatography was performed by counting of polymer-backed silica gel paper thin layer sheets using a thin layer chromatography scanner, Bioscan AR2000, Paris, France.

A high purity germanium (HPGe) detector coupled with a Canberra™ (model GC1020-7500SL) multichannel analyzer and a dose calibrator ISOMED 1010 (Dresden, Germany) were used for counting distributed activity in rat organs. Calculations were based on the 103 keV peak for ^{153}Sm. All values were expressed as mean ± standard deviation (Mean ± 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. Infra Red (IR) spectrum, Ultra Violet (UV) spectrum and NMR Analysis were performed to specify the formation, quality and state of compound.

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

Samarium-153 was produced by neutron irradiation of 1 mg of enriched [^{152}Sm]Sm_2O_3 (^{152}Sm, 98.7% from ISOTEC Inc.) according to reported procedures [15] in the 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 (12.76 GBq/mg). The irradiated target was dissolved in 100 µl of 1.0 M HCl, to prepare [^{153}Sm]$SmCl_3$ 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.

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}Sm]$SmCl_3$ was checked using 2 solvent systems for ITLC (A: 10 mM DTPA pH.4 and B: ammonium acetate 10%:methanol (1:1))

Preparation of [^{153}Sm]-AMD3100

The acidic solution (2 ml) of [^{153}Sm]$SmCl_3$ (111 MBq, 3 mCi) was transferred to a 5 ml-borosilicate vial and heated to dryness using a flow of N_2 gas at 50-60°C followed by the addition of acetate buffer (500µL, pH. 5).

Fifty microlitres of AMD3100 hexa hydrochloride dissolved in acetate buffer pH=5 (5 mg/ml ≈63 nmoles) was added to the samarium-containing vial and vortexed at 25, 50 and 80°C separately.

The active solution was checked for radiochemical purity by ITLC and HPLC methods at 0.5, 1, 2 and 24h after labeling. The final solution of radiolabeled compounds was then passed through a 0.22 µm filter and pH was adjusted to 5.5-7.

Quality control of [^{153}Sm]-AMD3100

Radio thin layer chromatography: A 5µl sample of the final fraction was spotted on the chromatography silica gel plates using 10% ammonium acetate:methanol (1:1) mixture as mobile phase.

High performance liquid chromatography: HPLC was performed with a flow rate of 1 ml/min, pressure: 130 kgF/cm^2 for 20 min. Radiolabeled compound was eluted using a mixture of two solutions (A: acetonitrile+0.1%TFA/water + 0.1% TFA, 90:10) using reversed phase column Whatman Partisphere C_{18} 4.6 × 250 mm.

Stability tests

The stability of the complex was checked according to the conventional ITLC method [16]. A sample of [^{153}Sm]-AMD3100 (37 MBq) was kept at room temperature for 4 days while being checked by ITLC at time intervals in order to check stability in final product using above chromatography system.

For serum stability studies, to 36.1 MBq (976 µCi) of [^{153}Sm]-AMD3100 was added 500µl of freshly prepared human serum and the resulting mixture was incubated at 37°C for 4d, aliquots (5-µl) were analyzed by ITLC.

Determination of Partition Coefficient

Partition coefficient (log P) of ^{153}Sm-AMD3100 complex was calculated followed by the determination of P (P = the ratio of radioactivity level 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 h and then left for 30 minutes in room temperature; alternatively, the mixture was centrifuged at 2500 rpm for 5 min. Then 5µl of the 2-octanol and aqueous phases were sampled and counted in HPGe detector for 20 seconds.

Serum AST/ALT assay after complex administration

In order to evaluate the liver toxicity of the complex, blood samples (2 ml pooled from 3 different mice, n=5) were taken 10 days (equal to five physical half-lives of ^{153}Sm) after injection of ^{153}Sm-AMD3100 through aorta and transferred to heparinized test tubes and the levels of AST/ALT were determined using clinical spectrophotometric assay kits in a reference laboratory in a double-blind manner.

Biodistribution of [^{153}Sm]-AMD3100 in wild-type rats

To determine biodistribution, [^{153}Sm]-AMD3100 and $^{153}SmCl_3$ were administered to normal rats separately. A volume (50-100 µl) of final radioactive solution containing 1.85±0.2 MBq radioactivity was injected intravenously to each rodent through their tail vein. The total amount of radioactivity injected into each rat was measured by counting the 1-ml syringe before and after injection in a dose calibrator with a fixed geometry.

