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# Preparation and biological evaluation of $^{99}Tc^{m}$-labelled fatty acids

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**Abstract** The aim of the present work was to develop radiolabelling fatty acids based on $^{99}Tc^{m}$ carbonyl chemistry for heart imaging. Undecanoic acids functionalised with iminodiacetatic acid and cysteine were radiolabelled with $[^{99}Tc^{m}(CO)_3(H_2O)_3]^+$ intermediates, and their radiolabelling conditions were carefully studied. Biodistribution of $^{99}Tc^{m}(CO)_3$-CYST FAC11 and $^{99}Tc^{m}(CO)_3$-IDA FAC11 were observed in normal mice. The results showed that two $^{99}Tc^{m}$-labelled compounds had similar profile in terms of high initial radioactivity uptake and rapid washout of radiotracers in the heart. $^{99}Tc^{m}(CO)_3$-IDA FAC11 was mainly excreted via hepatobiliary system in contrast to $^{99}Tc^{m}(CO)_3$-CYST FAC11, which was excreted from urinary system. It may be in part attributed to the more lipophilicity of $^{99}Tc^{m}(CO)_3$-IDA FAC11 than $^{99}Tc^{m}(CO)_3$-CYST FAC11.

**Key words** $^{99}Tc^{m}$-labelling, Fatty acid derivatives, Biodistribution
**CLC numbers** R817, O623.61

## 1 Introduction

Long free chain fatty acids are the major energy source of the normal myocardium. Approximately 60%~80% of adenosine triphosphate (ATP) produced in aerobic myocardium derived from fatty acid β oxidation.[1] The measurement of regional difference in the uptakes and retention of radiolabelled fatty acids using nuclear medicine techniques could provide significant information on myocardium energy metabolism *in vivo*, and thus could be a valuable approach in diagnoses of several heart diseases.[2]

A variety of fatty acid analogues radiolabelled with $^{11}C$ and $^{123}I$ have recently been investigated as useful radiopharmaceuticals for the estimation of myocardial fatty acid metabolism.[3] A key example of a diagnostic agent is $^{123}I$-labelled 15-(p-iodophenyl)-3-R,S-methylpentadecanoic acid (BMIPP), which has been successfully used in clinics for early detection of myocardial ischemia and assessment of the severity of ischemic heart disease. Considering excellent nuclide properties and wide availability of $^{99}Tc^{m}$, developments of $^{99}Tc^{m}$-labelled long chain fatty acid derivatives have also been pursued in this field.[4,5]

Recently, a convenient method for preparation of fac-$[^{99}Tc^{m}(CO)_3(H_2O)_3]^+$ under normal pressure was developed by Alberto et al.[6] $[^{99}Tc^{m}(CO)_3(H_2O)_3]^+$, a versatile organometallic precursor, could react with various chelating system and have been widely used for radiolabellings with $^{99}Tc^{m}$ of protein, peptides and small biomolecules [7,8]. The successful preparation and excellent biological properties of $[^{99}Tc^{m}(MIBI)_3(CO)_3]^+$ were attracting increasing interest in search of new optimal pharmaceuticals for myocardial metabolic imaging agents.[9] In this work, undecanoic acid functionalized with iminodiacetatic acid and cysteine were radiolabelled with $[^{99}Tc^{m}(CO)_3(H_2O)_3]^+$ precursor to test its radiobiological properties *in vivo* used as fatty acid analogue.

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## 2 Materials

CYST FAC11(S-cysteine undecanoic acid) and IDA FAC11 ((N,N-di(carboxymethyl) amino undecanoic acid) were provided by Ioannis Pirmettis, Institute of Radioisotopes-Radiodiagnostic Products, Athens, Greece. The Isolink® kits were available from Malinckrodt, Inc. $Na^{99}Tc^mO_4$ was eluted in saline solution from a $^{99}Mo/^{99}Tc^m$ generator (Atom Hitech Co. Ltd., China). Other chemicals obtained from commercial sources were of reagent grade and used without further purification.

