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Research Article

Synthesis and bio evaluation of a new fatty acid derivative labelled with technetium-99m

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Summary

^{123}I-iodophenylpentadecanoic acid (IPPA) and ^{123}I-beta-methyliodophenylpentadecanoic acid (BMIPP) are radiolabelled fatty acid derivatives used for assessment of myocardial viability. Because of limited accessibility of ^{123}I in the clinical scenario, a 99m-technetium-based agent would be more advantageous. In this context, a xanthate derivative of 15-hydroxypentadecanoic acid (HPDA) was synthesized for radiolabelling with $[^{99m}TcN]^{2+}$ intermediate.

Direct reaction of the HPDA with carbon disulphide in presence of crushed sodium hydroxide in dry tetrahydrofuran resulted in moderate yield of the desired xanthate product. The prepared ligand was radiolabelled with $[^{99m}TcN]^{2+}$ intermediate and the resultant complex was characterized by paper electrophoresis and HPLC. The labelled preparation was assessed for its myocardial extraction and retention characteristics using Swiss mice model.

The HPDA xanthate derivative was obtained in a low yield of $\sim$30%. Labelling via the $[^{99m}TcN]^{2+}$ intermediate gave more than 95% complexation. During *in vivo* studies with the $[TcN]^{2+}$ labelled complex maximum heart uptake observed was 3.10%ID/g at 5 min p.i., which cleared out rapidly, with retention of 0.79%ID/g of the activity at 60 min p.i.

The ^{99m}TcN-HPDA xanthate derivative showed some uptake in the heart but rapid wash out and substantial uptake in the background organs (blood, liver and lungs) led

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to unfavourable critical ratios at all the time points of study.

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Introduction

Fatty acids are taken up in the myocardium by passive diffusion and form the source of energy for normoxic cells. With the carboxylic acid terminus free to retain the biochemical properties of the molecule, the fatty acid chain length is the major determinant governing its uptake in the myocardium.[1] Once inside the normoxic cells, fatty acids are rapidly metabolized by β-oxidation. The differential fatty acid metabolism in normal and ischaemic cells becomes a tool for detection of cardiovascular diseases. A number of ^{123}I-labelled straight chain fatty acid derivatives were prepared earlier and tested *in vivo*.[2,3] However, *in vivo* instability and rapid metabolic washout were the limiting factors rendering them unsuitable for single photon emission computed tomography (SPECT) imaging, which is the preferred modality for clear delineation of the myocardium. To overcome the above-mentioned shortcomings, ^{123}I labelled Iodophenyl pentadecanoic acid (IPPA) and beta-methyl iodophenyl pentadecanoic acid (BMIPP)[4–8] were introduced. These agents showed good myocardial uptake with slow clearance making them suitable for SPECT imaging and convenient for use in patients who cannot undergo stress studies using ^{201}TlCl and ^{99m}Tc-MIBI.[9] These two ^{123}I radiolabelled fatty acids have therefore served as gold standards in myocardial functional imaging. However, the inherent drawbacks in the use of cyclotron-produced ^{123}I (limited availability and short half-life) contribute to unfavourable logistics, and hence the quest for ^{99m}Tc-based agents for this purpose continues to form a relevant field of research. In this regard, a number of ^{99m}Tc-labelled fatty acid derivatives have been prepared but showed poor myocardial extraction, which could be attributed to improper chemical modification in the parent molecule leading to altered biological behaviour. The ^{99m}Tc-fatty acid derivatives reported earlier made use of the common $[^{99m}Tc=O]^{3+}$ core with N_2S_2 as a chelator which was incorporated into the fatty acid chain. But these complexes showed poor myocardial extraction.[10–12] A modified '3 + 1' approach [1] using the same core was followed but lacked *in vivo* stability. Recently, $[^{99m}Tc(CO)_3(-H_2O)_3]^+$ core [13] has been used for labelling a fatty acid derivative via histidine moiety but this has shown no improvement in myocardial extraction, possibly due to the stereochemical changes in the molecule.

In the present study, the fatty acid was labelled with ^{99m}Tc using the $[^{99m}TcN]^{2+}$ core. This core has been reported to complex in high radiochemical yields and optimum stability with ligands containing soft donor

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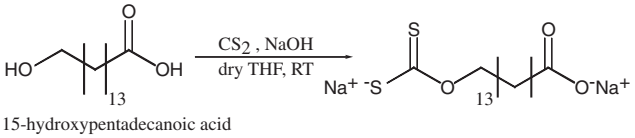
atoms (L) like S present in a desired array in dithiocarbamates, xanthates, etc.[14,15] They form tetradentate complexes of the type $^{99m}TcNL_2$. Here, a xanthate derivative of 15-hydroxy pentadecanoic acid was prepared and labelled using the $[^{99m}TcN]^{2+}$ core. The prepared complex was evaluated in Swiss mice for its biological behaviour.

