Synthesis, labelling and animal experiments of the derivative of phenylpentadecanoic acid (CACPPA)*

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Abstract The synthesis and biodistribution were discribed for ^{99m}Tc labelled fatty acid, *p*-[(N,N'-di-carboxymethyl) aminomethyl carboxyamino]-phenylpentadecanoic acid (CACPPA). ^{99m}Tc-CACPPA was prepared by the reduction of $Na^{99m}TcO_4$ with stannous chloride in aqueous solution at pH 8.5~9.5, the labelling yield and chemical purity were over 90% determined by TLC and HPLC. Biodistribution of ^{99m}Tc-CACPPA in mice demonstrated that the highest myocardial uptake of ^{99m}Tc-CACPPA was 18.17±2.67% ID/g. When blood disappearance of ^{99m}Tc-CACPPA was analysed with a biexponential model, an initial half time of 1.11 min and a late half time of 20.08 min were obtained. ^{99m}Tc-CACPPA exhibited high binding to HSA in vitro, and partition coefficients were 10.98 and 11.45 at pH7.00 and pH7.40, respectively.
Keywords ^{99m}Tc labelling, Fatty acid/derivative, Animal experiment, Myocardial imaging agent

1 Introduction

Fatty acids constitute the major energy source of heart tissue through β-oxidation catabolism. Radiolabelled fatty acids which display efficient myocardial uptake and adequate myocardial retention are attractive candidates for clinical evaluation of regional discrepancies in fatty acid metabolism, which occur in ischemic heart disease and cardiomyopathies.

p-radioiodinated phenylpentadecanoic acid (*p*-IPPA) and phenyl-β-methyl-pentadecanoic acid (*p*-BMIPP) have been proved to be potential myocardial imaging agents for clinical uses abroad. Since ^{123}I is produced from accelerator, it can not be put into clinical use in China at the present time. ^{99m}Tc is chosen as a radioisotope used in nuclear medicine due to its ideal physical properties and ready availability from ^{99}Mo-^{99m}Tc generators. Over the past 15 years, various researchers have explored the feasibility of incorporating ^{99m}Tc into fatty acid carrier molecules using a variety of ligands. Even though ^{99m}Tc complexes were formed, the myocardial profiles of the agents were disappointing.

Recently we have synthesized the derivative of phenylpentadecanoic acid, *p*-[(N,N'-di-carboxymethyl) aminomethyl carboxyamino]-phenylpentadecanoic acid (CACPPA). Detailed synthesis and biodistribution of ^{99m}Tc-CACPPA are given in this paper.

2 Materials and methods

2.1 Instruments

YANADIMOTO melting point instrument (made in Japan), FT-IR spectrometer (made in USA), PE2400 elemental analyser (made in USA), Varian Model AM-400 Proton Nuclear Magnetic Reasonance Spectrometer (made in USA), Pakard Cobra γ-counter (made in USA).

2.2 Reagents

Phenylpentadecanoic acid (PPA) was prepared by ourselves. Other reagents used for synthesis were from Shanghai Chemical Co. and all were of reagent grade.

2.3 The preparation of CACPPA

CACPPA was prepared by three-step sequences of reaction outlined in Fig.1.

2.3.1 *p*-nitrophenylpentadecanoic acid (*p*-NPPA)

Phenylpentadecanoic acid (PPA, 3.18 g, 0.01 mol) was added to a mixed acid (50 ml, H_2SO_4:HNO_3=1:1.3, V/V). The solution was stirred under 0°C for 30 min and then

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poured into ice-water. The white crystals were collected by filtration and recrystallized from methyl alcohol to obtain *p*-NPPA. mp: 85~87°C; IR(cm^{-1}):1515, 1352(-NO_2), 1704(-CO-), 1600, 1470(-ph); Theo Anal($C_{21}H_{33}NO_4$): C, 69.42%, H, 9.09%, N, 3.86%; Found: C, 69.06%; H, 9.51%; N, 3.41%; ^{1}HNMR($CDCl_3$): δ, 7.30 (d, 2H, Ar-H), 8.12 (d, 2H, Ar-H), 1.30 (s, 24H, CH_2), 2.57 (t, 2H, phCH_2), 2.30 (t, 2H, CH_2CO).

