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Synthesis, radiolabeling and evaluation of a new positively charged ^{99m}Tc-labeled fatty acid derivative for myocardial imaging

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^{123}I-labeled fatty acids and ^{18}F-FDG are used as metabolic markers for detecting myocardial abnormalities. However, a ^{99m}Tc-based molecule may find wider application. In the present work, a new ^{99m}Tc-labeled, uni-positively charged, 16-carbon fatty acid has been prepared and evaluated in normal Swiss mice. The results are then compared with the neutral analogue reported earlier.

A 16-cysteinyl hexadecanoic acid conjugate was synthesized in a six-step synthetic procedure starting with 16-bromohexadecanoic acid. The ligand upon incubation with $[^{99m}TcN(PNP6)]^{2+}$ core formed the required positively charged complex in ~85% yield. The complex, which was obtained as a mixture of syn-anti isomers, was purified by HPLC and the major fraction was used for *in vivo* studies in Swiss mice. The biodistribution studies in Swiss mice showed initial uptake similar to ^{125}I-IPPA followed by rapid clearance from the myocardium till 10 min p.i. Thereafter, the rate of clearance was significantly decreased, an observation reported earlier for positively charged fatty acid complexes. In terms of absolute uptake, the positively charged complex performed better than the neutral analogue reported earlier. The positively charged fatty acid complexes, prepared using $[^{99m}TcN(PNP)]^{2+}$ core, seems to be better candidates for the development of myocardial metabolic tracers than their neutral counterparts.

Keywords: $[^{99m}TcN(PNP)]^{2+}$ core; myocardial imaging; fatty acid; $[^{125}I]$-IPPA; PNP ligand

Introduction

Fatty acids are the major source of energy, contributing nearly ~90% of the energy needs, in normal myocardium under fasting condition.[1] They are transported across the lipid membrane either by passive diffusion or via protein transporter.[2] Once inside the myocardium, either they get metabolized via the β-oxidation pathway releasing energy required for the normal functioning of the heart or get stored in the form of triglycerides.[3]

An ischemic myocardium exhibits change in its normal metabolic pathway using fatty acids. The viable ischemic myocardial cells shift to anaerobic metabolic pathway using glucose for their energy needs and survival. It has been seen even after establishing normal oxygenation levels in previously ischemic cells, the cells show predominant glucose metabolism (ischemic memory) before reverting to normal state. Thus, radiolabeled fatty acids provide important information for early identification of cardiac abnormalities in high-risk patients.[1,4,5]

The currently used radiolabeled fatty acids are ^{123}I-labeled Iodophenylpentadecanoic acid (^{123}I-IPPA) and beta-methyl iodophenyl pentadecanoic acid (^{123}I-BMIPP).[1,6,7] Both are metabolic substrates but ^{123}I-IPPA is rapidly metabolized, resulting in rapid clearance from the heart, whereas ^{123}I-BMIPP being a branched chain fatty acid does not readily undergo β-oxidation and is retained. Thus, ^{123}I-IPPA imaging involves study of washout kinetics, which reflect fatty acid metabolism, with ischemic areas showing low uptake and delayed washout.[3]

The ^{123}I-labeled fatty acids are based on cyclotron produced radioisotope, ^{123}I. Although number of cyclotrons is increasing every year, cyclotron-based isotopes are currently not widely available in all parts of the world. Hence, molecules labeled with ^{99m}Tc, which is a more widely available isotope, are preferable. Several reports are available in the literature on the synthesis and evaluation of ^{99m}Tc-labeled fatty acid derivatives based on various metal cores albeit with suboptimal properties.[8–18]

Among the different ways of labeling molecules with ^{99m}Tc, the use of $[^{99m}TcN(PNP)]^{2+}$ core has shown excellent *in vivo* characteristics suitable for myocardial imaging.[19,20] This core forms *pseudo*-octahedral complexes, where the two cis-positions in the square basal plane are being occupied by phosphorus

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atoms of the long chain PNP ligand and the other two cis-positions occupied by the π-donor bidentate ligand having donor groups such as SS/SO/SN. Thus, the amino acid cysteine is a useful bi-functional chelator, where a bio-molecule, like fatty acid, can be attached either at –COOH or $–NH_2$ group, and the other two groups, SH and NH_2/COOH, can be used for labeling with $[^{99m}TcN(PNP)]^{2+}$ core.[21] The overall charge of the complex depends on the groups in cysteine that are coordinated to the $[^{99m}TcN(PNP)]^{2+}$ core, with the overall charge being positive, if –SH and $–NH_2$ group of cysteine are involved in coordination, or neutral, if –SH and –COOH groups are coordinated to the $[^{99m}TcN(PNP)]^{2+}$ core.

The desired characteristics of a ^{99m}Tc-labeled fatty acid are high uptake in the normal myocardium with retention long enough to carry out SPECT imaging and rapid clearance from background tissue/organs such as blood, liver and lungs.[5] The presence of PNP ligand in the complex while increasing the lipophilicity of the complex, and thus the initial uptake in the myocardium, also helps in fast clearance of the activity from the non-target organs due to its hydrolysable ether linkages.

