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Synthesis and evaluation of ^{68}Ga labeled palmitic acid for cardiac metabolic imaging

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HIGHLIGHTS

- NOTA-hexadecanoic acid synthesized and characterized by ^{1}H NMR and ESI-MS.
- NOTA-hexadecanoic acid radiolabeled with ^{68}Ga in > 95% yields.
- Formation of ^{68}Ga-conjugate confirmed by characterization of its ^{nat}Ga surrogate.
- Myocardial uptake of ^{68}Ga-conjugate was 3.7 ± 1.34%ID/g at 2 min p.i.
- Heart metabolite analysis showed metabolic transformation of fatty acid conjugate.

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ABSTRACT

This work evaluates the potential of a ^{68}Ga labeled long chain 16C fatty acid for cardiac metabolic imaging. For radiolabeling with ^{68}Ga, hexadecanedioic acid was coupled with the chelator *p*-NH_2-Bn-NOTA. Under the optimized conditions, NOTA-hexadecanoic acid could be radiolabeled with ^{68}Ga in ≥95% yields. In biodistribution studies carried out in Swiss mice, ^{68}Ga-NOTA-hexadecanoic acid showed low myocardial uptake at 2 min p.i. (3.7 ± 1.3%ID/g). While ^{68}Ga-NOTA-hexadecanoic acid cleared rapidly from non-target organs such as blood, lungs, intestine and kidney, wash out from liver was slow. Radio-HPLC analyses of myocardial extracts of rats injected with ^{68}Ga-NOTA-hexadecanoic acid confirmed its metabolic transformation in the myocardium.

1. Introduction

Long chain fatty acids serve as the major source of energy for the normal myocardium and are metabolized by β-oxidation in heart. In ischemic heart, a shift in myocardial metabolism is observed from fatty acids to glucose. Thus, alteration in the metabolism of fatty acids has been identified as a sensitive marker of ischemia and myocardial damage (Corbett, 1999; Liedtke, 1981; Neely et al., 1972). In this context, development of radiolabeled fatty acids for imaging of myocardial metabolism is highly advantageous for the early detection of abnormalities. Many Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET) based radiotracers have been developed for the purpose (Tamaki et al., 2000). Amongst these, ^{123}I-IPPA (15-(*p*-iodophenyl)pentadecanoic acid), ^{123}I-BMIPP (15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid) and ^{11}C-pamitate are the widely used radiotracers for early detection of ischemic myocardium (Peterson and Gropler, 2010; Tamaki et al., 2000). Many fluorine (F-18) labeled fatty acids such as ^{18}F-FTP (16-fluoro-4-thiapalmitate) and ^{18}F-FCPHA (trans-9(RS)-fluoro-3,4-(RS,RS)methylene heptadecanoic acid) have also been studied extensively for the purpose previously (DeGrado et al., 2006; Shoup et al., 2005). All the above PET radiotracers suffer from the disadvantage of being dependent on the cyclotron for their production which limits their availability. Development of ^{68}Ga labeled fatty acids would facilitate on-demand availability of the radiopharmaceutical as ^{68}Ga is accessible through a portable ^{68}Ge/^{68}Ga generator system obviating the need for a cyclotron (Röesch and Riss, 2010).

Towards the synthesis of ^{68}Ga based fatty acids, the fatty acid chain has to be modified so as to introduce a suitable bifunctional chelator. The acid group of the fatty acid is kept intact, so as to have minimal effect on the biological behaviour of the parent molecule. Our group working on such ^{68}Ga-labeled fatty acids in the past have evaluated

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^{68}Ga labeled 11 carbon and 12 carbon chain fatty acids conjugated with different bi-functional chelators (Jain et al., 2016; Jindal et al., 2014). While one of the evaluated ^{68}Ga labeled fatty acids *viz.* ^{68}Ga-NOTA-undecanoic acid exhibited good uptake in the heart (Jindal et al., 2014), all other ^{68}Ga labeled fatty acid conjugates suffered from low retention in the heart and high uptake in non-target organs which diminished their potential as myocardial imaging agents. Since it is well known that increase in the length of the carbon chain of the fatty acid moiety improves the myocardial uptake (Otto et al., 1981), the long chain 16 carbon palmitic acid was pursued towards the development of a new ^{68}Ga labeled fatty acid radiotracer. It was envisaged that an improved retention of the fatty acid in the heart might increase the heart/ non-target organ ratios of the compound which might lead to well defined images of the heart during dynamic scanning.