Results and discussion

Fatty acid xanthate derivative of 15-hydroxypentadecanoic acid (HPDA) was prepared using HPDA, carbon disulphide and crushed sodium hydroxide in dry THF at room temperature. The reaction is schematically shown in Figure 1. The sodium salt of fatty acid xanthate was synthesized in a moderate yield of ~30% and the product was characterized by C, H, N, S and mass spectral analysis. C, H, N, S: observed (calculated) 43.96 (44.06), 4.3 (4.52), 5.66 (5.71), 26.45 (26.14). MS (ESI): Mass (M) (calculated) $C_{16}H_{28}O_3S_2Na_2$ 379; m/z(observed) (M-2Na) 333.

$[^{99m}TcN]^{2+}$ intermediate for radiolabelling of the synthesized HPDA xanthate ligand was prepared using commercially available kit vial and the formation was characterized by TLC using ethanol:chloroform:toluene:0.5 M ammonium acetate (6:3:3:0.5 v/v) as solvent system. ^{99m}TcN-intermediate (~99%) was found to be concentrated near point of application (R_f = 0–0.25), while $^{99m}TcO_4^-$ in the same solvent system has R_f = 0.4–0.6.

The final ^{99m}TcN-HPDA-xanthate complex was obtained on addition of HPDA xanthate ligand to the prepared $[^{99m}TcN]^{2+}$ intermediate and incubating the reaction mixture at room temperature for 30 min. The ^{99m}TcN-complex was characterized by paper electrophoresis and HPLC. Electrophoresis pattern of the nitrido intermediate and the complex using radioactive scanner is shown in Figure 2. It was observed that more than 95% of the activity corresponding to ^{99m}TcN-complex showed a small movement towards anode with peak at 1.8 cm and when ^{99m}TcN-intermediate alone was spotted (~98% activity) under identical conditions showed rapid movement with peak at 7.2 cm.This slight anodic movement of the complex towards anode may be due to the dianion of the final complex at the physiological pH due to free carboxylic acid group present. The HPLC chromatograms of ^{99m}TcN-intermediate and ^{99m}TcN-HPDA-xanthate complex are shown in



15-hydroxypentadecanoic acid

Figure 1. Scheme for synthesis of xanthate derivative of 15-hydroxy pentadecanoic acid

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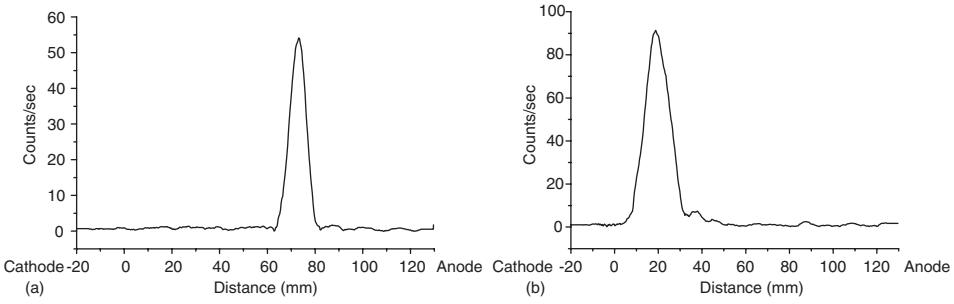

Figure 2. Paper electrophoresis pattern of (a) ^{99m}TcN-intermediat; (b) ^{99m}TcN-HPDA-xanthate complex

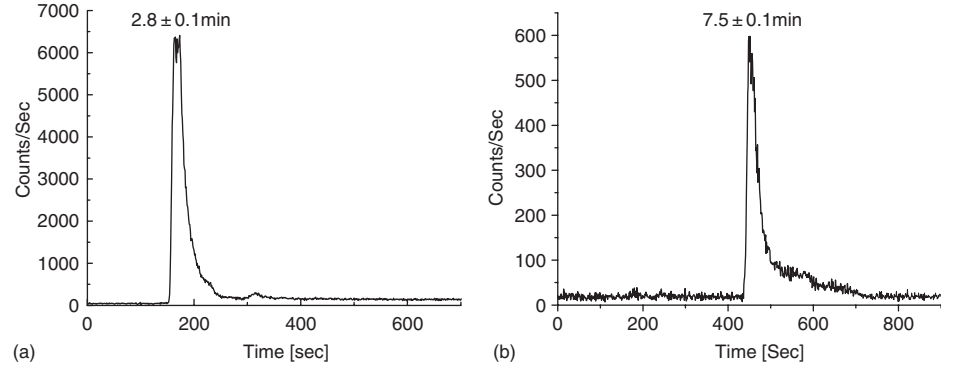

Figure 3. HPLC pattern of (a) ^{99m}TcN-intermediate; (b) ^{99m}TcN-HPDA-xanthate complex

Figure 3. The retention time of the radiolabelled complex was found to be 7.5 $\pm$ 0.1 min while that of ^{99m}TcN-intermediate was 2.8 $\pm$ 0.1 min.