The animals were killed by CO_2 asphyxiation (after anesthesia induction using propofol/xylazine

mixture) at selected times after injection at the exact time intervals and the specific activities of different organs were calculated. Dissection began by drawing blood from the aorta, followed by collecting heart, spleen, kidneys, liver, intestine, stomach, lung, bone muscle and skin samples.

The samples were weighed and their specific activities were determined with an HPGe detector counting the area under the curve of the 103 keV peak. The tissue uptakes were calculated as the percent of area under the curve of the related photo peak per gram of tissue (% ID/g).

RESULTS AND DISCUSSION

Radionuclide production

The radionuclide was prepared in a research reactor according to regular methods with a specific activity of 350mCi/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. The 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.

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 ammonium acetate: methanol, the free samarium cation in $^{153}Sm^{3+}$ form remains at the origin (R_f= 0.0) and while other Sm-153 species migrate to higher R_f = 0.8. Another eluent for Sm^{3+} detection was 10 mM DTPA aqueous solution at pH 3 (R_f = 0.8).

Radiochemical purity of the final complex dissolved in aqueous medium was checked by ITLC using a mixture of 10% ammonium acetate: methanol (1:1) as mobile phase and a silica-gel sheet (10x15 cm) as solid phase (Figure 2).

Radiolabeling

In the radiolabeling of the complex, high polarity solvent containing ammonium acetate 10%: methanol was used to distinguish $^{153}Sm^{3+}$ from the radiolabeled complex (Figure 3).

At room temperature no detectable complex was formed. The best temperature was found to be 55-60°C. At this temperature, when freshly prepared Sm-153 was used, all the radio-samarium was inserted into the complex, while heating the reaction mixture over 100°C or for more than 1h, the radiochemical yield dropped. The solution was stable at room temperature up to 4 days post-formulation, allowing performance of biological experiments. Before experiments, the solution passed through a 0.22µm filter (Millipore).

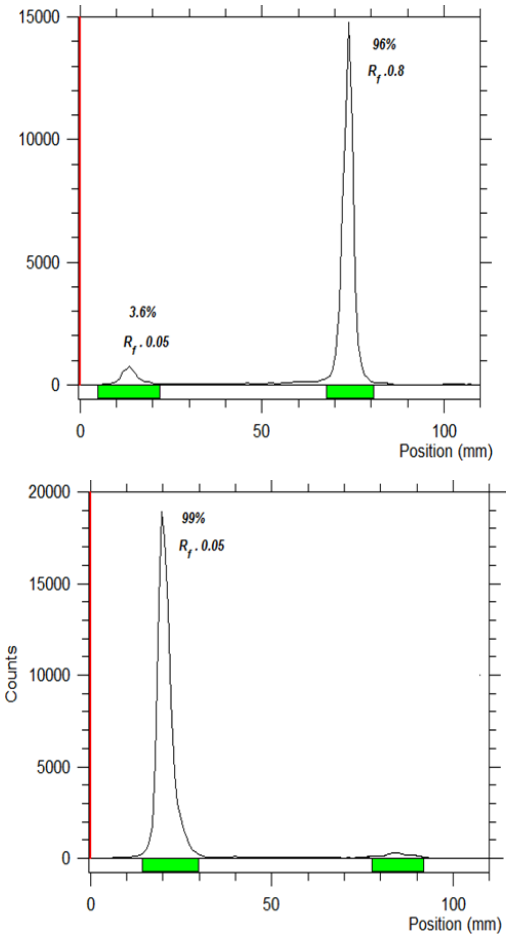

Fig 2. ITLC chromatograms of ^{153}Sm-AMD3100 solution (above) and [^{153}Sm]$SmCl_3$ solution (below) on silica-gel paper using 10% ammonium acetate: methanol (1:1).

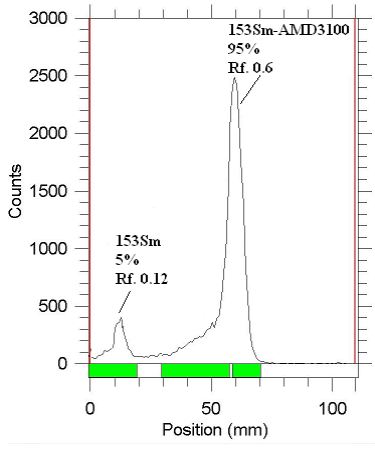

Fig 3. ITLC of [^{153}Sm]-AMD3100 in ammonium acetate 10%:methanol (1:1) mixture on silica gel sheets.

