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A systematic evaluation of the potential of PCTA-NCS ligand as a bifunctional chelating agent for design of ^{177}Lu radiopharmaceuticals

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Abstract This paper explores the utility of para isothiocyanato benzyl 3,6,9,15-tetraazabicyclo [9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid (PCTA-NCS) as a bifunctional chelating agent for ^{177}Lu in comparison to para isothiocyanato benzyl 1,4,7,10-tetra aza cyclododecane tetraacetic acid (DOTA-NCS). The ^{177}Lu-PCTA-NCS complex could be obtained in high radiolabeling yields at ambient temperature and exhibited excellent stability in vitro. Influence of trace metal ions on the radiolabeling yields was also evaluated. Biodistribution studies revealed no significant retention of ^{177}Lu-PCTA-NCS in any vital organ at 24 h p.i. It could be concluded from our study that PCTA-NCS could be a potential BFCA for design of ^{177}Lu radiopharmaceuticals.

Keywords Therapeutic radiopharmaceuticals · ^{177}Lu · Bifunctional chelating agent · Trace metal ions

Introduction

Therapeutic radiopharmaceuticals comprising β^- emitters are increasingly used to target a number of disease conditions, most importantly cancer. Amongst the various β^- emitters which are in use currently, ^{177}Lu has received noteworthy attention in the past decade and is one of the extensively used radionuclides in recent times for in vivo targeted therapy [1–4]. The favorable nuclear properties and the well-established co-ordination chemistry of ^{177}Lu have led to the speedy translation of ^{177}Lu radiopharmaceuticals from laboratories to the nuclear medicine clinics. ^{177}Lu labeled receptor-targeted peptides and antibodies are regularly used in the nuclear medicine clinics for targeting a number of cancers, demonstrating its dominant role in cancer therapy. ^{177}Lu emits medium energy β^- particles (78.6 % of 497 keV β^-) thereby decaying to stable ^{177}Hf [5–7]. Scintigraphic imaging and dosimetric evaluation during therapy is possible due to the emission of two low energy γ radiations with low abundances (11 % of 208 keV and 6.4 % of 113 keV) [5–7]. Radiolabeling of biomolecules such as peptides and antibodies with ^{177}Lu is accomplished through the use of bifunctional chelating agents. The macrocyclic chelator DOTA (1,4,7,10-tetraaza cyclododecane tetraacetic acid) and its analogs are universally employed chelating agents for cross-linking biomolecules with many radiometals, including ^{177}Lu [7–9]. DOTA remains the chelating agent of choice for sequestering many radionuclides including ^{177}Lu due to the achievement of high radiolabeling yields in moderately high specific activities as well as superior stability of the radiolabeled complexes in vivo [7–9]. In pursuit of newer chelating agents which demonstrate excellent thermodynamic and kinetic stabilities on radiolabeling with the diagnostic and therapeutic radioisotopes, a wide range of ligands with varied donor atoms as well as chelating networks have been synthesized and investigated [10–12]. Selection of a ligand for use with a particular radioisotope is based on radiolabeling yields, reaction conditions including temperature, influence of trace metallic impurities in solution, in vitro stability, kinetic inertness in vivo preventing the release of the radiolabel in vivo and the extent of accumulation of the radiolabeled complexes in non-target organs. Although DOTA and its bifunctional derivatives are considered to be very efficient