In this work, the palmitic acid chain, hexadecanedioic acid was conjugated with the chelator *p*-NH_2-Bn-NOTA (2-*S*-(4-aminobenzyl) − 1,4,7-triazacyclononane-1,4,7-triacetic acid) for radiolabeling with ^{68}Ga. Bioevaluation studies of the synthesized ^{68}Ga-NOTA-hexadecanoic were performed and the results obtained were compared with that of ^{68}Ga-NOTA-undecanoic acid conjugate (Jindal et al., 2014) which was previously reported by our group. Further, the results were also compared with that of the SPECT agent ^{125}I-IPPA (Mathur et al., 2008), an analogue of the clinically used metabolic marker ^{123}I-IPPA.

2. Materials and methods

2.1. Materials

16-Hexadecanedioic acid, O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium- hexafluorophosphate (HATU) and diisopropylethylamine (DIPEA) were procured from Sigma Aldrich, USA while *p*-NH_2-Bn-NOTA was procured from Macrocyclics, USA. All other reagents used were of analytical grade. Gallium-68 for the work was eluted in 0.6 N HCl from a 925 MBq ^{68}Ge/^{68}Ga generator purchased from iThemba Labs, South Africa. Silica gel plates (Silica Gel 60 F254) used for thin layer chromatography were obtained from Merck, India. High Performance Liquid Chromatography (HPLC) characterization was carried out on a JASCO PU 2080 Plus dual pump HPLC system (JASCO, Japan) equipped with a JASCO 2075 Plus tunable absorption detector and a Gina Star radiometric detector system (Raytest, Germany) using a C18 reversed phase HiQSil (5 μm, 4 × 250 mm) column. Water (A) and acetonitrile (B), each containing 0.1% TFA, were used as the mobile phase at a flow rate of 1 mL/min. The following gradient elution method was adopted for monitoring the progress of the reaction: 0 min 70% A, 15 min 0% A, 20 min 0% A, 25 min 70% A. Purification of the synthesized NOTA-hexadecanoic acid conjugate was carried out on a JASCO-PU-2086 PLUS Intelligent Prep Pump semi-preparative HPLC system (JASCO, Japan) equipped with a Mega Pak SIL C18-10 column (10 × 250 mm) connected with a JASCO UV-2075 Plus absorption detector. The same solvent system and method were used in both analytical HPLC as well as semi-preparative HPLC for the synthesis and purification of NOTA-hexanoic acid conjugate as well as for the characterization of the $^{68/nat}$Ga labeled NOTA-hexanoic acid conjugate. ^{1}H NMR spectrum was recorded on a 500 MHz Varian spectrophotometer. Mass spectra of the samples were recorded on a Varian Prostar mass spectrometer using ESI in positive mode. $^{68}GaCl_3$ was eluted in 0.6 N HCl from a 925 MBq (25 mCi) ^{68}Ge/^{68}Ga radionuclide generator procured from iThemba Labs (South Africa).

2.2. Synthesis of NOTA-hexadecanoic acid

16-hexadecanedioic acid (30 mg, 0.1 mmoles) was dissolved in dimethyl formamide (DMF, 1 mL). HATU (40 mg, 0.1 mmoles) and DIPEA (13.4 mg, 0.1 mmoles) were added to the reaction mixture and stirred for 10 min under nitrogen atmosphere. Further, *p*-NH_2-Bn-NOTA (54 mg, 0.1 mmoles) was added to it and then left for overnight stirring. Progress of the reaction was monitored using analytical HPLC following the UV peak at 254 nm. On completion of the reaction, the product was purified using semi-preparative HPLC.