^{153}Sm cation is retained to the origin while the radiolabeled complex [^{153}Sm]-AMD3100, eluted to the higher R_fs (Rf. 0.6). In HPLC radio analysis, we used a reverse phase column in our settings and it worked with a tolerable difference in the retention times, enough for analytical measurements. The HPLC experiments using acetonitrile/water + 0.1% TFA with a gradient protocol (10:90 to 90:10) was applied. In this system, free Sm eluted at 0.85 minutes while the complex was eluted at 3.77 minutes (scintillation detector) demonstrating a radiochemical purity of higher than 99 percent using optimized conditions without further purifications (Figure 4).

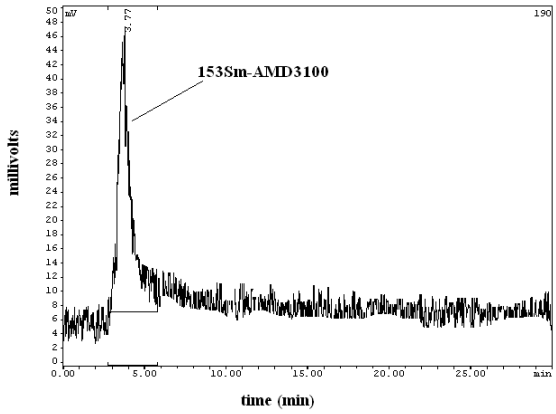

Fig 4. HPLC chromatogram of [^{153}Sm]-AMD3100 solution on a reversed phase column using acetonitrile+0.1%TFA/water + 0.1% TFA, gradient from 10:90 to 90:10.

Stability

Incubation of [^{153}Sm]-AMD3100 in freshly prepared human serum for 2 days at 37°C showed no loss of ^{153}Sm from the complex. The radiochemical purity of complex remained at 99% for 2 days under physiologic conditions.

Liver enzymes test

Liver toxicity is a potential side effect of chemicals and liver enzymes which are normally found within the cells may release into blood circulation in case of liver cell injuries. An initial step in detecting liver damage in evaluation of new drugs is the determination of liver enzymes in the circulation. Among the most sensitive and widely used liver enzymes are the aminotransferases including aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Due to the development of a possible therapeutic compound in this work and quite high mass of carrier added complex usually applied using reactor products, determination of liver toxicity of the complex was performed. Table 1 demonstrates an 11±3% increase of the AST enzyme in the test groups and 14±4% increase in case of ALT.

Table 1: Result of liver enzymes test.

	AST (U/L)	ALT (U/L)
Control Group	156	66
Group 1	192	100
Group 2	166	80
Group 3	172	60
Group Average	176.66±13.61	80±20

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 h and left for 30 minutes in room temperature. Then 5µl 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 liver and gastrointestinal tract uptake of the complex and a high excretion through kidneys. (Log P -1.09).

Biodistribution

Sm^{3+} cation: 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 5). The liver uptake of the cation is comparable with many other radio-lanthanides mimicking calcium cation accumulation; about % 3-4 of the activity accumulates in the liver after 4 h and remains constant in 48 h.

The blood content is low at all time intervals and this shows the rapid removal of activity from the circulation. The lung, muscle and also skin do not demonstrate significant uptake which is in accordance with other cations accumulation. A 1% bone uptake is observed for the cation which remains almost constant after 48 h. The spleen also has significant uptake possibly related to reticuloendothelial uptake. The kidney plays an important role in ^{153}Sm cation excretion during 48 h.

[^{153}Sm]-AMD3100 complex: CXCR4 is abundantly expressed in normal tissues such as lungs, liver, and bone marrow and much less in other tissues [17]. Interestingly CXCR4 is absent in most of healthy tissue cell surfaces and as observed in Figure 6, just the excretion tissues contain the activity. Unlike other radiolabeled AMD3100 complexes already reported, [^{153}Sm]-AMD3100 is not highly water soluble and its wash out is delayed and does not mainly excrete through the urinary tract. The high stability of the complex predicted by *in vitro* methods does not allow the detachment of the radio cation into blood and other organs, thus the kidneys are the second important excretion organs after liver and also possible critical organ in the dosimetry calculations.