## 3 Methods

### 3.1 Preparation of $[^{99}Tc^m(CO)_3(H_2O)_3]^+$ intermediate

$[^{99}Tc^m(CO)_3(H_2O)_3]^+$ intermediate was prepared as described by Alberto et al.[6] Typically, a 1.0 mL saline solution containing $^{99}Tc^mO_4^-$ (0.74~1.85 GBq) was added to a 10 mL serum vial containing 4.0 mg $Na_2CO_3$, 5.5 mg $NaBH_4$, 20 mg Na/K-Tartrate and $1\times10^5$Pa carbon monoxide (headspace volume). The mixture was heated for 20 min at 95℃ while shaking periodically, allowed to cool to room temperature, and pH adjusted to 7.0 with 1.0mol/L hydrochloric acid.

$[^{99}Tc^m(CO)_3(H_2O)_3]^+$ was also prepared from a commercial Isolink® kits according to the package insert instructions.

The solution was analyzed by reverse phase HPLC using a C-18 analytical column (Hypersil ODS, $\phi$4.6mm×250mm) with a 0.1% TFA/acetonitrile gradient. The applied gradient was (A: 0.1% TFA in water, B: 0.1% TFA in acetonitrile): 0% to 5 min, 0% B; 5 to 15 min, from 0 to 80 % B; 15 to 30 min, 80% B; 30 to 32 min, from 80% to 0% B; 32 to 38 min, 0% B. Chromatography was carried out at a flow rate of 1.0 mL/min. In each HPLC analysis, recovery of the radioactivity was determined by comparing the total activities of all fractions to a standard prepared from the injectate to verify no activity on the HPLC column.

### 3.2 Labelling of CYST FAC11 and IDA FAC11

To a 10 mL serum vial purged with $N_2$ was added 0.1 mL of IDA FAC11 (1.0 mg/mL in aqueous 0.1mol/L phosphate buffer solution) followed by 0.2 mL of aliquot of the $[^{99}Tc^m(CO)_3(H_2O)_3]^+$ intermediate solution and the mixture was heated at 95℃ for 30 min. After cooling to room temperature, the radiotracer was analyzed by HPLC using conditions identical to those described above for $[^{99}Tc^m(CO)_3(H_2O)_3]^+$ analysis. For improving the radiolabelling yield, reaction time, temperature, concentrations and the pH value of the radiolabelling reactions were investigated.

Labelling of CYST FAC11 with $[^{99}Tc^m(CO)_3(H_2O)_3]^+$ was carried out using the procedure described above.

### 3.3 Purification of radiolabelled products by SPE method

To facilitate biological assays, the products were purified from $^{99}Tc^mO_4^-$, $[^{99}Tc^m(CO)_3(H_2O)_3]^+$ and other radioactive impurity by solid-phase extraction. In short, preparations containing labelled compounds were loaded onto a C18 Sep-Pak® Light (Waters) solid-phase extraction cartridge, pre-flushed with 10 mL of 1.0 mol/L HCl. After rinsing with another 10 mL of 1.0 mol/L HCl until no detectable amount of radioactivity was released from the column, the labelled compounds were eluted in 2.0 mL of methanol, dried in a $N_2$ stream, reconstituted in saline, and filtered through a sterile 0.22 μm Millipore filter. The purity of the products was checked by radio-HPLC analysis.

### 3.4 *In vitro* stability

The *in vitro* stability of $^{99}Tc^m(CO)_3$-CYST FAC11 and $^{99}Tc^m(CO)_3$-IDA FAC11 was assessed by challenging with cysteine and histidine. The 0.1 mL of radio-complexes each was placed into 0.2 mL of 10 mmol/L cysteine or 10 mmol/L histidine in 0.1 mol/L phosphate buffer solution respectively, and then incubated for 30 min, 1 h, 2 h and 3 h at 37℃. After incubation, the percentage dissociation of each complex was determined by radio-HPLC analysis, and compared with the sample in saline used as a control.

### 3.5 Biodistribution studies in normal mice

Specific pathogen-free, female Kunming mice, weighing 18~22g, for biodistribution studies were housed with free access to food and water, and fasted for 12 h before experiments. $^{99}Tc^m(CO)_3$-CYST FAC11 and $^{99}Tc^m(CO)_3$-IDA FAC11 in saline solution

(0.74~1.10 MBq in 0.1 mL) were administrated via the tail vein of mice. At appropriate time points after the administration, the animals were sacrificed by decapitation. Samples of blood and organs of interest were excised and weighed, and counted in a NaI(Tl) gamma scintillation counter. The results were expressed as the percent injected dose/gram of blood or organs after decay correction.

