Development of [153]Sm-DTPA-SPION as a theranostic dual contrast agents in SPECT/MRI

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ARTICLE INFO	ABSTRACT
	Objective(s): This study describes the preparation, biodistribution of [153]Sm-DTPA-SPION after intravenous injection in rats. ***Materials and Methods:*** The chelator DTPA dianhydride was conjugated to SPION using a small modification of the well-known cyclic anhydride method. Conjugation was done at a 1: 4 (SPION: ccDTPA) molar ratio. Conjugation reaction was purified with magnetic assorting column (MACs) using high gradient magnetic field following incubation, the radio labeled conjugate was checked using RTLC method for labeling and purity checked. ***Results:*** The RTLC showed that labeling yield was above 99% after purification and the compound have good *in vitro* stabilities until 48 hr post injection in the presence of human serum. The biodistribution of [153]Sm-DTPA-SPION in rats showed dramatic uptake in the reticuloendothelial system (RES) and their clearance is so fast in other organs especially in the blood. Biodistribution results show that after 30 min post injection more than 84% of injected activities were taken up by the liver and spleen (about 64% and 20%, respectively). ***Conclusion:*** Due to magnificent uptakes of this radiotracer in the liver and spleen and their fast clearance from other tissues, especially in blood, it is suggested that this radiotracer would be a potential candidate for RES theranostic purposes.

Introduction

Super paramagnetic iron oxide nanoparticles (SPION) with a cross-linked dextran coating are used as negative contrast agents in MRI and have flexible uses in different medical fields such as imaging (1, 2), drug delivery (3), cell tracking (3, 4), therapy(5, 6) and lymph node detection (7). Previous studies confirmed that dextran coated NPs with the hydrodynamic size of 60-80 nm had a great tendency to take up via reticuloendothelial system (RES) after intravenously injection (5, 8, 9). Dextran coated NPs maintained in the liver up to several days post injection and they are suitable carriers with high potential for liver and spleen therapies (9, 10). in our prior study we radiolabeled dextran coated SPION with ^{99m}Tc and due to their magnificent uptakes in RES (55% in Liver and 20% in spleen) and their fast clearance from other tissues we suggested that that these labeled nanoparticles (NPs) (with beta emitter radioisotopes) could be suitable to be used as treatment agents for spleen and liver malignancies (5).

Samarium-153 shows promising radiation characteristics, medium-energy beta particle emissions (Emax= 808.20 keV) which is appropriate for treatment, medium energy gamma photon (103.18 keV) which is also suitable for imaging, and short half-life (46.28 hr). Samarium -153 decays 17 beta (β^-) particles which the most frequents beta are 705.02 keV, 635.35 keV and 808.20 keV with the intensities of 49.6%, 32.2% and 17.5%, respectively (11). [153]Sm also decays 80 gamma and X rays, the dominating gamma rays have energies of 103.18 keV (30%) and 69.67 keV (4.85%), respectively. This radionuclide is the most widely used pain palliation radiopharmaceutical in the United States in form of EDTMP complex (Lexidronam) (12).

In this study, we try to develop a [153]Sm-DTPA-SPION as a theranostic radiotracer, which can be applied as dual contrast agents in single photon emission computer tomography (SPECT), and MRI.

Materials and Methods

Nanomag®-CLD-SPION NPs were provided from Micromod (Micromod Partikeltechnologie GmbH) with lot number Product No. 77-01-201. These nanoparticles were cross linked dextran-coated iron