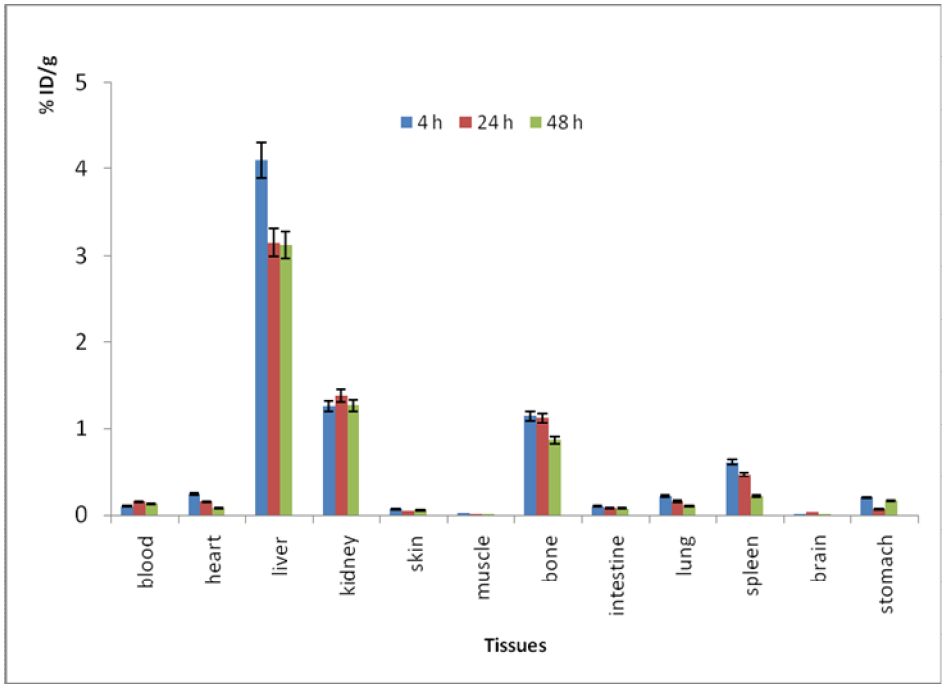

Fig 5. Biodistribution of [153]Sm-free in different organs of normal mice

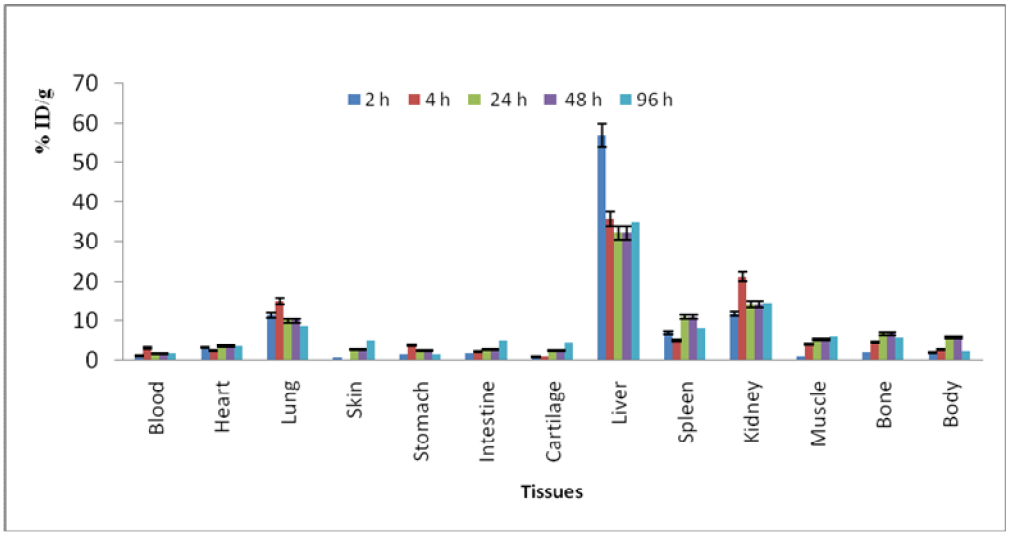

Fig 6. Biodistribution of [153]Sm-AMD3100 in organs of wild-type mice (n=5) (%ID/g tissue).

Kidney, liver, spleen and lungs are only significant uptake targets. From the data it can be suggested that [[153]Sm]-AMD3100 is metabolized and/or excreted through the hepatic and kidneys metabolism respectively. However no metabolic study performed to identify the natures of metabolite(s).