Recently, a series of neutral and positively charged fatty acid derivatives labeled with $[^{99m}TcN(PNP)]^{2+}$ core have been prepared and evaluated in biological systems.[8] Our group working on similar complexes has earlier reported the synthesis and bio-evaluation of a neutral complex of a long chain 16-carbon fatty acid derivative.[9] In the present work, a positively charged structural analogue of the previously reported 16-carbon fatty acid derivative was synthesized, radiolabeled and evaluated in normal Swiss mice. The results are compared with the neutral analogue as well as ^{125}I-IPPA reported earlier.[9]

Experimental

The 16-bromo hexadecanoic acid, triethyl silane, succinic dihydrazide and stannous chloride were obtained from Aldrich, USA. *N*-Boc *S*-trityl cysteine, *N*-ethyl, *N*′-(3-dimethylamino) carbodiimide hydrochloride (EDCI) and potassium carbonate were purchased from Fluka, Germany. Hydrazine hydrate (80%) was purchased from Riedel-de-Haën, Germany. Phthalimide was obtained from Loba Chemicals, India. All other reagents used were of analytical grade. Sodium pertechnetate ($Na^{99m}TcO_4$) was eluted with normal saline just before use from a ^{99}Mo-^{99m}Tc gel generator, supplied by Board of Radiation and Isotope Technology, India. Bis[(diethoxypropylphosphanyl) ethyl]ethoxy ethylamine (PNP6) was obtained as a gift from Prof. Adriano Duatti, University of Ferrara, Italy. Silica gel plates (Silica Gel 60 F_{254}) were obtained from Merck, India. The HPLC of the prepared complex was carried out on a JASCO PU 2080 Plus dual pump HPLC system, Japan, with a JASCO 2075 Plus tunable absorption detector and Gina Star radiometric detector system, using a C18 reversed phase HiQ Sil (5 μm, 4 × 250 mm) column. The IR spectra of all the compounds were recorded on a JASCO FT-IT/420 spectrophotometer, Japan. The ^{1}H-NMR spectra were recorded either on 200 or 300 MHz Bruker Spectrophotometer, USA.

Synthesis

Ethyl 16-bromohexadecanoate (1)

The 16-bromo hexadecanoic acid (500 mg, 1.5 mmol) was dissolved in ethanol (20 mL) and concentrated sulphuric acid (0.1 mL) was added. The reaction mixture was then refluxed overnight and cooled. Excess ethanol was removed under vacuum and ice-cold water (20 mL) was added to the oily residue. The aqueous layer was extracted with chloroform (3 × 10 mL). The chloroform extracts were pooled, dried over anhydrous sodium sulphate, filtered and evaporated to obtain the target compound. Yield: 97% (∼525 mg). IR (neat, cm^{-1}) 2919 (s); 2849 (m); 1740 (s).

Ethyl 16-phthalimidohexadecanoate (2)

The compound **1** (500 mg, 1.37 mmol) and phthalimide (203 mg, 1.37 mmol) were dissolved in acetonitrile (15 mL) and anhydrous potassium carbonate (209 mg, 1.5 mmol) was added. The reaction mixture was then refluxed overnight. The progress of the reaction was monitored by TLC in ethyl acetate:hexane (1:9 v/v) mixture. Upon completion of the reaction, the reaction mixture was cooled, filtered and solvent was removed under vacuum to obtain the crude product. The target compound was obtained by silica gel chromatography (10% ethyl acetate: 90% chloroform). Yield ∼87% (511 mg). R_f = 0.8 (ethylacetate/hexane, 1:9 v/v). IR (neat, cm^{-1}) 2919 (s); 2849 (m); 1740 (s); 1704 (s); 1464 (m); 1406 (m); 1174 (m); 1064 (m); 714 (s). ^{1}H-NMR ($CDCl_3$, δ ppm) 7.790–7.853 ($C_6\underline{H}_4$C-, 2H, m); 7.672–7.733 ($C_6\underline{H}_4$C-, 2H, m); 4.057–4.164 (-COOC$\underline{H}_2$CH$_3$, 2H, q, J = 7.2 Hz); 3.626–3.698 (-CH$_2$C$\underline{H}_2$N(CO)$_2$-, 2H, t, J = 7 Hz); 2.235–2.309 (-CH$_2$C$\underline{H}_2$COOEt, 2H, t, J = 7.4 Hz); 1.622 (-C$\underline{H}_2$CH$_2$COOEt and -C$\underline{H}_2$CH$_2$N(CO)$_2$-, 4H, m); 1.206–1.277 [(C$\underline{H}_2$)$_{11}$ and -COOCH$_2$C$\underline{H}_3$, 25H, m].