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chelators for ^{177}Lu, they reportedly suffer from slow complex formation at mild conditions requiring the performance of the radiolabeling reaction at elevated temperatures [13, 14]. This is definitely a drawback especially while radiolabeling temperature-sensitive biological vectors such as antibodies. Hence, extensive research has been carried out in pursuit of ligands which can enable instantaneous radiolabeling at room temperature along with imparting high kinetic stability in vivo to the radiolabeled complexes. In this respect, the development of backbone substituted derivatives of the acyclic ligand DTPA such as the cyclohexyl derivative of DTPA (CHX-A″-DTPA) which form stable complexes with ^{177}Lu is an important advancement [15]. However, macrocyclic ligands which can sequester the radiometals at rates faster than DOTA with the additional benefit of kinetic inertness in vivo are highly desirable. Recently, Chong et al. have reported the synthesis of the ligand NETA which consists of a macrocyclic triaza cyclononane ring linked to an iminodiacetic acid moiety while Baranyai and co-workers have reported the synthesis of AAZTA, a cyclic heptadentate ligand forming instantaneous complexes with many metals including radiolanthanides [16, 17]. Tircsó G et al. have reported the synthesis of the ligand [*S*-5*p*-(para nitrobenzyl) 3,6,9,15-tetraazabicyclo [9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid] (*p*-NO_2-Bz-PCTA) which forms complexes with several metals of particular interest to nuclear medicine including lutetium, indium and yttrium, at an order of magnitude quicker than DOTA [18, 19]. The authors observed that the acid catalyzed dissociation of the lanthanide-PCTA complexes was slower even though the thermodynamic stabilities of the complexes were low. However, they reportedly exhibit high kinetic inertness analogous to the DOTA complexes [19]. Recently, the potential of this ligand for use with ^{64}Cu was examined by Ait-Mohand et al. [20] while our group has evaluated its ^{68}Ga and ^{90}Y complexation properties [21–23]. The objective of the present study is to investigate the use of para isothiocyanato benzyl 3,6,9,15-tetraazabicyclo [9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid (PCTA-NCS) as a BFCA for preparation of ^{177}Lu radiopharmaceuticals with respect to the complexation kinetics, amenability for room temperature labeling, influence of trace metal ions on the radiolabeling yields, in vitro and in vivo stabilities and the biodistribution pattern in normal Swiss mice, in comparison to ^{177}Lu-DOTA-NCS.

Experimental

Materials

^{177}Lu of specific activity 25.7 ± 1.4 Ci/mg (at 24 h after the end of irradiation) was produced by irradiation of enriched Lu_2O_3 target (82 % in ^{176}Lu) at a thermal neutron flux of ~1.4 × 10^{14} n/cm^2 s for a period of 21 days and further processed at the Radiochemicals Section of Isotope Production and Applications Division, BARC [6, 24]. The bifunctional chelating agents viz. PCTA-NCS (Fig. 1) and DOTA-NCS were purchased from M/s. Macrocyclics, Dallas, USA. Lutetium chloride used as a carrier in the experiments, calcium (II), copper (II), iron (III) and zinc (II) salts as well as sodium acetate were purchased from M/s. Aldrich, USA. HPLC grade water (Merck, India) was used for preparation of all reagents. Radioactivity measurements were performed using a well-type NaI (Tl) detector (ECIL, India) while radioactivity measurements during biodistribution experiments were performed using a flat-geometry NaI(Tl) detector. HPLC analyses were carried out on a dual pump HPLC system (JASCO, Japan) equipped with a reversed phase C-18 HiQ Sil column (5 µm, 4 × 250 mm), coupled to a PU 1575 UV/visible detector (JASCO, Japan) and a NaI (Tl) radioactivity detector (Raytest, Germany). Whatman 3 mm chromatography paper (20 mm width) was purchased from M/s. Whatman International, UK. Approval from the institutional animal ethics committee was obtained before carrying out the biodistribution studies. All the reactions were carried out in acid washed glass vials to minimize the presence of trace metallic impurities.

Methods

Preparation and characterization of ^{177}Lu-PCTA-NCS complex

The ability of the ligand PCTA-NCS to sequester ^{177}Lu effectively was ascertained by carrying out its radiolabeling at various Lu to PCTA-NCS molar ratios (1:20, 1:10, 1:4, 1:2 and 1:1) in 0.1 M sodium acetate buffer at pH ~5.5 at 80 °C for 30 min. The reaction volume was kept constant at