Xanthates are known to complex with $[^{99m}TcN]^{2+}$ intermediate leading to complexes of $^{99m}TcNL_2$ type [15] having square pyramidal geometry with an apical $^{99m}Tc{\equiv}N$ bond and four sulphur atoms occupying the basal plane. It may be presumed that the fatty acid xanthate complex prepared above would have a similar structure.

Biological studies

The prepared complex was tested for *in vitro* serum stability and *in vivo* biological evaluation was carried out in Swiss mice. Using HPLC analysis, the ^{99m}Tc-labelled fatty acid derivative was found to be stable in serum for at least 60 min at 37°C. A negligible amount of activity was associated with serum-precipitated protein. As can be seen from Table 1 the biodistribution showed that the maximum myocardial uptake of this preparation was 3.1%ID/g at 5 min p.i., with a rapid wash out leading to a meagre 0.79%ID/g of the activity

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Table 1. Biodistribution of ^{99m}TcN-HPDA-xanthate complex in Swiss mice ($n = 3$)

Organ	% ID/g ± SD			
	5-min	10-min	30-min	60-min
Liver	48.16 ± 0.02	29.02 ± 2.57	27.07 ± 1.03	29.69 ± 5.67
Intestine + GB*	1.23 ± 0.08	0.83 ± 0.13	3.66 ± 0.31	5.23 ± 0.64
Kidney	21.57 ± 3.89	17.06 ± 1.60	16.29 ± 1.38	16.89 ± 4.29
Heart	3.10 ± 0.08	1.38 ± 0.19	0.92 ± 0.06	0.79 ± 0.18
Lungs	5.84 ± 0.28	2.99 ± 0.67	2.03 ± 0.05	2.96 ± 1.14
Blood	8.21 ± 1.10	3.06 ± 0.38	1.96 ± 0.23	1.85 ± 0.28
Heart/Blood	0.38	0.45	0.47	0.43
Heart/Lungs	0.53	0.46	0.45	0.27
Heart/Liver	0.06	0.05	0.03	0.03

GB*- Gall Bladder

remaining in the heart at 60 min p.i. The per cent uptake characteristics obtained in the present study are far from that observed in the standard ^{123}I –IPPA agent reported earlier.[16] Blood pool activity while initially high (8.21%ID/g at 5 min p.i.) also showed clearance with time. The heart/blood ratio remained within a reasonably narrow range throughout the period of study. The complex showed high liver and lung uptake with slow clearance. Hepatic clearance of the complex to a small extent is evident from the gradually increasing activity in the intestinal region. An appreciable fraction of activity can be seen in the kidneys, which could be possibly due to metabolism of the complex in liver and heart.

The test compound is twice the mass that of monoacid leading to increased lipophilicity, which can possibly alter the uptake and clearance characteristics from both target and background organs. But the authors feel that using smaller fatty acid ligands (<15 C-chain length) towards formation of a $^{99m}TcNL_2$ complex may adversely affect the myocardial uptake, since the complex may not behave as a single straight chain. Hence, it was decided to use a 15 C-chain length fatty acid ligand, this being the minimum chain length for favourable uptake characteristics, [8] although it significantly increases the mass of the overall complex as compared to radioiodinated monoacid agents. The other aim of the study was to observe the effect on retention that comes from the modification effected by the presence of the ^{99m}TcN core, regardless of initial uptake. However, as our experiments show, the proposed molecule exhibited limited uptake with rapid clearance from myocardium, decreasing its utility for the intended application.

Experimental

15-hydroxypentadecanoic acid (HPDA) was obtained from Aldrich, USA. Carbon disulphide, tetrahydrofuran and sodium hydroxide were purchased

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from S.D. Fine Chemicals Ltd., Mumbai, India. Tetrahydrofuran used for the reaction was dried as per the standard procedure. All chemicals were of analytical grade and used without further purification unless otherwise mentioned. Sodium pertechnetate ($Na^{99m}TcO_4$) was eluted with saline just before use from a ^{99}Mo-^{99m}Tc alumina column generator prepared in-house. The $[^{99m}TcN]^{2+}$ intermediate kit was obtained from Cis Bio International, France. Silica gel plates (Silica Gel 60 F_{254}) were obtained from Merck, Mumbai, India. Whatman chromatography paper (Whatman 3 mm Chr, 20 mm width, Maidstone, England) was used for paper electrophoresis. The radioactivity profile of TLC and Paper electrophoresis strips were determined using GINA-Star TLC chromatography evaluation system, Germany. HPLC Chromatograms were obtained on a JASCO PU 1580 HPLC system, with a JASCO 1575 tunable absorption detector and a radiometric detector system, using a C18 reversed phase HiQ Sil (5 μm, 4×250 mm) column and acetonitrile:water mixture as the mobile phase. Acetonitrile and water used for HPLC were filtered through Millipore filter paper and 0.1% of trifluroacetic acid was added to both. Elemental analysis was performed on C, H, N, S elemental analyser, Thermofinnigan, Flash EA 1112 series. Mass spectra were recorded on QTOF Micromass Instrument using electron spray ionization (ESI) in positive mode.