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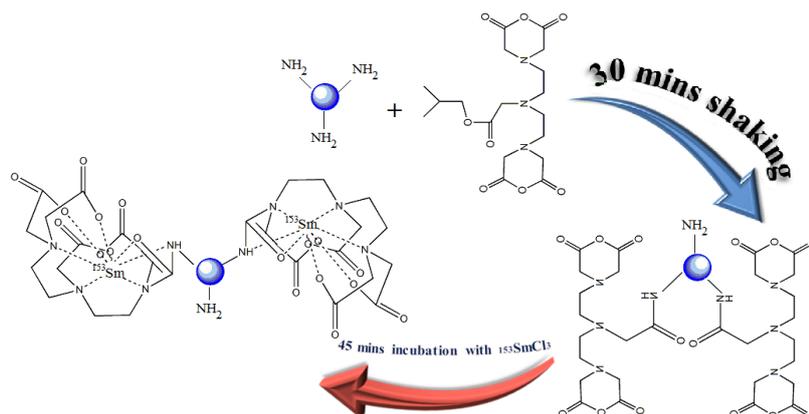
Figure 1. Schematic scheme of labeling procedure of Dextran coated iron oxide NPs with amine group conjugated with ccDTPA and labeled with ¹⁵³Sm finally

oxide particles with NH_2 functional group on their surface. The mean core diameter of 8.86±1.61 nm with the Fe concentration of the nanoparticles 2.4 mg/ml. Production of ¹⁵³Sm was performed at the Tehran research reactor using ¹⁵²Sm (n, gamma) ¹⁵³Sm nuclear reaction. ¹⁵²Sm with purity of >98% was obtained from ISOTEC Inc., USA. Radiochromatography was performed by counting of Whatman no. 2 (GE, England) using a thin layer chromatography scanner, Bioscan AR2000 (Paris, France). All other chemical reagents were purchased from Sigma-Aldrich Chemical Co UK Whatman No 2 paper was obtained from Whatman (Maidstone, UK). Radio-chromatography was performed by Whatman paper using a thin layer chromatography scanner, Bioscan AR2000, Paris, France. A high-purity germanium (HPGe) detector, coupled with a Canberra™ multichannel analyzer (model GC1020-7500SL, canberra Industries Inc, CT, USA) and a dose calibrator ISOMED 1010 (Elimpex-Medizintechnik, Austria) was used for measuring the distributed activity in rat organs. Calculations were based on the 103.18 keV peak for ¹⁵³Sm. All values were expressed as mean ± standard deviation (Mean ± SD). 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 (13).

Particles characterizations

The particle core size and structure of the SPIONs were checked with a TEM (transmission electron microscope, Philips, CM 30) (14). Photon correlation spectroscopy (PCS) was used to determine the hydrodynamic particle diameter of the particle samples. The PCS measurements were performed with a Malvern Zetasizer Nano ZS-90 (Malvern Instruments Ltd, Worcestershire, UK) (3). Iron concentration of suspensions was acquired with inductively coupled plasma atomic emission spectroscopy (ICP-AES, Varian-

Liberty, 150 AX Turbo, USA) of digested samples with boiling HNO_3 (7).

Radiolabeling of SPION with ¹⁵³Sm
*Conjugation of cyclic DTPA di-anhydride with SPION*

The chelator diethylenetriamine penta-acetic acid dianhydride was conjugated to SPIONs using a small modification of the well-known cyclic anhydride method (15, 16). Conjugation was performed at a 1:4 (SPION:ccDTPA) molar ratio. Concisely, NPs (1 mg, 4.3 µM) and DTPA anhydride (2.10 mg, 13.76 µM) were mixed in 0.3 ml of 0.1M phosphate buffer solution (pH = 9.0) and 0.3 ml of normal saline and stirred at room temperature (RT) under N_2 for 30 min.

Radiolabelling of the conjugated SPIONs

The SPIONs was labeled with ¹⁵³Sm using an optimization protocol according to the literature (17). Typically, 102-105 MBq of ¹⁵³Sm -chloride (in 0.2 M HCl) was added to a conical vial and dried under a flow of nitrogen. The conjugated fraction was added to the ¹⁵³Sm containing vial, in 1 ml of phosphate buffer (0.1M, pH 8) and mixed gently for 2 min. The resulting solution was incubated at RT for 45 min. Following incubation, the radio-labeled conjugate was checked using RTLC method for labeling and purity checked. The complex reaction was purified with magnetic assorting column (MACs) using high gradient magnetic field (8, 18).