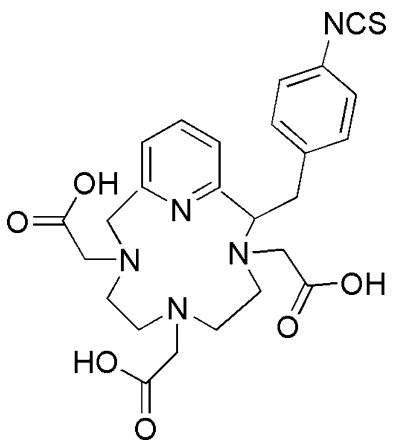

Fig. 1 Chemical structure of PCTA-NCS

1 ml. All the experiments were carried out with trace activity of $^{177}LuCl_3$ (~37–74 MBq) with deliberate addition of ^{176}Lu carrier equivalent to 100 mCi of ^{177}Lu (~4 μg, based on the specific activity of ^{177}Lu as ~25 Ci/mg). The trace level activity of $^{177}LuCl_3$ (~37–74 MBq) contained very small amounts of carrier ^{176}Lu (~40–80 ng) which was much less in comparison to the carrier ^{176}Lu deliberately added in the reaction. Complexation of ^{177}Lu with DOTA-NCS was also carried out at the above-mentioned molar ratios, under identical reaction conditions, for comparison. After ascertaining the complexation yields at the above-mentioned Lu to PCTA-NCS molar ratios, complexation kinetics was determined by carrying out the radiolabeling reaction at ambient temperature (25 °C) and at 80 °C at the lowest ^{177}Lu:PCTA molar ratio (1:2) at which >95 % radiolabeling yield was achieved. For comparison, complexation was also carried out with DOTA-NCS by adopting identical reaction conditions using 37 MBq of $^{177}LuCl_3$ and 4 μg of ^{176}Lu carrier.

Characterization of ^{177}Lu-PCTA-NCS and ^{177}Lu-DOTA-NCS complexes as well as the determination of radiolabeling yields was performed by HPLC and paper chromatography techniques. For HPLC characterization, 20 μl aliquots of the ^{177}Lu labeled complexes were injected into a C18 reverse phase column eluted using water (solvent A) and acetonitrile (solvent B) both containing 0.1 % trifluoroacetic acid in a gradient mode of elution (0–4 min 5 % B, 4–20 min 5–95 % B, 20–30 min 95–5 % B) at a flow rate of 1 ml/min and the radioactivity was monitored using a flow-through NaI(Tl) scintillation detector. Paper chromatography was performed using 50 % aqueous acetonitrile as the mobile phase to differentiate between free and complexed ^{177}Lu.

Role of trace metal impurities on yields of ^{177}Lu-PCTA-NCS

It is well recognized that trace metallic contaminants play a major role in reducing the yields of ^{177}Lu radiopharmaceuticals, particularly the receptor-targeted radiopharmaceuticals synthesized using small amounts of ligands. For instance, Fe(III) ions may be present in trace levels even in highly pure reagent solutions [25, 26]. Hence, the potential role of trace metal ions such as Ca(II), Cu(II), Fe(III) and Zn(II) on the radiochemical yield of ^{177}Lu-PCTA-NCS was studied in comparison to that of ^{177}Lu-DOTA-NCS. For this purpose, the absence of trace metal ions in the $^{177}LuCl_3$ solution was ascertained by carrying out ICP-AES analysis of decayed ^{177}Lu samples, as reported elsewhere [27]. All the experiments were carried out using 37–74 MBq of $^{177}LuCl_3$ with deliberate addition of ^{176}Lu carrier (4 μg) equivalent to 100 mCi of ^{177}Lu, as explained previously. Standard solutions of the trace metal metal ions (Ca(II), Cu(II), Fe(III) and Zn(II)) were prepared and diluted to the appropriate concentrations corresponding to trace metal to ^{176}Lu molar ratios of 0.1:1, 1:1 and 10:1 respectively, keeping the Lu:PCTA-NCS molar ratio constant at 1:2 for all the studies). In a typical complexation reaction, 37–74 MBq of $^{177}LuCl_3$ was taken in 0.1 M sodium acetate buffer to which carrier Lutetium (^{176}Lu) (4 μg/23 μM) equivalent to 100 mCi of ^{177}Lu (3.7 GBq) was added along with the corresponding trace metal solution followed by the ligand PCTA-NCS (46 μM). The reaction volume was kept constant at 1 ml and was buffered at pH 5–5.5. The radiolabeling reaction was carried out for 15 min at ambient temperature. A reference reaction was also set-up simultaneously in the absence of trace metal ions. All the reactions were carried out with DOTA-NCS under identical reaction conditions, for comparison. All the reactions were repeated four times and the results correspond to the mean ± SD of four replicates ($n = 4$).