Synthesis of xanthate derivative of HPDA

A mixture of 0.5 g (1.94 mmol) of HPDA and 0.78 g (5.81 mmol) of crushed sodium hydroxide were stirred vigorously in 20 ml dry tetrahydrofuran (THF) for 5 min at room temperature. To the stirred solution, ~0.14 ml (2.13 mmol) of carbon disulphide was added. Stirring was continued overnight at room temperature. The product was obtained as a yellow precipitate and was seen to settle at the bottom of the flask. After completion of the reaction THF was removed under vacuum, and the product was washed with ether and re-crystallized from methanol-ether.

Radiolabelling studies

The $[^{99m}TcN]^{2+}$ intermediate was prepared by adding 1 ml of freshly eluted $^{99m}TcO_4^-$ (15-30MBq) to a $[^{99m}TcN]^{2+}$ intermediate kit vial. The kit vial was vortexed for 1 min and kept at room temperature for 20 min. About 5 mg of the ligand was dissolved in 0.5 ml of saline and added to 0.5 ml of freshly prepared $[^{99m}TcN]^{2+}$ intermediate in another vial. The pH of the mixture was adjusted to 7–8 using phosphate buffered saline of pH 7. The reaction mixture was vortexed for 1 min and incubated at room temperature for 30 min.

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Quality control techniques

The characterization of $[^{99m}TcN]^{2+}$ intermediate was carried out by thin layer chromatography. The ^{99m}TcN-xanthate complex was characterized by paper electrophoresis and HPLC.

Thin layer chromatography (TLC). The $[^{99m}TcN]^{2+}$ intermediate was characterized by TLC using ethanol:chloroform:toluene:0.5 M ammonium acetate (6:3:3:0.5 v/v) as developing solvent.

Paper electrophoresis and high-performance liquid chromatography (HPLC). Radiolabelling yield of the complex was determined using paper electrophoresis and HPLC. Paper electrophoresis was carried out in 0.05 M phosphate buffer (pH 7.5) for 1 h under a potential gradient of ~10 V/cm.

In HPLC, about 25 µl of the test solution was injected into the column and elution was monitored by observing the radioactivity profile. Water (A) and acetonitrile (B) each containing 0.1% trifluoroacetic acid were used as the mobile phase in the following gradient (0–2 min 98% A, 3–10 min 2% A).

Biological studies

Serum stability studies. Stability of the ^{99m}TcN-HPDA-xanthate complex in serum (source Swiss mouse) was assayed *in vitro*. About 50 µl of the radiolabelled preparation was added to 500 µl serum and the mixture was incubated at 37°C. Aliquots were taken at two intervals, at 30 and 60 min. Protein precipitation was done with equal volume of cold ethanol and the precipitate was removed after centrifugation at 5000 rpm (~1957 g) for 20 min. The respective supernatants were subjected to HPLC by the above-described protocol.

Biodistribution studies. Normal Swiss mice (20–25 g body weight) were used for the biodistribution studies. Each animal was intravenously injected about 0.1 ml of the labelled compound (~2.5 MBq) via the tail vein. Four different sets (3 each) of animals were kept under normal conditions for various time periods (5, 10, 30 and 60 min p.i.). The animals were sacrificed immediately at the end of the respective time points and the relevant organs and tissues were excised for measurement of associated activity. Radioactivity measurements were carried out in a flat-bed-type NaI(Tl) scintillation counter with suitable energy window for ^{99m}Tc. Accumulated activity was expressed in terms of percentage of the total injected dose per gram associated with the specific organ/tissue. All the procedures performed herein were in accordance with the national laws pertaining to the conduct of animal experiments.

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Conclusion

The xanthate derivative of 15-hydroxypentadecanoic acid (HPDA) was synthesized and labelled with the $[^{99m}TcN]^{2+}$ core yielding more than 95% complexation. The study indicated that a xanthate derivative of a fatty acid makes a feasible route for labelling biomolecules with ^{99m}Tc tracer under mild conditions. Biodistribution study carried out in Swiss mice showed limited extent of uptake in myocardium with rapid clearance. There was significant uptake in the surrounding organs leading to unfavourable target to non-target ratios, which would affect the quality of the images. But these studies have opened a new route for radiolabelling fatty acids via $[^{99m}TcN]^{2+}$ core.

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