Ethyl 16-aminohexadecanoate (3)

To compound **2** (300 mg, 0.69 mmol) dissolved in ethanol (15 mL), 80% hydrazine hydrate (219 μL, 3.49 mmol) was added and the reaction mixture refluxed for 3 h. Thereafter, the reaction mixture was brought to room temperature and excess hydrazine hydrate and ethanol were removed under vacuum. The precipitate obtained was re-dissolved in ethanol (15 mL) and treated with 2 N HCl (5 mL). The reaction mixture was again refluxed overnight, cooled and then excess solvent was removed under vacuum to obtain a white powder. The crude product obtained was again refluxed overnight in excess ethanol and concentrated H_2SO_4 to esterify the fatty acid hydrolyzed in the previous step. The reaction mixture was cooled to obtain a white precipitate of phthalhydrazide, which was removed by filtration. The filtrate was then evaporated under vacuum to obtain an oily residue, which was dissolved in cold water and the pH was brought above 7 with 5% sodium bicarbonate solution. The alkaline solution was extracted with chloroform (3 × 10 mL) and the pooled extracts were dried over anhydrous sodium sulphate. The chloroform was removed to give the pure product in quantitative yield (∼230 mg). IR (neat, cm^{-1}) 3197 (w); 2916 (s); 2848 (m); 1738 (s); 1577 (m); 1518 (s); 1463 (m); 1182 (s); 721 (m). ^{1}H-NMR ($CDCl_3$, δ ppm) 4.056–4.162 (–COOC$\underline{H}_2$CH$_3$, 2H, q, J = 7 Hz); 3.000 (–CH$_2$C$\underline{H}_2$NH$_2$, 2H, m); 2.234–2.308 (–CH$_2$C$\underline{H}_2$COOEt, 2H, t, J = 7.4 Hz); 1.895 (–C$\underline{H}_2$CH$_2$NH$_2$ and –CH$_2$CH$_2$N$\underline{H}_2$, 4H, m); 1.602 (–C$\underline{H}_2$CH$_2$COOEt, 2H, m); 1.239–1.274 [(C$\underline{H}_2$)$_{11}$ and –COOCH$_2$C$\underline{H}_3$, 25H, m].

Ethyl 16-(*N*-Boc, *S*-trityl cysteinyl)hexadecanoate (4)

The compound **3** (150 mg, 0.5 mmol) and *N*-Boc, *S*-trityl cysteine (233 mg, 0.5 mmol) were dissolved in dry dichloromethane (15 mL) and cooled to 0°C with continuous stirring. To the

cooled reaction mixture, EDCI (106 mg, 0.55 mmol) was added and stirred for 1 h after which the reaction was continued overnight at room temperature. The progress of the reaction was monitored by TLC. On completion of the reaction, the reaction mixture was washed with water (3×10 mL) and the organic phase was dried over anhydrous sodium sulphate. The crude product was obtained after removing dichloromethane and purified using silica gel column chromatography (ethyl acetate:chloroform, 5:95 v/v) to obtain the desired product **4**. Yield ~72% (267 mg). $R_f = 0.6$ (ethyl acetate/chloroform, 5:95 v/v). IR (neat, cm^{-1}) 3316 (b); 3057 (w); 2975 (w); 2925 (s); 2853 (s); 1713 (bs); 1681 (m); 1488 (m); 1366 (m); 1168 (s); 1032 (w); 743 (s); 700 (s). ^{1}H-NMR ($CDCl_3$, δ ppm) 7.221–7.436 [$(C_6\underline{H}_5)_3C$–, 15H, m]; 5.996 (–CHCONH$\underline{C}$H$_2$–, 1H, s); 4.825 (–N$\underline{H}$CHCH$_2$S–, 1H, m); 4.057–4.164 (–COO$\underline{C}$H$_2$CH$_3$, 2H, q, $J = 6.9$ Hz); 3.817 (–NHC$\underline{H}$CH$_2$S–, 1H, m); 3.142–3.190 (–CH$_2$C$\underline{H}_2$NHCO–, 2H, m); 2.67–2.74 (–CHC$\underline{H}_A$H$_B$S–, 1H, m); 2.45–2.53 (–CHCH$_A$H$_B$S–, 1H, m); 2.265–2.314 (–CH$_2$C$\underline{H}_2$COOEt, 2H, t, $J = 7.4$ Hz); 1.617 (–C$\underline{H}_2$CH$_2$COOEt and –C$\underline{H}_2$CH$_2$NHCO–, 4H, m); 1.415 [$(C\underline{H}_3)_3$C–, 9H, s]; 1.15–1.35 [$(C\underline{H}_2)_{11}$ and –COOCH$_2$C$\underline{H}_3$, 25H, m].

16-(*N*-Boc, *S*-trityl cysteinyl) hexadecanoic acid (5)