In vitro stability studies and determination of octanol/water partition co-efficients (log P)

In vitro stability of ^{177}Lu-PCTA-NCS complex (which has a Lu:PCTA-NCS molar ratio of 1:2) was determined in saline and human serum for 7 days at 37 °C. For determining the in vitro stability in saline, 100 μl of the complex was incubated in 1.9 ml of saline and incubated at 37 °C. Aliquots of ~100 μl were taken at specific time intervals up to 7 days post-incubation and the radiochemical purity of the complex was determined by paper chromatography using 50 % aqueous acetonitrile as mobile phase. For determination of stability in human serum, ~100 μl of the complex was incubated in 1 ml of human serum at 37 °C for 7 days. At end of every 24 h, ~100 μl was taken and 2 ml of acetonitrile was added. The precipitate was removed by centrifugation and the supernatant was analysed by paper chromatography using 50 % aqueous acetonitrile as mobile phase. A similar experiment was carried out with ^{177}Lu-DOTA-NCS for comparison (Lu:DOTA-NCS molar ratio of 1:2).

The octanol to water partition coefficient of ^{177}Lu-PCTA-NCS was determined by adding 100 μl of the complex to a biphasic solution consisting of 900 μl saline and 1 ml of *n*-octanol. The resulting solution was shaken for ~5 min after which the aqueous and octanol layers were separated. Equal aliquots of both the layers were counted. 100 μl of the octanol phase was again mixed with 900 μl of octanol and 1 ml of saline and the experiment was repeated once again. Logarithm of the ratio of the counts in the octanol phase to that in the aqueous phase gave the partition coefficient values. Similar experiment was also carried out with ^{177}Lu-DOTA-NCS for comparison.

Biodistribution studies

For biodistribution studies, ^{177}Lu-PCTA-NCS complex was prepared at a Lu:PCTA-NCS molar ratio of 1:2 (using 37–74 MBq of $^{177}LuCl_3$, 4 μg of $^{176}LuCl_3$ carrier) and injected via the lateral tail vein in normal healthy Swiss mice. In brief, each mice, weighing 20–25 g, was injected with 0.1 ml of ^{177}Lu-PCTA-NCS complex (~370 kBq per animal, $n = 4$ per time point). The animals were kept in separate cages and were sacrificed by carbon dioxide asphyxiation at 1, 3 and 24 h p.i., blood was collected, the organs of interest (liver, lungs, kidney, intestine, spleen, stomach, muscle and bone) were excised and weighed. The associated radioactivity was measured in a flat-bed NaI (Tl) scintillation counter. The organ activities were corrected for decay and percentage injected activity (%ID) and percentage injected activity per gram (%ID g^{-1}) were determined. Percentage activity excreted (urine and feaces) was determined by counting the cage paper. Blood, bone and muscle uptakes were calculated by assuming their weights to be 6.5, 10 and 40 % of the body weight respectively [28]. Biodistribution study of ^{177}Lu-DOTA-NCS was also carried out under identical experimental conditions (at a Lu:DOTA-NCS molar ratio of 1:2, using 37–74 MBq of $^{177}LuCl_3$ and ~4 μg of $^{176}LuCl_3$ carrier) for comparison.