Purification of ¹⁵³Sm-DTPA-SPIONs

In order to purify the final solution the final solution were run through a strong stationary magnetic field using a magnetic-assorting cell separation columns (MACS®), in order to trap the iron oxide NPs in the magnetic column. All the magnetic materials (included labeled and non labeled SPIONs) were trapped via an LS-MACS column (Miltenyi Biotec). The LS-MACS column washed 3 times with PBS, while the column attached
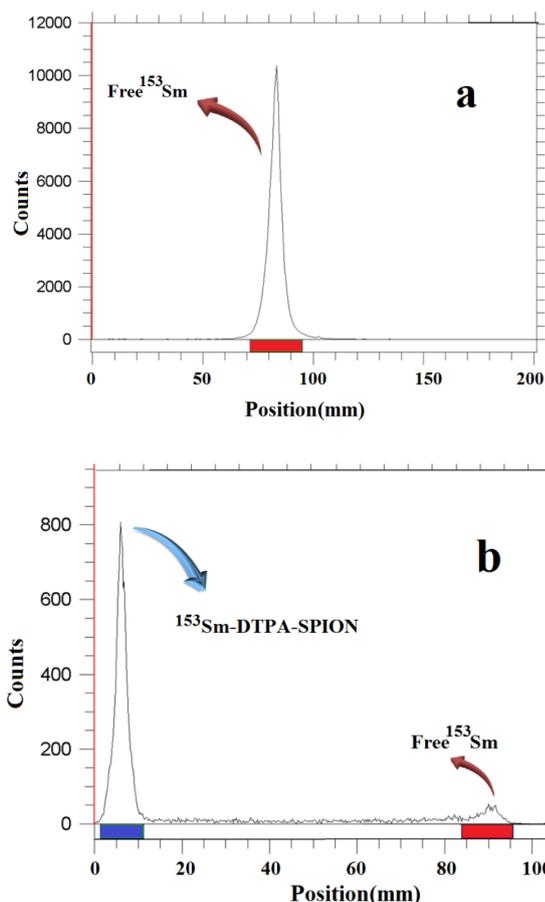
Figure 2. RTLC of free ¹⁵³Sm (a) and (b) ¹⁵³Sm-DTPA-SPIONs after passing through the MACs column in presence of 10 mM DTPA solution as mobile phase and Whatman paper as stationary phase

to high gradient magnet (MACS® Separation Unit, Miltenyi Biotec Inc, Germany), and after separating the column the trapped NPs were washed with the 1 ml of PBS (5).

Quality control of ¹⁵³Sm-DTPA-SPIONs

Radiochemical purity and radiolabeling yield were tested with a 1 μl sample of the ¹⁵³Sm-DTPA-SPION complex that spotted on a chromatography paper (Whatman No 2), and developed in a 10 mM DTPA as the mobile phase. The Rf values of free ¹⁵³Sm and ¹⁵³Sm-DTPA-SPION complex were 0.9 and 0.0, respectively.

Labeling efficiency and stability study

The labeling efficiency of ¹⁵³Sm to SPION NPs by ascending RTLC using Whatman sheets (Whatman, NJ, USA). The RTLC was performed using a 5 μl sample of the final fraction (after passing the MACs column) was spotted on a chromatography sheet paper, and developed in a 10 mM DTPA solution as the mobile phase. Labeling efficiency was calculated using the following equation 1 (16):
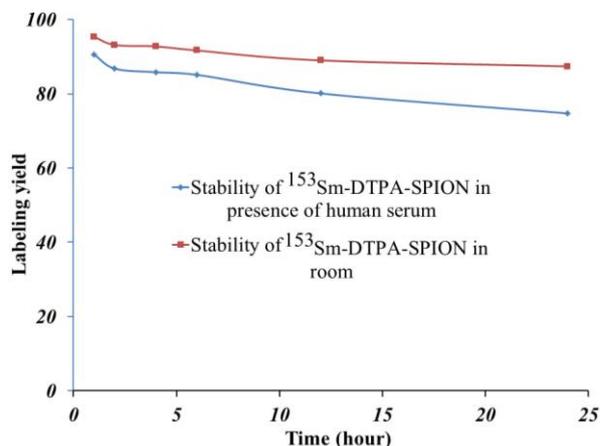
Figure 3. RTLC of ¹⁵³Sm-DTPA-SPION at different time points, in presence of human serum and RT in 10 mM DTPA as mobile phase and Whatman paper as stationary phase

$$Labeling\,efficiency\% = \frac{Total\,counts - counts\,of\,free\ ^{153}Sm}{Total\,counts} * 100$$