The compound **4** (60 mg, 0.08 mmol) dissolved in methanol (320 μL) was treated with KOH solution (1 M, 160 μL, 0.16 mmol). The reaction mixture was stirred at room temperature for 48 h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, methanol was removed under vacuum, 5 mL of water was added and the pH of the reaction mixture was adjusted to 3 using 2 N HCl. The white precipitate of the target compound obtained was filtered and dried under vacuum. Yield ~80% (57 mg). $R_f = 0.2$ (ethyl acetate/chloroform, 1:9 v/v). IR (neat, cm^{-1}) 3313 (b); 3057 (w); 2924 (s); 2852 (s); 1685 (s); 1656 (s); 1530 (b); 1491 (m); 1444 (m); 1366 (m); 1248 (w); 1167 (s); 1033 (w); 742 (s); 699 (s). ^{1}H-NMR ($CDCl_3$, δ ppm) 7.209–7.430 [$(C_6\underline{H}_5)_3$C–, 15H, m]; 6.178 (–CHCONH$\underline{C}$H$_2$–, 1H, s); 5.092 (–N$\underline{H}$CHCH$_2$S–, 1H, m); 3.849 (–NHC$\underline{H}$CH$_2$S–, 1H, m); 3.083–3.171 (–CH$_2$C$\underline{H}_2$NHCO–, 2H, m); 2.667 (–CHC$\underline{H}_A$H$_B$S–, 1H, m); 2.523 (–CHCH$_A$H$_B$S–, 1H, m); 2.321–2.345 (–CH$_2$C$\underline{H}_2$COOH, 2H, t, $J = 7.2$ Hz); 1.620 (–C$\underline{H}_2$CH$_2$COOH and –C$\underline{H}_2$CH$_2$NHCO–, 4H, m); 1.405 [$(C\underline{H}_3)_3$C–, 9H, s]; 1.259 [$(C\underline{H}_2)_{11}$, 22H, s].

16-Cysteinyl hexadecanoic acid (6)

The compound **5** (40 mg, 0.06 mmol) was stirred with trifluoroacetic acid (2 mL) for 2 h at room temperature. To the yellow solution, triethyl silane was added drop-wise until it becomes colorless and stirring was continued for another 15 min. Upon removal of the solvent, the target compound was obtained as a sticky white solid, which was used as such for radiolabeling.

Radiolabeling

In a typical labeling procedure, succinic dihydrazide (5 mg), stannous chloride (0.1 mg) and ethanol (250 μL) were taken in a vial to which freshly eluted Na^{99m}TcO$_4$ (50 mCi, 750 μL) was added. Upon keeping the reaction mixture at room temperature for 20 min $[^{99m}TcN]^{2+}$ intermediate was formed. To this intermediate, PNP6 ligand (~2.5 mg) and fatty acid cysteine conjugate (5 mg), each dissolved in nitrogen purged ethanol (250 μL), were added simultaneously and the reaction mixture heated at 90°C for 30 min. Thereafter, the reaction mixture was cooled and then characterized by HPLC.

Quality control

HPLC

The radiochemical purity of the $[^{99m}TcN(PNP)]^{+2}$ core prepared as well as the complex was assessed by HPLC using a C18 reversed phase column. Water (A) and methanol (B) were used as the mobile phase and the following gradient elution technique was adopted for the separation (0 min 50% A, 15 min 0% A, 50 min 0% A). Flow rate was maintained at 1 mL/min. About 25 μL of the test solution was injected into the column and elution was monitored by observing the radioactivity profile.

The purification of the complex was carried out in the same analytical column used for characterization. The ^{99m}Tc-pertechnetate with high radioactive concentration (50 mCi/mL) was used for the radiolabeling. The methanolic fraction containing the pure complex obtained upon HPLC purification was evaporated under vacuum and reformulated in 2 mL of 10% ethanolic solution. About 500 μCi of the pure radiolabeled fatty acid could be obtained by this method, which was sufficient to carry out further studies.

Partition coefficient (Log $P_{o/w}$)

The HPLC purified labeled compound (100 μL) was mixed with water (0.9 mL) and octanol (1 mL) on a vortex mixer for about 1 min and then centrifuged for 5 min to effect the separation of the two layers. Equal aliquots of the two layers were withdrawn and measured for the radioactivity. The readings thus obtained were used to calculate the Log $P_{o/w}$ value of the complex.

Stability studies

Cysteine challenge: For cysteine challenge studies, purified fatty acid complex (50 μL, 10 μCi), 10 mM cysteine solution (50 μL) and saline (400 μL) were mixed in a 5 mL vial and incubated at 37°C for 30 min. Thereafter, the sample was analyzed by TLC (EtOH:$CHCl_3$:benzene:0.5 M ammonium acetate (1.5:2:1.5:0.5) for possible degradation of the original complex (Fatty acid complex: $R_f = 0.1$–0.2).

Serum stability: To assess the stability of the fatty acid complex in human serum, purified fatty acid complex (50 μL, 10 μCi) was incubated with human serum (450 μL) at 37°C for 30 min. Thereafter, the serum proteins were precipitated by addition of ethanol (500 μL), the solution was centrifuged and the supernatant was analyzed by TLC to determine the stability of the complex in serum.

In vivo evaluation studies

All procedures performed herein were in accordance with the national laws pertaining to the conduct of animal experiments. Normal Swiss mice (20–25 g body weight) were used for the *in vivo* distribution assays of the prepared fatty acid complexes. All the mice involved in the study were kept under fasting for 6–7 h prior to the experiment, although water was given *ad libitum*. The HPLC purified radiolabeled preparation (100 μL, 20 μCi) was administered intravenously through tail vein of each animal. Individual sets of animals ($n = 3$) were utilized for studying the biodistribution at different time points (2, 5, 10 and 30 min). The animals were sacrificed immediately at the end of the respective time point and the relevant organs and tissue

were excised for measurement of associated activity. The organs were weighed and the activity associated with each was measured in a flat-bed type NaI(Tl) counter with suitable energy window for ^{99m}Tc (140 keV ± 10%). For the sake of comparison, the activity retained in each organ/tissue was expressed as a percent value of the injected dose per gram (% ID/g).