Results

Preparation and characterization of ^{177}Lu-PCTA-NCS complex

For a bifunctional chelating agent to be considered suitable for preparation of radiopharmaceuticals for in vivo targeted therapy, it must preferentially form complexes with the radiometals at low radiometal to ligand ratios thereby reducing the amount of ligand used for preparing the radiopharmaceuticals. Herein, experiments were carried out to determine the amount of PCTA-NCS ligand required for preparation of ^{177}Lu-PCTA-NCS complex at high radiochemical yields using carrier $^{176}LuCl_3$ equivalent to 100 mCi of $^{177}LuCl_3$ and ~37 MBq of $^{177}LuCl_3$ activity. Complexation reactions were carried out at various ^{177}Lu to PCTA-NCS molar ratios. Table 1 depicts the radiolabeling yields at various ^{177}Lu:PCTA-NCS molar ratios, in comparison to ^{177}Lu-DOTA-NCS, when reaction was carried out at 80 °C for 30 min, using trace levels of ^{177}Lu and ~4 μg of Lu carrier equivalent to ~100 mCi of ^{177}Lu. It is evident that at a 1:1 molar ratio of ^{177}Lu to PCTA-NCS, the radiolabeling yield was only 80 ± 6 %. Radiolabeling yields in excess of 95 % could be obtained even at 1:2 (lutetium to PCTA) ratios, similar to that of ^{177}Lu-DOTA-NCS. Based on the results of the above experiments, all further experiments (complexation kinetics, influence of trace metal ions, in vitro stability studies and biodistribution studies) were carried out using ^{177}Lu-PCTA-NCS complex having a Lu:PCTA molar ratio of 1:2. The ^{177}Lu-PCTA-NCS and ^{177}Lu-DOTA-NCS complexes were characterized by paper chromatography and HPLC techniques. In paper chromatography using 50 % aqueous acetonitrile as the mobile phase, R_f value of ^{177}Lu-PCTA-NCS and ^{177}Lu-DOTA-NCS complexes was about 0.8–1.0 while uncomplexed ^{177}Lu remained at $R_f = 0.0$. HPLC analysis employing a C18 reverse phase column with a gradient elution of water and acetonitrile both containing 0.1 % TFA using the optimized protocol revealed the formation of ^{177}Lu-PCTA-NCS complex having the major peak at a retention time of 19.1 min (Fig. 2) while unbound ^{177}Lu(III) eluted at 3.2 min. Results of the experiments carried out to determine the complexation kinetics at ambient temperature (25 °C) and 80 °C showed that high radiolabeling yields (>95 %) could be achieved even within 15 min of incubation at ambient temperature for 15 min, for both ^{177}Lu-PCTA-NCS and ^{177}Lu-DOTA-NCS (at Lu:Ligand molar ratio of 1:2). Hence, all subsequent radiolabeling reactions were carried out for 15 min at ambient temperature. This finding demonstrates that it was not essential to perform the radiolabeling reaction at elevated temperature to obtain high radiolabeling yields of ^{177}Lu-PCTA-NCS.

Role of trace metal impurities on yields of ^{177}Lu-PCTA-NCS

Results of the studies carried out to determine the role of trace metal ions on the ^{177}Lu labeling yields are summarized in Table 2 in which the percentage yield of ^{177}Lu-PCTA-NCS and ^{177}Lu-DOTA-NCS is given in terms of trace metal to lutetium molar ratios of 0.1, 1.0 and 10 respectively. In control experiments carried out in the absence of any trace metallic contaminant, radiolabeling yields exceeding 95 % could be readily obtained. It is very well evident from the Table 2 that the adverse effects exerted by Fe(III) ions on the yields of ^{177}Lu-PCTA-NCS and ^{177}Lu-DOTA-NCS is similar with reduced yields only at higher trace metal to lutetium ratios (10:1). The yield of

Table 1 Complexation of ^{177}Lu with PCTA-NCS and DOTA-NCS at various Lu to ligand molar ratios (pH ~5.5, added ca. Lu ~4 μg, 30 min at 80 °C, $n = 3$)

^{177}Lu-BFC complex	Lu:ligand molar ratio				
	1:20	1:10	1:4	1:2	1:1
^{177}Lu-PCTA-NCS	98 ± 2	98 ± 1	97 ± 1	97 ± 1	80 ± 6
^{177}Lu-DOTA-NCS	99 ± 1	99 ± 1	99 ± 1	98 ± 1	97 ± 2