## 4 Results and discussion

### 4.1 Radiolabelling

The $[^{99}Tc^{m}(CO)_3(H_2O)_3]^+$ precursor, either using the commercial kits or employed carbon monoxide gas, was prepared reproducibly with a yield of more than 97% as determined by radio-HPLC analysis.

$^{99}Tc^{m}(CO)_3$-CYST FAC11 and $^{99}Tc^{m}(CO)_3$-IDA FAC11 could be formed with yields better than 95% under the described specific conditions. HPLC analysis showed that $[^{99}Tc^{m}(CO)_3(H_2O)_3]^+$ had been quantitatively transformed into the desired product (Fig.1 and Fig.2). In the reaction solution, there were the main products (retention time of $^{99}Tc^{m}(CO)_3$-CYST FAC11 and $^{99}Tc^{m}(CO)_3$-IDA FAC11, 20.3 min and 25.1 min, respectively) apart from small amount of $^{99}Tc^{m}O_4^-$ (retention time, 4.6 min) and negligible amount of $[^{99}Tc^{m}(CO)_3(H_2O)_3]^+$ (retention time, 7.4 min). Recovery of the radioactivity in HPLC analysis was conducted and more than 95% of the injected activity was eluted from the column.

Radiolabelling of two fatty acids was investigated under different conditions, namely, pH value, ligand concentration and heating time. The experimental results showed that radiolabelling yields were not significantly affected by pH values within the range of 6.8-11(Fig.3), and preferably from 7.8 to 9.0. The two radio-complexes could be synthesized in high yields with more than 50μg of ligand each (amount less than 50μg was not investigated) at 95℃ at pH 7.8 (Fig.4). Studies on influence of temperature on reaction kinetics demonstrated that $[^{99}Tc^{m}(CO)_3(H_2O)_3]^+$ could react rapidly with 100μg of each fatty acid derivatives at pH 7.8 (Fig.5). After heating at 95℃ for 10 min, yields larger than 95% could be achieved.

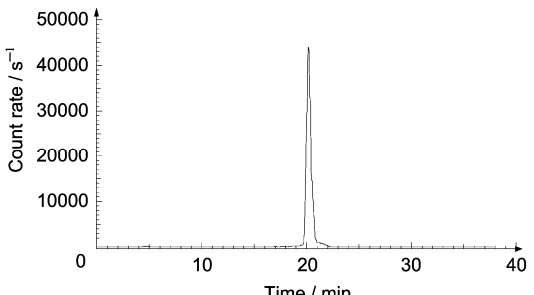


**Fig.1** Radio-HPLC chromatograms of $^{99}Tc^{m}(CO)_3$-CYST FAC11.

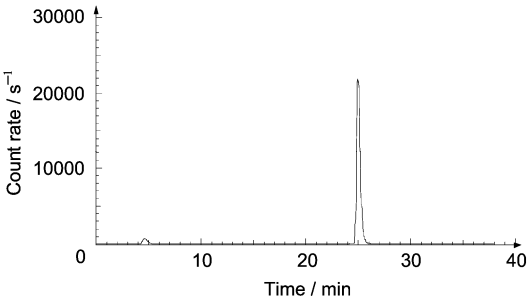


**Fig.2** Radio-HPLC chromatograms of $^{99}Tc^{m}(CO)_3$-IDA FAC11.

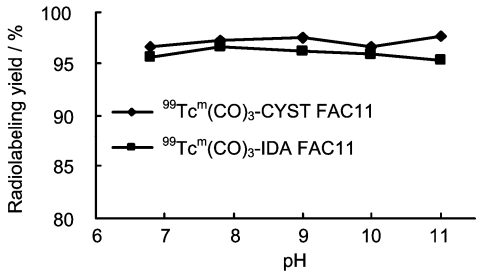


**Fig.3** Influence of pH value on the radiolabelling yields of $^{99}Tc^{m}(CO)_3$-CYST FAC11 and $^{99}Tc^{m}(CO)_3$-IDA FAC11 at 95℃.