Results and discussion

The scheme followed for the synthesis of cysteine conjugated fatty acid derivative is shown in Figure 1. The target compound **6** was synthesized following a six-step synthetic procedure. The first step involved the protection of the carboxylic group of bromo fatty acid followed by conversion of the halide to amine via the Gabriel phthalimide synthesis. The free amino group of 16-amino hexanoic ester derivative was then conjugated with acid group of *N*-Boc, *S*-Trt cysteine using EDCI to obtain compound **4**. The compound **4** was then de-esterified using methanolic KOH to generate the free acid **5**. All the intermediates were characterized using FT-IR and ^{1}H-NMR. Finally, the target compound **6** was obtained by simultaneous deprotection of Boc- and trityl-groups using trifluoroacetic acid/triethyl silane combination and used as such without further characterization.

The $[^{99m}TcN]^{2+}$ core forms exceptionally inert complexes when π-donor (N and S^- atoms from cysteine) as well as π-acceptor (two P atoms from PNP6) ligands are present together in the complex.[21] The radiolabeling of the prepared fatty acid derivative was carried out as shown in Figure 2. The formation of $[^{99m}TcN(PNP)]$-fatty acid complex involved prior preparation of $[^{99m}TcN]^{2+}$ core followed by simultaneous addition of PNP6 ligand and fatty acid derivative. The addition of long chain PNP6 ligand to the $[^{99m}TcN]^{2+}$ core sterically orients P donor groups (good π-acceptor) to occupy the basal cis-positions in a square pyramidal complex leaving the other two cis-positions, occupied by labile groups, available for coordination with a suitable π-donor bi-dentate ligand. The fatty acid cysteine ligand now occupies this position forming a unipositively charged [2+2] asymmetric *pseudo*-octahedral complex. The fatty acid complex prepared herein is similar to the complexes reported earlier.[21] Therefore, it is logical to presume the present complex also to have similar structure.

The prepared complex was characterized by HPLC. The asymmetric centre in cysteine residue leads to a final $[^{99m}TcN(PNP6)]$-fatty acid complex as a mixture of syn and anti-isomers.[22] Consequently, two peaks were observed at 25 min (64.8%) and 26.6 min (19.7%) respectively (Figure 3). The stereochemistry of the isomers can be ascertained only after preparing the complex in macroscopic level. The major fraction was isolated by HPLC and used for carrying out the stability and *in vivo* biological studies.

The labeled complex did not show any significant degree of trans-chelation upon challenging with cysteine. However, the complex was observed to degrade in human serum (about 10% over a period of 30 min). The Log $P_{o/w}$ value of the present fatty acid complex was found to be 1.2, which was much lower compared to the neutral analogue (1.8)[9] and ^{125}I-IPPA (1.7).

Figure 4 shows the myocardial uptake and clearance pattern of the complex under evaluation. The results obtained with ^{125}I-IPPA[9] as well as earlier reported neutral analogue[9] are also shown for comparison. It could be observed that initial uptake shown by the present complex (9.88 ± 2.99% ID/g) in the myocardium at 2 min post injection (p.i.) is similar to that of ^{125}I-IPPA. However, initially accumulated activity was not retained as

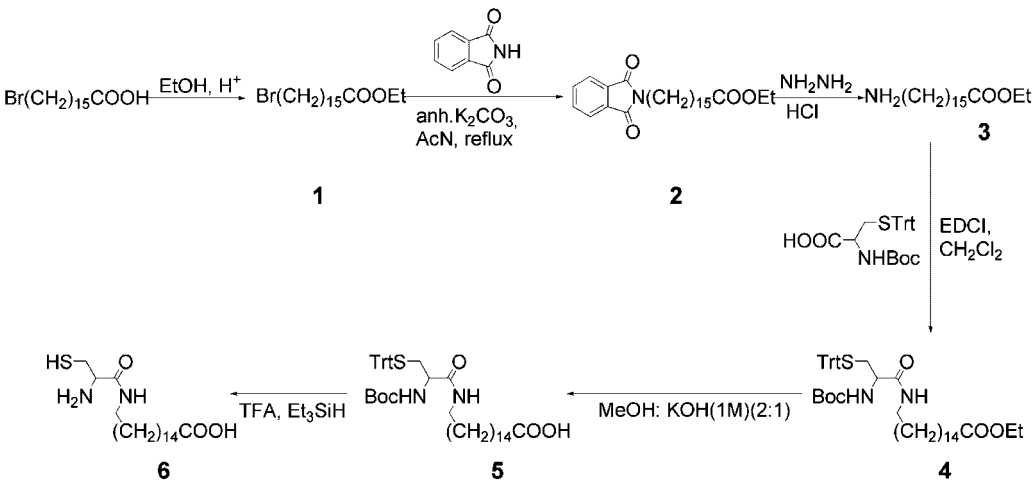

Figure 1. Synthesis of 16-cysteinyl hexadecanoic acid.