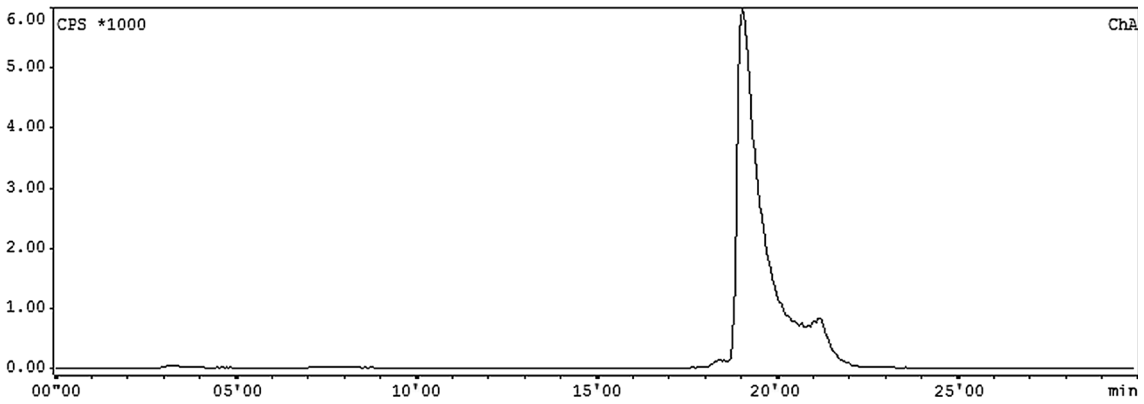

Fig. 2 HPLC radiochromatogram of ^{177}Lu-PCTA-NCS

^{177}Lu-PCTA-NCS was reduced only at ten times molar excess of Cu(II) ions over Lu(III) ions. In contrast, the yields of ^{177}Lu-DOTA-NCS were reduced at equimolar ratios of Cu(II) and Lu(III) ions. Zn(II) ions reduced the radiochemical yields of ^{177}Lu-PCTA-NCS at equimolar ratios with Lu(III) ions. A similar trend was also observed in the formation of ^{177}Lu-DOTA-NCS in presence of Zn(II) ions. Ca(II) ions did not have any adverse effect on the radiochemical yields of the ^{177}Lu complexes at lower Ca(II) to Lu(III) molar ratios (0.1 and 1.0).

In vitro stability studies and determination of octanol/water partition co-efficients (log *P*)

The radiochemical purity of ^{177}Lu-PCTA-NCS when incubated in saline and serum respectively was determined at various time intervals up to 7 days (at 37 °C) in comparison to ^{177}Lu-DOTA-NCS. The results are given in Fig. 3. It is evident that the radiochemical purity of ^{177}Lu-PCTA-NCS did not decrease significantly with time and was more or less similar that of ^{177}Lu-DOTA-NCS complex. Even at 7 days post-incubation, >95 % of the ^{177}Lu-PCTA-NCS complex remained intact, both in saline and serum.

Based on the results of the Octanol:water partition experiments, log *P* values for ^{177}Lu-PCTA-NCS and ^{177}Lu-DOTA-NCS were determined to be -1.4 ± 0.2 and -2.4 ± 0.4. Based on the log *P* values obtained herein, it is evident that ^{177}Lu-PCTA-NCS is less hydrophilic than the corresponding DOTA-NCS complex.

Biodistribution studies

Biodistribution of ^{177}Lu-PCTA-NCS was carried out in normal Swiss mice in comparison to that of ^{177}Lu-DOTA-NCS, the results of which are shown in Table 3 as the percentage administered activity per gram of tissue/organ (%ID g^{-1}). It is evident from the data that most of the ^{177}Lu-PCTA-NCS complex is excreted by 24 h p.i., similar to that of ^{177}Lu-DOTA-NCS complex. Activity in the blood was not very high

Table 2 Influence of trace metal ions on the radiochemical yields of ^{177}Lu-PCTA-NCS and ^{177}Lu-DOTA-NCS (Lu:ligand molar ratio of 1:2, $n = 4$)

Trace metal impurity	Trace metal/Lu molar ratio	% Radiochemical yield	
		^{177}Lu-PCTA-NCS	^{177}Lu-DOTA-NCS
Ca(II)	0.1	93 ± 4	96 ± 3
	1.0	89 ± 1	94 ± 0
	10	84 ± 5	89 ± 4
Cu(II)	0.1	94 ± 2	94 ± 3
	1.0	92 ± 4	86 ± 1
	10	90 ± 0	82 ± 3
Fe(III)	0.1	97 ± 1	96 ± 0
	1.0	95 ± 1	94 ± 3
	10	91 ± 1	89 ± 3
Zn(II)	0.1	95 ± 3	94 ± 4
	1.0	89 ± 1	92 ± 7
	10	85 ± 1	91 ± 3

in both cases. However, the uptake of ^{177}Lu-PCTA-NCS complex in the liver and intestine was higher than that of the ^{177}Lu-DOTA-NCS complex at 1 h and 3 h p.i. which decreased significantly by 24 h p.i., as shown in Table 4. The absence of noteworthy uptake in the bone affirmed the in vivo stability of the ^{177}Lu-PCTA-NCS complex.