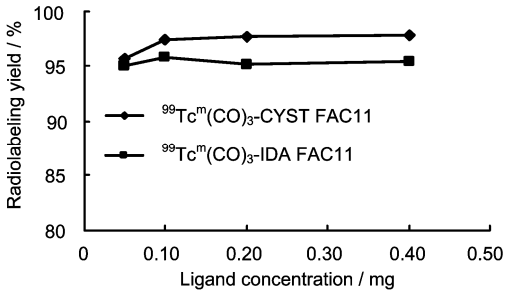


**Fig.4** Influence of concentration of ligand on the radiolabelling yields of $^{99}Tc^{m}(CO)_3$-CYST FAC11 and $^{99}Tc^{m}(CO)_3$-IDA FAC11 at 95℃, pH=7.8.

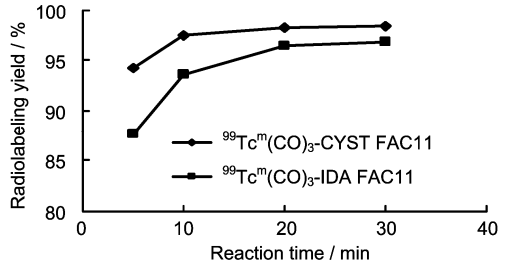


**Fig.5** Influence of heating time on the radiolabelling yields of $^{99}Tc^{m}(CO)_3$-CYST FAC11 and $^{99}Tc^{m}(CO)_3$-IDA FAC11 at 95℃, pH=7.8.

### 4.2 *In vitro* stability

*In vitro* stability studies revealed that $^{99}Tc^{m}(CO)_3$-IDA FAC11 was more stable than $^{99}Tc^{m}$(CO)3-CYST FAC11 (Figs.6,7). $^{99}Tc^{m}(CO)_3$- IDA FAC11 remained stable in saline, and no much degradation was observed up to 3h when challenged with large excess of histidine and cysteine at 37℃. In case of $^{99}Tc^{m}(CO)_3$-CYST FAC11, the complex has moderate stability either in saline or in challenging studies.

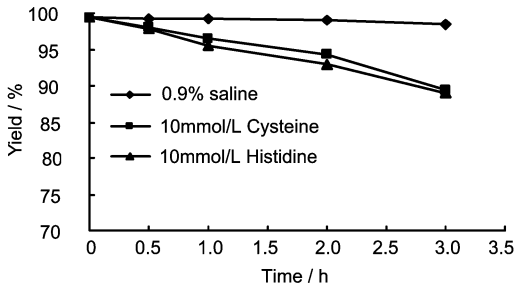


**Fig.6** Stability of $^{99}Tc^{m}(CO)_3$-IDA FAC11 in saline, cysteine and histidine solution.

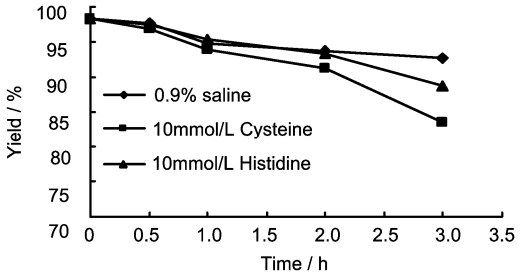


**Fig.7** Stability of $^{99}Tc^{m}(CO)_3$-CYST FAC11 in saline, cysteine and histidine solution.

### 4.3 Biodistribution

The formulated solution has enough radiopurity to be used to animal test. To further remove less than 5% of pertechnetate, it was isolated by SPE method to give only one single species with a radiochemical purity of at least 98% as revealed by radio-HPLC (Figs.1,2).

Biodistribution experiments of $^{99}Tc^{m}(CO)_3$-CYST FAC11 and $^{99}Tc^{m}(CO)_3$-IDA FAC11 were performed in normal mice. Based on the experimental data (Table 1 and Table 2), two radiolabelled compounds had similar profile in terms of high radioactivity initial uptake and rapid washout of radiotracers in the heart. The uptakes of $^{99}Tc^{m}(CO)_3$-CYST FAC11 were 3.11% and 3.44% ID/g at 5min, then dropping to 0.80% and 0.86% ID/g after 30 min, respectively. Meanwhile, the radioactivity in the blood was also cleaned rapidly, being reasonable for the high lipophilicity of the fatty acid complexes. Comparing with in the heart, high uptakes and retentions of the above compounds in the liver and kidney were observed.