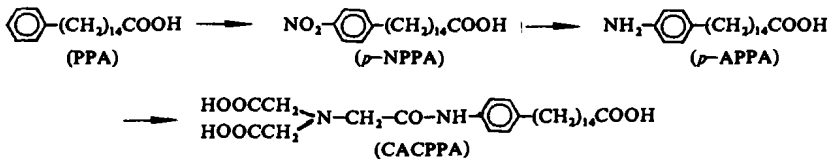

Fig.1 Preparation scheme of CACPPA

2.3.2 *p*-amino-phenylpentadecanoic acid (*p*-APPA)

To a solution of *p*-NPPA (3.63 g, 0.01 mol) in water (100 ml) containing a portion of HCl, iron powder reduced (2g) was added in portions, the solution was maintained under refluxing for 4 h, then filtered, the residue was crystallized from CH_3OH, producing a pale yellow crystal (*p*-APPA), mp:110~112°C; IR (cm^{-1}): 3373, 3250 (-NH_2), 1704 (-CO-), 1600, 1430 (-ph); Theo Anal ($C_{21}H_{35}NO_2$): C, 75.68%, H, 10.51%, N, 4.20%; Found: C, 75.44%, H, 10.79%, N, 3.88%; ^{1}HNMR($CDCl_3$): δ, 6.95 (d, 2H, Ar-H), 6.61 (d, 2H, Ar-H), 4.64 (b, 2H, NH_2), 1.30 (s, 24H, CH_2), 2.57 (t, 2H, phCH_2), 2.30 (t, 2H, CH_2CO).

2.3.3 *p*-CACPPA

A mixture of nitrilotriacetic acid (2.5 g, 0.013 mol) and acetic anhydride (10 ml) in pyridine (50 ml) was kept refluxing for 30 min, then *p*-APPA (1 g, 0.003 mol) was added. The resulting solution was stirred at 100°C for 2 h. The solvents were removed under vacuum, and the residue was dissolved in a small volume of water, the pH was adjusted with concentrated $NH_3 \cdot H_2O$. After filtration, the filtrate was adjusted with HCl to afford a pale brown crystal (CACPPA). mp: 194~196°C; IR(cm^{-1}): 3323 (-CONH), 1690 (-CO-), 1600, 1430 (-ph): Theo Anal ($C_{27}H_{42}N_2O_7$): C, 64.03%, H, 8.30%, N. 5.53%; Found: C, 64.11%; H, 8.48%, N, 5.94%; ^{1}HNMR (d_6–DMSO): δ, 10.30 (b, 1H, COOH), 7.85 (b, 1H, Ar-NH), 7.27 (m, 4H, Ar-H), 3.50 (s, 6H, NCH_2), 1.30 (s, 24H, CH_2), 2.30 (t, 2H, CH_2CO), 2.57 (t, 2H, phCH_2).

2.4 The preparation of ^{99m}Tc-CACPPA

To a 10 ml vial were added 0.5~1.0 ml of an aqueous solution of CACPPA(1~10mg/ml) at pH8.5~9.5, 50~200μg $SnCl_2 \cdot 2H_2O$ dissolved in 25μl HCl(0.05 mol/L), and 1~2 ml $^{99m}TcO_4^-$ eluate. The vial was allowed to stand for 10 min.

2.5 Determination of radiochemical purity (RCP) and radiolabelling yield (RLY)

Thin-layer chromatography (TLC): silica gel plates, with developing system of 80% (V/V) CH_3CN. The R_f values of $^{99m}TcO_4^-$, $^{99m}TcO_2 \cdot xH_2O$ and ^{99m}Tc-CACPPA are 1.0, 0.0 and 0.4~0.6, respectively.