Discussion

The successful clinical deployment of ^{177}Lu labeled peptides and antibodies has led to the widespread recognition of ^{177}Lu as an important therapeutic radionuclide. The pursuit for chelating agents which enable near-complete complexation at ambient temperature while imparting high stability to the complex in vivo has resulted in the synthesis and evaluation of a number of ligands in recent times. One such ligand PCTA is reportedly of potential application due to its fast complexation rate with a number of medically important radioisotopes [18, 19]. Although there are a few reports on the use of this ligand for preparation of ^{177}Lu labeled monoclonal antibodies and peptides [29], to the best of our knowledge, detailed studies as regards the influence of trace metal ions, in vitro stability and the biodistribution pattern of ^{177}Lu-PCTA-NCS complex are not yet reported. Herein we report detailed studies carried out to evaluate the suitability of PCTA-NCS as a BFCA for design of ^{177}Lu radiopharmaceuticals. The results of our work reported here show that under the optimized conditions, high radiolabeling yields could be achieved with PCTA-NCS even at 1:2 lutetium to PCTA-NCS molar ratios. However, the radiolabeling yields at equimolar ratios of Lu and PCTA-NCS was less than 90 %, in contrast to ^{177}Lu-DOTA-NCS complex having equimolar ratios of Lu and DOTA-NCS. Experiments carried out to determine the detrimental effects of trace metal contaminants on ^{177}Lu-PCTA-NCS complex formation showed that while Fe(III) and Cu(II) ions had minimum influence on the radiolabeling yields at low concentrations, Zn(II) ions reduced the yields at equimolar ratios with respect to lutetium. A similar trend was observed for DOTA-NCS ligand. However, none of the trace metallic impurities studied reduced the radiochemical yields significantly even at high metal to lutetium molar concentrations, although all the studied cations such as Cu(II), Zn(II), and Fe(III) also

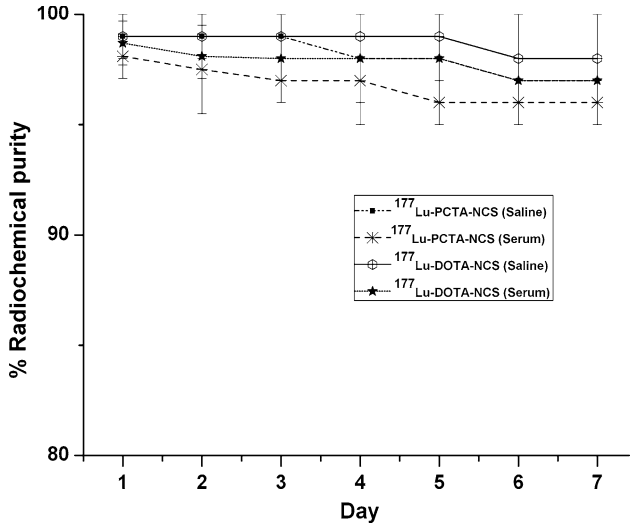

Fig. 3 In vitro stability of ^{177}Lu-PCTA-NCS and ^{177}Lu-DOTA-NCS in saline and serum (incubated for 7 days at 37 °C)

Table 3 Biodistribution pattern of ^{177}Lu-PCTA-NCS in normal Swiss mice ($n = 4$)