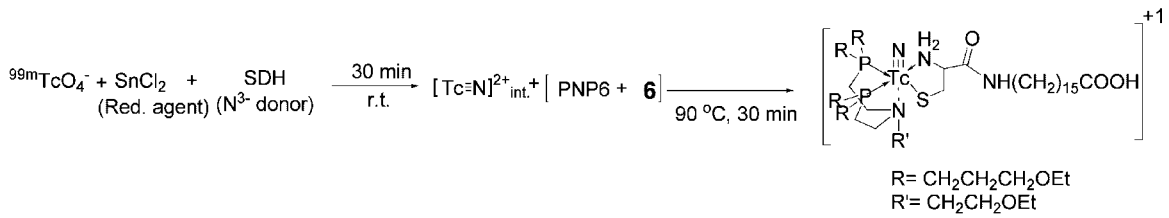

Figure 2. Synthesis of $[^{99m}TcN(PNP)]$-fatty acid complex.

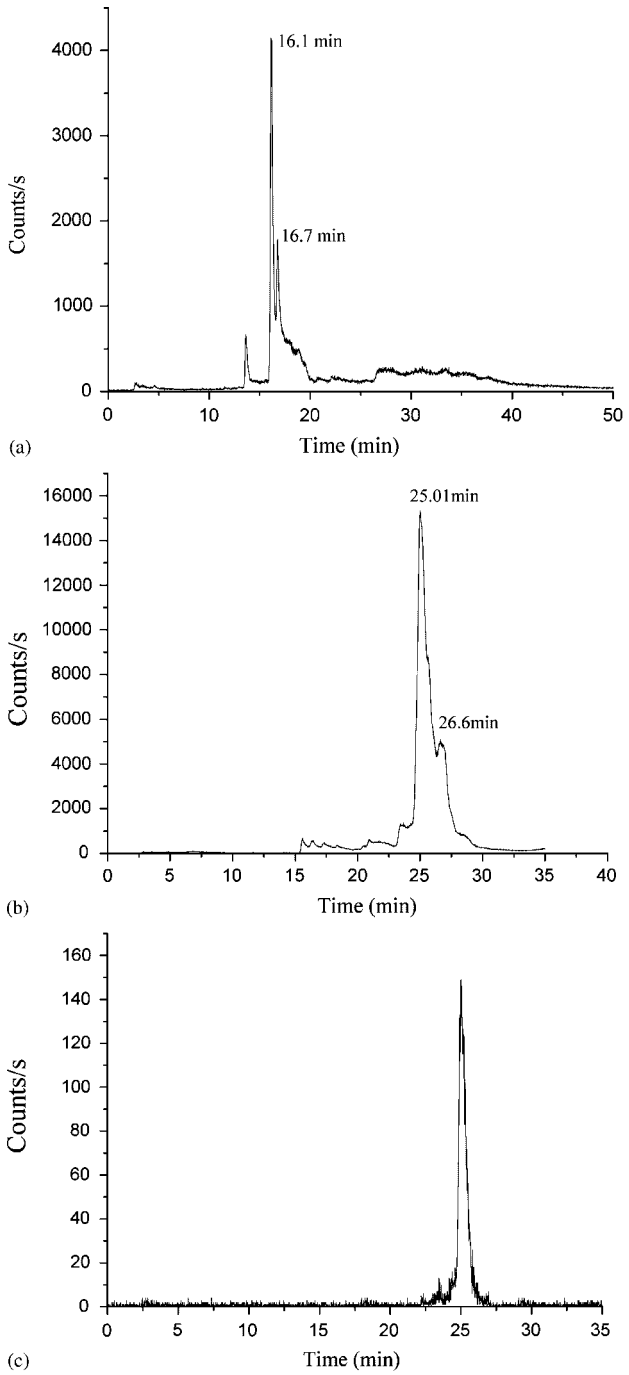

Figure 3. HPLC chromatograms of (a) [^{99m}TcN(PNP)] core, (b) [^{99m}TcN(PNP)]-fatty acid complex and (c) Purified [^{99m}TcN(PNP)]-fatty acid complex.

indicated by the subsequent clearance from the myocardium. This probably is an indication that the [^{99m}TcN(PNP)]-cysteine moiety is not functioning like the ^{125}I-iodophenyl ring in ^{125}I-IPPA. Although clearance of activity was observed from the myocardium, however, it was not uniform. The initial rapid clearance phase up to 10 min p.i. was followed by slow phase with $1.39 \pm 0.36\%$ ID/g remaining after 30 min p.i. It could be noted that activity retained at the end of 30 min p.i. is around 50% of the activity observed at 5 min p.i. This is similar to the observation made by Cazzola *et al.*[8] The positive charge on the complex may lead to the retention of activity in the