High performance liquid chromatography (HPLC): C-18 PRP reverse-phase column (Φ4mm×300 mm) using the eluent 70% CH_3OH at a flow rate of 1.0 ml/min. The retention time of $^{99m}TcO_4^-$ and ^{99m}Tc-CACPPA are 1.56 min and 1.96 min at 20°C, respectively.

2.6 Biodistribution in mice

^{99m}Tc-CACPPA (0.2ml, 5.5 MBq) was injected through a tail vein into NIH mice (18~22 g, divided into 6 groups, 5 for each). The mice were killed at regular intervals (1, 5, 10, 20, 30, 60 min) postinjection. The organs of interest (blood, heart, liver, spleen, lung, etc) were dissected, weighed, prepared for counting,

and the uptake in each organ was expressed in fraction of injection dosage of per gram organ (%ID/g). Blood disappearance of ^{99m}Tc-CACPPA was analysed with a biexponential model.

2.7 Determination of partition coefficient (PC)

To a mixture of phosphate buffer (3 g, 0.1 mol/L, pH7.00 and pH7.40) and *n*-octanol (3g) was added ^{99m}Tc-CACPPA(RCP>95%, approximately 500000 cpm), Vortexed each for 3×1 min, centrifuged for 5 min at approximately 1500 rpm. Took out 1 ml of buffer solution and 1 ml of *n*-octanol solution, weighed and counted.

2.8 Determination of binding yield(BY) with HSA in vitro

^{99m}Tc-CACPPA (RCP>95%, approximately 50000 cpm) was added into 1 ml of HSA (10%, pH7.40). The solution was incubated at 37°C, trichloride acetic acid (1 ml, 10%) was added at a regular interval to stop the reaction. Separated the solution and the precipitate, counted them, respectively.

3 Results and discussions

CACPPA containing the successful functional group of PPA was synthesized with good yield. The purity of every synthesized product obtained was characterized.

The ^{99m}Tc labeling of CACPPA was easily carried out in the presence of $SnCl_2 \cdot 2H_2O$ at room temperature. The RCP and RLY of ^{99m}Tc-CACPPA were over 95% determined by TLC and HPLC. pH is the most important factor of successfully labeling. Labeling at pH8.5~9.5 is required to form the desired complex with RCP over 95%. From a chemical point of view CACPPA is, in fact, insoluble at low pH(pH<7), thus the preparation becomes turbid. At high pH(pH>10), labeling yields are not stable due to the colloids which $SnCl_2 \cdot 2H_2O$ might form. The results of labeling experiments confirm that high RLY and RCP can be obtained with amounts of CACPPA between 0.5 and 10 mg, $SnCl_2 \cdot H_2O$ between 0.05~0.2 mg, pH between 8.5 and 9.5. In all conditions mentioned above, the complex of ^{99m}Tc occurs almost immediately. Temperature does not obviously affect radiolabeling in this study.

^{99m}Tc-CACPPA was stable at room temperature for 24 h postpreparation. The partition coefficients were 10.98 and 11.45 at pH7.00 and pH7.40, respectively. Table 1 gives the binding yield of ^{99m}Tc-CACPPA with HSA in vitro.

Table 1 The binding yields of ^{99m}Tc-CACPPA to HSA in vitro (n=3)

Time/min	1	5	10	20	30	60	120
BY	48.95±0.91	52.15±0.49	69.55±1.49	76.50±0.28	78.50±0.42	77.10±1.69	76.45±1.91

Table 2 Biodistribution of ^{99m}Tc-CACPPA in mice (%ID/(g·organ), n=5)