Tissue/organ	%ID g^{-1}		
	1 h p.i.	3 h p.i.	24 h p.i.
Blood	1.2 ± 0.1	0.9 ± 0.1	0.4 ± 0.1
Liver	1.3 ± 0.2	1.2 ± 0.1	0.4 ± 0.1
Int + Gb	9.3 ± 1.8	8.2 ± 3.1	0.1 ± 0.0
Stomach	0.2 ± 0.1	0.2 ± 0.1	0.01 ± 0.0
Heart	0.4 ± 0.1	0.3 ± 0.0	0.1 ± 0.0
Lung	0.7 ± 0.2	0.5 ± 0.2	0.4 ± 0.1
Spleen	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Tibia[#]	0.05 ± 0.01	0.03 ± 0.02	0.01 ± 0.01
Muscle	1.3 ± 0.3	1.3 ± 0.2	1.0 ± 0.1
Kidneys	0.7 ± 0.2	0.6 ± 0.1	0.5 ± 0.1
Excreta (urine + feaces)[*]	52 ± 5	58 ± 6	74 ± 8

[#] (%ID/organ)

[*] (%ID)

Table 4 Biodistribution pattern of ^{177}Lu-DOTA-NCS in normal Swiss mice ($n = 4$)

Tissue/organ	%ID g^{-1}		
	1 h p.i.	3 h p.i.	24 h p.i.
Blood	1.0 ± 0.1	0.7 ± 0.1	0.3 ± 0.2
Liver	1.0 ± 0.2	0.8 ± 0.1	0.2 ± 0.1
Int + Gb	4.8 ± 0.1	5.2 ± 1.1	0.1 ± 0.0
Stomach	0.2 ± 0.1	0.2 ± 0.1	0.0
Heart	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Lung	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.1
Spleen	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.0
Tibia[#]	0.01 ± 0.0	0.01 ± 0.0	0.0
Muscle	1.2 ± 0.1	0.5 ± 0.2	0.3 ± 0.2
Kidneys	0.7 ± 0.0	0.7 ± 0.1	0.9 ± 0.2
Excreta (urine + feaces)[*]	56 ± 6	62 ± 3	79 ± 6

[#] (%ID/organ)

[*] (%ID)

form thermodynamically stable complexes with the BFCAs used for complexation with ^{177}Lu due to their suitable ionic size, charge and co-ordination chemistry [18, 30, 31]. Asti et al. and Pawlak et al. have previously reported a detailed study of the influence of trace metal ions on ^{90}Y and ^{177}Lu labeling of DOTA-TATE [26, 32]. They have reported that the radiochemical yields of ^{177}Lu-DOTA-TATE is drastically reduced in presence of trace metal ions such as Cu, Fe and Zn. However, a direct comparison of our results of ^{177}Lu-DOTA-NCS with that reported by them for ^{177}Lu-DOTA-TATE is difficult due to the difference in the experimental conditions. Asti et al. have reported that the exclusion of one carboxylate group as in DOTA-TATE (DO3A) has a major impact on the stability of the complexes with Y^{3+} or Lu^{+3} [26]. In addition, the influence of trace metal ions would also depend on the lutetium to ligand molar ratios used for the studies. With higher lutetium to ligand molar ratios, the interference of the trace metal ions is expected to be lower [26]. ^{177}Lu-PCTA-NCS complex also exhibited high in vitro stability, both in saline and serum. Results of the biodistribution studies carried out in Swiss mice show that ^{177}Lu-PCTA-NCS did not show any significant uptake in any vital organs, except the liver and intestine. The dose to the liver and intestine was more with ^{177}Lu-PCTA-NCS than ^{177}Lu-DOTA-NCS at 1 and 3 h p.i. Similar results have been also reported for ^{90}Y and ^{68}Ga labeled PCTA-NCS complexes by other researchers [21, 22]. The higher liver uptake may be attributed to the lower hydrophilicity of ^{177}Lu-PCTA-NCS complex in comparison to the ^{177}Lu-DOTA-NCS complex. The kinetic stability of ^{177}Lu-PCTA-NCS in vivo could be confirmed from the absence of notable uptake in the bone. These results establish the utility of PCTA-NCS ligand as a bifunctional ligand for use with ^{177}Lu, especially while radiolabeling temperature-sensitive biomolecules as the radiolabeling is complete within few minutes at ambient temperature.

Conclusion

In this study, the potential use of PCTA-NCS ligand as a BFCA for ^{177}Lu was evaluated. The high labeling efficiency, in vitro stability and results of the biodistribution studies show that PCTA-NCS ligand could be a suitable BFCA for use with ^{177}Lu.

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