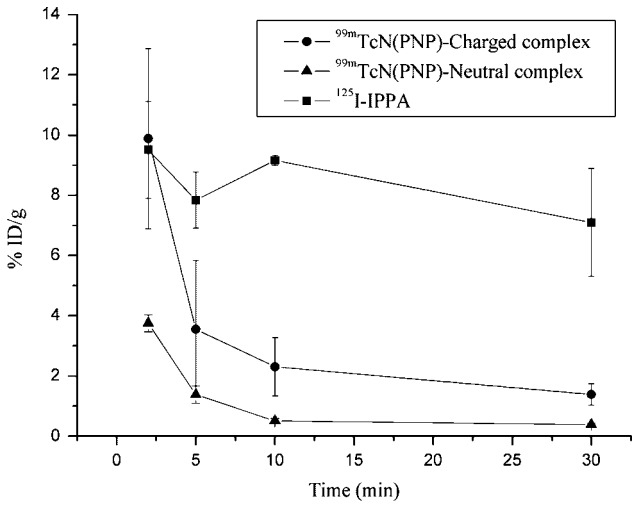

Figure 4. Myocardial uptake pattern of charged fatty acid complex compared with ^{125}I-IPPA and neutral fatty acid complex.

mitochondria. And that such a situation will complicate the interpretation of the results. Though, in the present study, no attempt was made to determine the extent of such interaction, there are few points which indicate that mitochondrial retention of the present cationic complex is insignificant, if not completely absent. The retention of activity in the myocardium through the Coulombic attraction with the mitochondria is often characterized by steady retention of activity for prolonged period as can be seen in the case of the cationic perfusion agents, *viz*. Sestamibi and Tetrofosmin. However, the clearance pattern of the cationic fatty acid complex from the myocardium, evaluated in the present study, showed similarity to neutral fatty acid complex rather than the cationic perfusion agents.

Variation of heart/blood, heart/lung and heart/liver ratio with time observed with positively charged fatty acid complex, ^{125}I-IPPA and neutral fatty acid complex is shown in Figure 5. It can be seen that there is no significant difference in heart/lung and heart/liver ratio among the three different radiolabeled compounds. The ^{125}I-IPPA, however, showed slightly better heart/blood ratio compared to the other two complexes.

The clearance of activity from different organs exhibited by the three radiolabeled compounds is shown in Figure 6. For the positively charged fatty acid complex injected activity cleared mostly through hepatobiliary route. Similar observation could be made for the neutral analogue as well. Increase in activity in intestine with time indicates fast clearance of activity from liver. This is due to the hydrolysis of the ether groups of PNP ligand in the liver, which facilitates easy clearance of the activity.[23] Also, a different mechanism based on P-glycoproteins (Pgp) or multi-drug resistance-associated protein (MDR)-Pgp has been suggested to be the probable mechanism for rapid elimination of [^{99m}TcN(PNP)] lipophilic compounds from the tissues.[24] Contrary to this it could be observed that in the case of ^{125}I-IPPA clearance of activity from the liver is very slow, which resulted in low heart/liver ratio.

Conclusions

A new 16-carbon fatty acid cysteine derivative was synthesized and labeled with [^{99m}TcN(PNP)]$^{2+}$ core in good yields. The