Equation (1)

Stability testing of the radio-labeled compound in presence of human serum

For serum stability studies, 7 ml of blood is centrifuged at 3500 rpm for 5 min and then 1 ml of serum is withdrawn. 100 μl of the ¹⁵³Sm-DTPA-SPIONs was added to 1 ml of serum and incubated at 37 ℃ up to 24 hr (16). Aliquots of the reaction mixture were diluted 1:10 with PBS (pH 7.4) and analyzed by RTLC like the procedure described above at 1, 2, 6 and 24 hr. The labeling efficiency and radiochemical purity were determined (Figure 3).

Biodistribution of ¹⁵³Sm-DTPA-SPION NPs in normal rats

To determine its biodistribution, NPs was administered to normal rats (NMRI) purchased from Razi Institute (Karaj, Iran). A volume (50 μl) of the final ¹⁵³Sm-DTPA-SPION solution containing an activity of 4.4-5.2 MBq was injected intravenously to rats through their tail veins. Five rats were sacrificed at specific time intervals (1, 5, 15, 30, 60 min and 2, 6 and 24 hr and 2 and 4 days post injections.), and the specific activity was calculated for various organs as a percentage of the injected dose per gram tissue (ID/g %) (Based on the area under the 103.18 keV peak obtained by an HPGe detector)(19).

Measurement of activity

The activity in the syringes was measured before and after administration of the radiopharmaceutical with well-type ionization chamber (CRC-15R, Capintec, USA N.J.). All samples were background subtracted, the decay correction was not performed, and then similar samples were averaged together (20).

For each of these measurements, three samples (from each organ) were weighed and then counted

by HPGe to determine the percentage of injected dose per gram (which was equivalent to the percentage of injected activity per gram %IA/g≡%ID/g) (21); all the organ activity measurements were normalized to injected activity. Uncertainties in the determinations were minimal, because each assay collected at least 10,000 counts, which results the standard deviation (SD) of less than 1%. In all the measurements, we try to keep same geometry and same volume in order to prevent overestimation and underestimation in dose measurements (22). All samples were background subtracted and decay correction was not considered for all measurements, and the similar samples were averaged together (5, 22).

The HPGe detector give us count therefore we had used the below formula to convert the counts into the activity (22, 23):

$$A(Bq)_{Tissue} = \frac{Area}{t * Eff * Br} \quad \text{Equation (2)}$$

Where t is the time of counts and Eff is the efficiency of the detector for the selected energy and Br is the decay yield of selected energy (103.18 keV) for the ^{153}Sm.

The ^{153}Sm activity concentration at time t, %ID/g (t), was then calculated as the percentage of injected activity per gram of tissue (%IA/g):

$$\%ID/g = \frac{A_{Tissue}/M_{Tissue}}{A_{Total}} * 100 \quad \text{Equation (3)}$$

Where A_{tissue} is the ^{153}Sm activity in the sample, M_{tissue} is the mass of the sample and A_{total} is the total activity of ^{153}Sm injected into the rat (24, 25).

Statistical analysis

Data have been represented as the mean of four individual observations with standard error of mean. Significance has been calculated using Student's *t*-test.

Results

Figure 4 shows size information of the prepared SPIONs. As demonstrated in Figure 4, the core size distribution of the NPs is narrow.

The PCS histogram of SPIONs indicating their hydrodynamic magnitudes of about 69.98 nm. The radionuclide purity of 153Sm-DTPA-SPION was about 81% post labeling but after passing through the MACs column, it increased to 99%. The accumulation of ^{153}Sm-DTPA-SPION in the various organs are shown in Table 1. The results showed that most of the activity was accumulated in the reticuloendothelial system (liver and spleen). Nearly all excretion of activity occurred by the renal system, and hepatobiliary excretion was insignificant.