NOTA-hexadecanoic acid:

Yield: 50% (35 mg)

^{1}H NMR (500 MHz, DMSO)δ ppm: 7.68 (d, 2 H, benzene, *J = 7.5 Hz*), 7.25 (d, 2 H, benzene, *J = 7.5 Hz*), 3.49–3.48 (m, 7 H, -NCH_2COOH and –C_6H_4-CH_2CHN-), 2.93–2.84 (m,12 H, NOTA ring -NCH_2CH_2N- and -NCH_2CHN-, C_6H_4-CH_2CHN-), 2.40 (t, 2 H, -CH_2CONH-, *J = 7 Hz*), 2.30 (t, 2 H, -CH_2COOH, *J = 7 Hz*), 1.68–1.67 (m, 2 H, -CH_2CH_2CONH-), 1.58–1.57 (m, 2 H, -CH_2CH_2COOH), 1.29 (s, 20 H, -$(CH_2)_{10}$)

MS (ESI, + ve mode): Mass (calculated) [$C_{35}H_{56}N_4O_9$] 676.4; *m/z* (observed) 676.9

2.3. Radiolabeling of NOTA-hexadecanoic acid with ^{68}Ga

For radiolabeling, NOTA-hexadecanoic acid (100 μL, 1 mM in ethanol) was mixed with 0.4 mL of 2 M sodium acetate solution (pH = 7). 1 mL of $^{68}GaCl_3$ (74–185 MBq) eluted in 0.6 N HCl from a commercial ^{68}Ge/^{68}Ga generator was added to it and the reaction mixture (pH = 4) was incubated for 10 min at ambient temperature. The radiochemical yield of ^{68}Ga-NOTA-hexadecanoic acid was determined by radio-HPLC as per the method given in the Materials Section. The stability of ^{68}Ga-NOTA-hexadecanoic acid was periodically evaluated using radio-HPLC up to 4 h post-preparation at ambient temperature.

2.4. Preparation of ^{nat}Ga-NOTA-hexadecanoic acid

To the NOTA-hexadecanoic acid conjugate (100 μL, 0.1 M in ethanol), excess gallium nitrate solution (1 mL water, 0.1 M) was added. The pH of the reaction mixture was adjusted to 4 with dilute NaOH and it was incubated at ambient temperature for 30 min. The product was characterized by ESI-MS and HPLC following UV profile at 254 nm using the gradient elution technique as described in the Methods section.

MS (ESI, + ve mode): Mass (calculated) [$C_{35}H_{53}N_4O_9Ga$] 742.3; *m/z* (observed) 743.4 g/mol [$M+H^+$]

2.5. Partition coefficient and in vitro serum stability studies

Partition Coefficient (log $P_{o/w}$) of ^{68}Ga-NOTA-hexadecanoic acid was determined by mixing 0.1 mL of the radioconjugate (1.85 MBq) with 0.9 mL of water and 1 mL of octanol on a vortex mixer. Further, the aqueous and organic layers were separated by centrifugation of the reaction mixture. Equal aliquots from both the layers were counted in a well-type NaI(Tl) counter and the partition coefficient (log $P_{o/w}$), expressed as the logarithm of the ratio of the counts in n-octanol *versus* that of water was determined. Further, the n-octanol layer was re-partitioned with equal volumes of water followed by the separation and counting of the aqueous and organic layers to obtain consistent log $P_{o/w}$ values.

The *in vitro* stability studies of ^{68}Ga-NOTA-hexadecanoic acid were performed in human serum as well as in EDTA solution. Briefly, 50 μL of the radiolabeled fatty acid conjugate (1 MBq) was incubated in human serum (450 μL) for 2 h at 37 °C. Thereafter, the serum proteins were precipitated by addition of 0.5 mL acetonitrile followed by the centrifugation of the solution. The supernatant was analyzed by radio-HPLC to determine the stability in serum.

Further, in order to determine the extent of trans-chelation in presence of EDTA, 50 μL of ^{68}Ga-NOTA-fatty acid conjugate (1 MBq) was incubated with 10 mM EDTA solution (450 μL) for 2 h and the stability of the radiolabeled fatty acid was evaluated by radio HPLC.