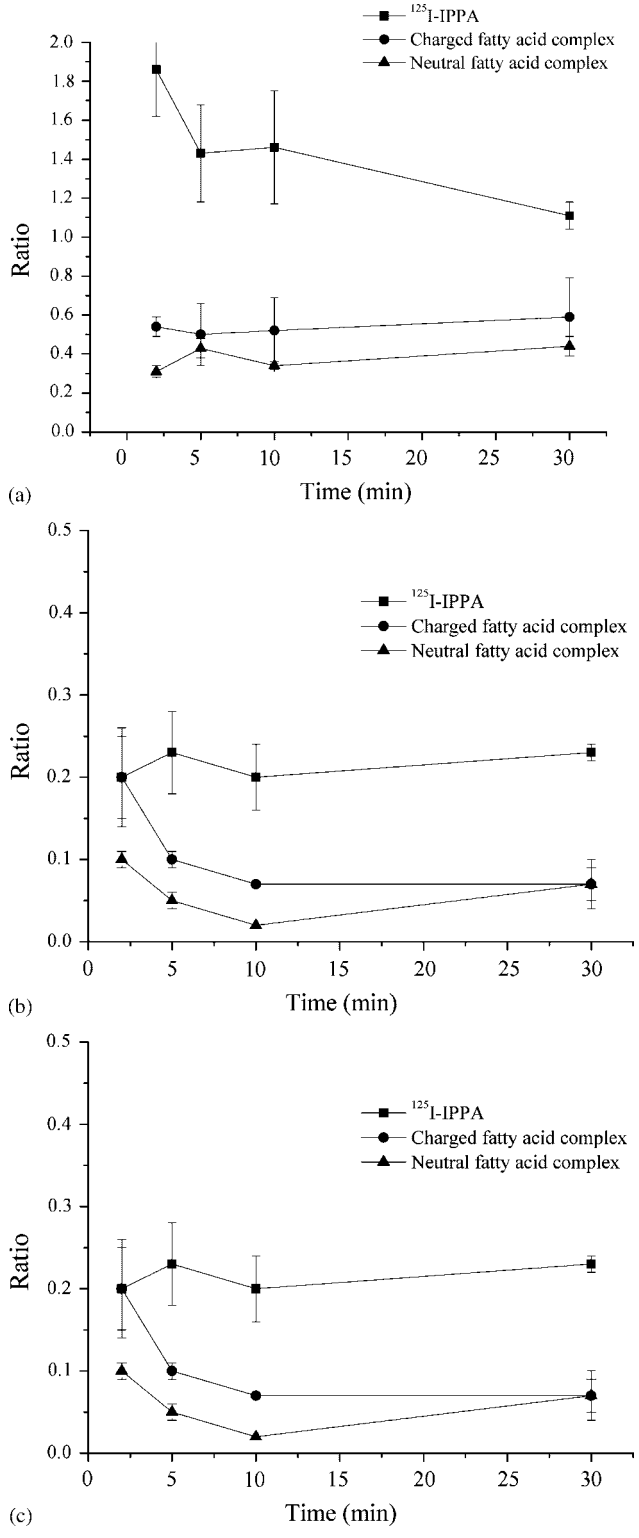

Figure 5. Comparison of (a) Heart/Blood, (b) Heart/Lung and (c)Heart/Liver ratios of charged fatty acid complex with ^{125}I-IPPA and neutral fatty acid complex.

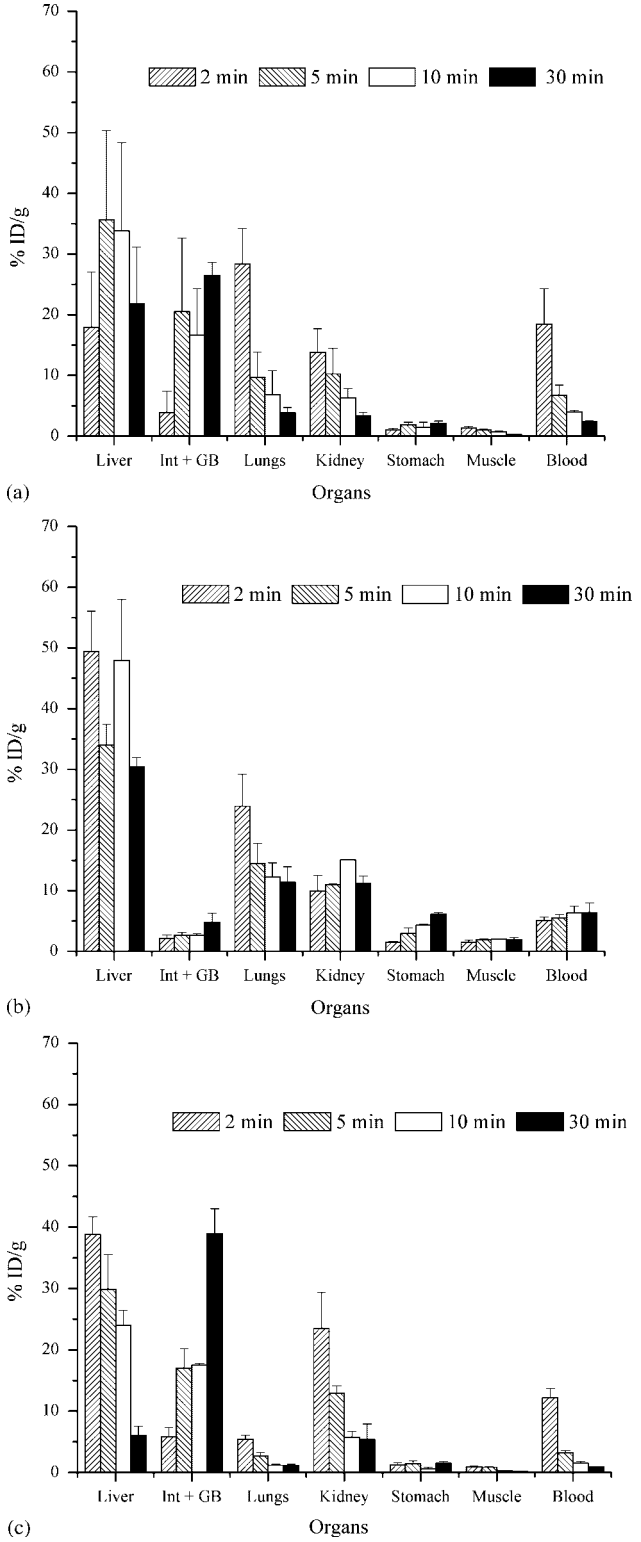

Figure 6. Comparison of clearance pattern of (a) charged fatty acid complex, (b) ^{125}I-IPPA and (c) neutral fatty acid complex from non-target organs.

biodistribution studies in Swiss mice showed high myocardial extraction similar to the ^{125}I-labeled standard agent IPPA at 2 min p.i., however, with poor retention subsequently. The complex showed better uptake characteristics than the neutral analogue. Positively charged fatty acid derivatives, prepared using $[^{99m}TcN(PNP)]^{2+}$ core, seems to be better candidates for the development of myocardial metabolic tracers than their neutral counterparts.

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