Discussion

Development of new contrast agents as an theranostic agents with the potential of using in multimodalities such as single photon emission computer tomography SPECT and MRI and positron emission tomography (PET) is now emerging (26, 27). The labeling process of SPION-DTPA is took about 1 hr (include the purification). Although

Table 1. Biodistribution of ^{153}Sm-DTPA-SPION in various time points (represented as injected dose per gram)

Tissues	Time								
	1	5	15	30	60	360	1440	2880	5760
Blood	46.5±1.4	13.1±1.4	3.5±0.8	0.9±0.1	0.2±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Intestine	0.1±0.0	0.2±0.0	0.3±0.1	0.2±0.1	0.2±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Stomach	0.1±0.0	0.4±0.1	0.5±0.2	0.2±0.1	0.2±0.1	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Brain	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Muscle	0.1±0.0	0.2±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Liver	22.5±2.6	45.4±3.4	57.9±2.7	63.9±3.7	61.5±2.9	50.6±2.2	34.6±1.9	20.2±1.2	12.9±0.8
Spleen	3.9±0.4	15.8±1.2	16.8±1.1	19.6±1.3	16.9±1.4	8.0±0.7	3.1±0.4	1.0±0.2	0.3±0.0
Bone	0.4±0.0	0.3±0.0	0.3±0.1	0.3±0.0	0.3±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0
Heart	2.4±0.2	1.3±0.1	0.9±0.1	0.3±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Kid ney	3.7±0.6	4.6±0.9	3.3±0.4	1.9±0.2	1.2±0.1	0.7±0.1	0.2±0.0	0.0±0.0	0.0±0.0
Pancreas	1.1±0.1	0.4±0.1	0.4±0.0	0.3±0.0	0.2±0.0	0.1±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Lung	5.2±0.3	2.6±0.3	1.7±0.1	1.3±0.1	1.2±0.3	0.4±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Skin	0.2±0.0	0.1±0.0	0.1±0.0	0.3±0.0	0.1±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.0±0.0
Feces	0.0±0.0	0.1±0.0	0.2±0.0	0.3±0.0	0.4±0.0	1.1±0.1	2.1±0.3	2.3±0.2	1.6±0.1
Cascade	3.0±0.2	1.9±0.1	1.1±0.1	0.7±0.1	0.3±0.0	0.2±0.0	0.2±0.0	0.1±0.0	0.03±0.0
urine	0.0±0.0	0.1±0.0	1.8±0.1	2.1±0.3	3.6±0.2	2.9±0.2	1.9±0.1	1.1±0.1	0.3±0.0

Data are presented as (mean±SD) and the mean values as the percentage of administered activity per gram (decay corrected)
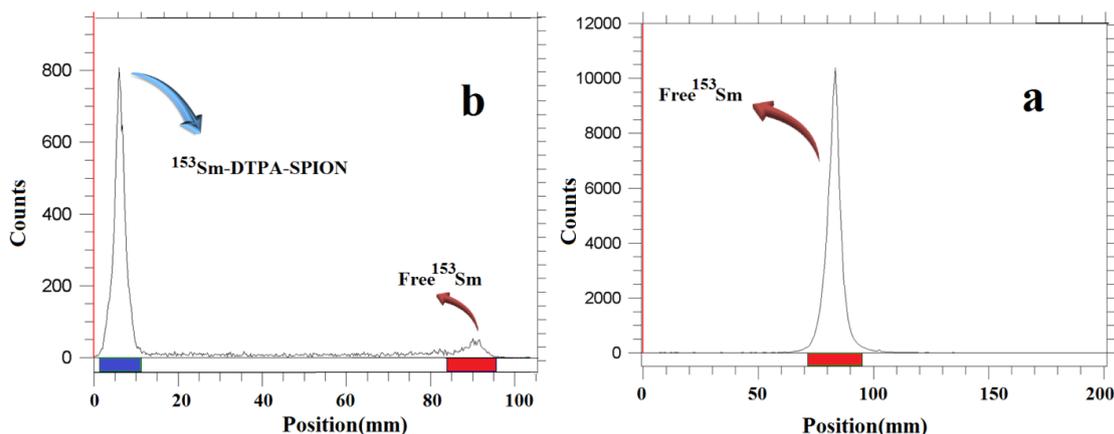
Figure 4. right)TEM of a SPIONs shows that the crystal core size of the particles is about 10.4±1.98 nm similar to our previous study (21) and left) The PCS histogram of SPIONs indicating their hydrodynamic magnitudes of about 69.98 nm