The significant kidney uptake can cause extra dose to surrounding critical tissues including gonads, this can be an obstacle, especially when using therapeutic radionuclides for therapy. However, in case of liver, the decrease in uptake in 4 hours would minimize this effect while the activity is almost constant in 96h.

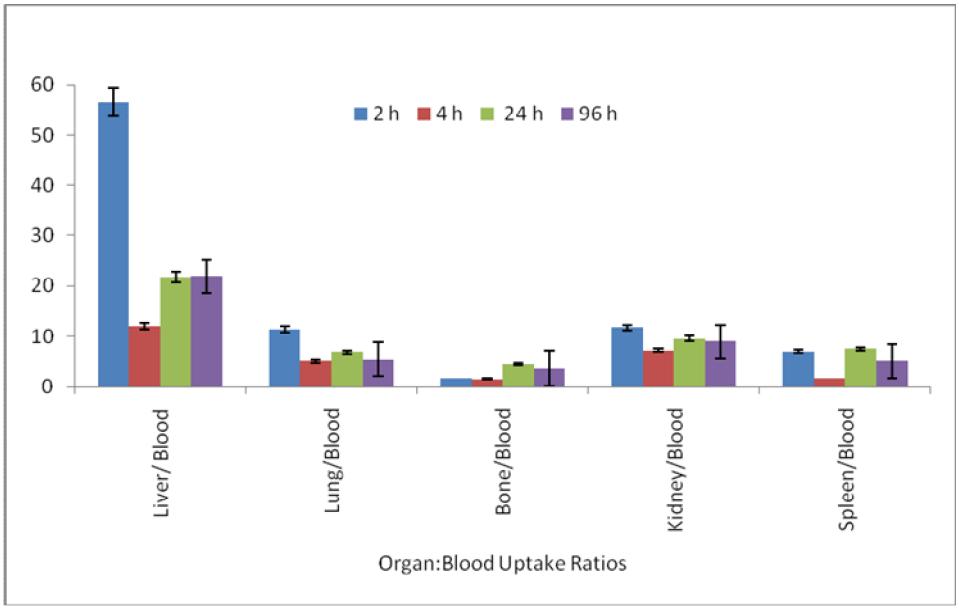

Fig 7. Various critical oragn:blood uptake ratios for ^{153}Sm-AMD3100 2-96 h post injection.

In a single report using ^{125}I-anti CXCR4, spleen has been shown to be a major site of accumulation, possibly due to the presence of CXCR4-containg blood cells. Also lungs and liver contain medial receptor sites at their cell surfaces [18]. With respect to this work, the major receptor rich organs (%ID/g=14 at 4 h post injection for lungs and 12 at 24 h for spleen), directly or indirectly can be considered lungs and spleen. However the high kidney and liver uptake is a result of being the major excretion organs due to high water solubility of the complex, and not the receptor mediated uptake (Figure 6).

For better comparison of target/non target ratios for the labeled compound various ratios for organ:blood are calculated and shown in Figure 7. Lung and spleen can be considered as receptor rich organ and at the best condition lung/blood ratio at 24 post injection is 12 and in case of spleen the best ratio is almost 7 at 24 h post injection.

It should be mentioned that in the case of tumor treatment with ^{153}Sm-AMD3100 in some precautions are needed to avoid normal organs damage such as liver, lung and kidney. For instance the application of diuretics would minimize the kidney exposure dose. On the other hand while tested in tumor-bearing animals the optimized dose for possible human dose should be calculated based on animal data.

CONCLUSION

In this report the development of a possible CXCR4 targeted therapy agent, i.e. [^{153}Sm]-AMD3100 is reported. The radiolabeled complex was prepared in high radiochemical purity (>95%; RTLC and >99% HPLC) and specific activity of 278 GBq/mmol in 12 h at 50°C in acetate buffer and demonstrated significant stability up to 48h and 37 °C (in presence of human serum). Partition coefficient determination was calculated Log P= -1.09. Hepatotoxicity experiments demonstrated no distinguishable effect on hepatic enzymes in 10 days post injection. Since lung and spleen are considered as CXCR4 rich organs, the best lung/blood and spleen/blood ratios were 12 and 7 at 24 h post injection. Further investigation on the therapeutic properties of this agent must be conducted on tumor-bearing animal models.

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