However, a little difference could be found in the *in vivo* handling of two radiolabelled compounds. $^{99}Tc^{m}(CO)_3$-IDA FAC11 was mainly excreted *via* hepatobiliary system, whereas $^{99}Tc^{m}(CO)_3$-CYST FAC11 was mainly from urinary system. It could be in part attributed to the more lipophilicity of $^{99}Tc^{m}(CO)_3$-IDA FAC11 than that of $^{99}Tc^{m}(CO)_3$-CYST FAC11, and also may be from the structural difference in the coordination moieties of the two $^{99}Tc^{m}$ complexes. The overall results revealed that the two $^{99}Tc^{m}$-labelled compounds were not the optimal candidates for myocardial metabolic imaging agents.

**Table 1** Biodistribution of $^{99}TC^{M}(CO)_3$-cyst FAC11 in normal mice

| Organ/Tissue | Time after i.v. administration /min | | | |
|---|---|---|---|---|
| | 5 | 30 | 60 | 120 |
| Blood | 7.11±0.15 | 1.26±0.10 | 0.80±0.02 | 0.54±0.05 |
| Heart | 3.11±0.44 | 0.80±0.05 | 0.50±0.03 | 0.45±0.09 |
| Liver | 30.81±10.4 | 14.70±2.62 | 12.53±5.57 | 6.65±1.81 |
| Spleen | 1.96±0.18 | 0.76±0.07 | 0.42±0.03 | 0.37±0.07 |
| Lung | 6.13±0.68 | 2.37±0.22 | 1.76±0.29 | 1.37±0.31 |
| Kidney | 121.3±11.0 | 48.00±2.86 | 17.05±5.13 | 14.24±4.91 |
| Stomach | 1.81±0.11 | 0.67±0.15 | 0.63±0.02 | 0.46±0.06 |
| Intestine | 6.23±1.77 | 2.84±0.35 | 2.68±1.15 | 0.58±0.35 |
| Muscle | 1.56±0.21 | 0.49±0.11 | 0.66±0.18 | 0.41±0.06 |

All values shown are mean ± standard deviation of % ID/g ($n$=3)

**Table 2** Biodistribution of $^{99}TC^{m}(CO)_3$-IDA FAC11 in normal mice

| Organ/Tissue | Time after i.v. administration /min | | | |
|---|---|---|---|---|
| | 5 | 30 | 60 | 120 |
| Blood | 13.86±1.11 | 2.15±0.09 | 1.16±0.15 | 0.91±0.14 |
| Heart | 3.44±0.60 | 0.86±0.15 | 0.55±0.07 | 0.37±0.02 |
| Liver | 91.71±7.51 | 22.00±3.10 | 10.21±1.38 | 6.50±1.51 |
| Spleen | 3.80±0.23 | 1.43±0.15 | 0.95±0.10 | 1.26±0.12 |
| Lung | 6.19±1.92 | 1.99±0.28 | 1.23±0.40 | 0.83±0.06 |
| Kidney | 23.32±2.66 | 7.92±2.73 | 2.52±0.57 | 1.21±0.04 |
| Stomach | 3.34±1.15 | 1.92±0.10 | 2.36±0.75 | 1.64±0.45 |
| Intestine | 10.53±0.97 | 2.39±0.27 | 2.37±0.38 | 1.80±0.96 |
| Muscle | 1.10±0.07 | 0.61±0.11 | 0.34±0.02 | 0.47±0.13 |

All values shown are mean±standard deviation of % ID/g ($n$=3)

## 5 Conclusions

Radiolabelling of CYST FAC11 and IDA FAC11 was carried out with high yields using the $[^{99}Tc^{m}(CO)_3(H_2O)_3]^{+}$ intermediate under optimized conditions. The experimental results showed that after intravenous administration to normal mice, $^{99}Tc^{m}(CO)_3$-CYST FAC11 and $^{99}Tc^{m}(CO)_3$-IDA FAC11 were mainly handled by the hepatobiliary and urinary systems. Both radiocompounds have high initial uptake in the heart. Unfortunately, the relative rapid washout of radioactivity in the heart excluded them to be used as potential imaging agents for fatty acid metabolism.

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