Nosrati *et al* shows that 1 hr is the optimum and best time for conjugation of DTPA with SPIONs but due to some limitation, we incubate the DTPA with SPIONs for 30 min (28). As demonstrated in Figure 2 the labeling yield after purification was increased from 81% into %100. The main reason of high purity is that the MACs column trapped all magnetic materials and therefore the free radioisotopes were washed from the column. The final product had a good stability as shown in Fig 3 after 24 hr post labeling the labeling purity in the RT and human plasma were more than 90 and 74%, respectively. The hydrodynamic size examination of the NPs shows that the size was not significantly change after labeling. The size and the kinetic of the labeled NPs were in accordance to our previous studies (5, 9). As represented in Table 1 after 30 min post injection more than 84% of injected activities were taken up by the liver and spleen (about 64% and 20%, respectively). This unique characteristic of these NPs is due to their size and their coating. Previous studies confirmed that dextran coated particles with the size range between 60 to 80 nm were rapidly cleared from the blood and accumulated in the RES (29-31). We can deliver high quantities of dose to the targeted organs (liver and spleen) with this brilliant spectacular phenomenon and since the tracer cleared so fast from blood the other organs (except RES) uptakes were negligible.

Shanehsazzadeh *et al* radiolabeled the Dextran coated nanoparticles with the range of 40nm to 80nm via ⁹⁹ᵐTc, ⁶⁷Ga and ¹⁶⁶Ho. They got similar results in terms of short blood half-life and high RES uptakes and suggested that using theranostic radioisotopes such as ¹⁷⁷Lu or ¹⁵³Sm would be better for RES detection and therapeutic purposes (28, 32). Dr. Weissleder et al in 1988 used the (AMI-25) which was SPION with 80 nm mean size and they reported that after 1 hrr post *i.v.* injection in rats more than 82% and 6% of injected dose were accumulated in liver and spleen, respectively (33). While in our study, more than 64% and 20% of administrated dose were taken up by the liver and spleen, respectively. In agreement to Dr Weissleder et al the uptake of SPIONs was negligible in brain which shows that the NPs cannot pass through the blood brain barrier (BBB) (31, 33).

Due to splendid amount of cumulative activity in liver and spleen the effective absorbed dose in these two organs were so high compare to normal human diagnostic nuclear medicine studies. As exposed in Table 1 if we simply divided the spleen/blood and liver/blood uptakes we see that these ratios would be radically raised by the time increased this trend shows that the amount of spleen/blood and liver/blood uptakes ratios after 30 min post injection would be more than 22 and 71, respectively. These ratios were rise dramatically after 6 hr post injection into 80 and 506, correspondingly. Although the results of this study was promising in terms of liver and spleen uptakes but further studies still needed to calculate the internal dose and safety of this radiotracer (32, 34, 35).

This research evaluated the feasibility of SPIONs labeled with ¹⁵³Sm as potential theranostic dual contrast agents in nuclear medicine and MRI. We showed that the magnetic NPs based tracers are mostly accumulated in the liver and spleen, which are the major organs of accumulation throughout the study. Due to splendid uptakes of ¹⁵³Sm-DTPA-SPION in the liver and magnificent uptakes of this radiotracer in the liver and spleen and their fast clearance from other tissues especially in blood, it is suggested that this radiotracer would be a potential candidate for theranostic purposes.

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