Organ	Time/min					
	1	5	10	20	30	60
Heart	18.17±2.67	12.65±1.62	8.73±2.68	5.00±0.97	3.43±0.57	1.80±0.13
Liver	11.16±1.16	14.61±2.31	12.68±2.79	11.63±2.03	9.47±1.93	8.70±2.13
Spleen	6.06±1.40	14.74±2.39	8.72±2.98	8.44±2.01	7.14±1.93	4.00±0.40
Lung	22.27±8.31	14.53±5.38	10.25±1.97	6.80±3.00	5.35±1.01	3.36±0.50
Kidney	22.05±1.56	25.55±5.97	41.42±7.36	21.42±2.37	14.05±2.69	11.21±1.91
Blood	14.56±1.32	7.12±2.77	5.42±1.76	4.13±1.37	3.63±1.20	2.39±0.67
Heart/Liver	1.63	0.87	0.67	0.43	0.36	0.21
Heart/Lung	0.82	0.87	0.85	0.74	0.64	0.54

The biodistribution data in mice (see Table 2) show that CACPPA has rapid and substantial uptakes in heart muscle after intravenous injection. Maximal myocardial accumulation of 18.17±2.67%ID/g was achieved, poor retention was also found in the experiment. The uptake of ^{99m}Tc-CACPPA in heart muscle was only 8.73±2.68%ID/g 10 min postinjection, it might be influenced by an altered ^{99m}Tc-CACPPA to albumin binding.[1] All other organs display low uptake of radioactivity. Major excretion organ is kidney, and its peak concentration is 41.42±7.36%ID/g at 10 min. The elimination of the radioactivity from blood has a biexpo-

nential pattern. The first $T_{1/2}$ is 1.11 min and the second $T_{1/2}$ is 20.08 min, kinetic equation is C=13.19exp(-0.624t) + 7.76exp(-0.035t), apparent volume of distribution (V_d) is 11.78 ml, clearance (Cl) is 0.407 ml/min. Fig.2 shows a typical elimination curve of ^{99m}Tc-CACPPA in blood. Rapid clearance from peripheral blood, decreasing tissue concentration except kidney, indicated a low background for imaging.

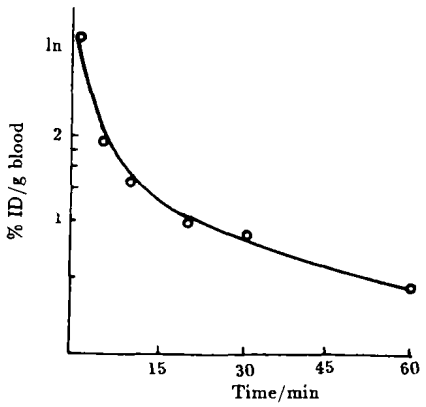

Fig.2 Blood radioactivity-time curve of ^{99m}Tc-CACPPA in mice

Deutsch *et al*[2] first demonstrated that cationic complexes of ^{99m}Tc(I) and ^{99m}Tc(III) are guite stable. They are easier to be extracted by myocardium than ^{99m}Tc(V) complexes. The ^{99m}Tc(I) and ^{99m}Tc(Ⅲ) complexes also exhibited excellent myocardial uptake in animal model.[3,4] It is necessary for a myocardial imaging agent to own a definite lipophilicity. The structure of ^{99m}Tc-CACPPA is the same as ^{99m}Tc-EHIDA, the presence of $[Tc{=}O]^{3+}$ core in the ^{99m}Tc complexes is identified.[5] From a chemical point of view, ^{99m}Tc-CACPPA has two free carboxy groups, it might be the reason for high water-solubility of ^{99m}Tc-CACPPA. So the partition coefficients (PC) are much lower than that of IPPA(PC are over 1000 at pH7.40 and pH7.00), ^{99m}Tc-CACPPA presents a high binding to HSA in vitro.

4 Conclusion

In summary ^{99m}Tc-CACPPA can be extracted by myocardium. Even though the rapid clearance of heart muscle makes it unable to be a myocardial imaging agent, it suggested the feasibility of incorporating ^{99m}Tc into PPA derivatives. In the study of ^{99m}Tc-labeled fatty acids, since the ^{99m}Tc-chelating agent occupies a large fraction, it is important for us to choose an appropriate chelating agent. A further study will be carried out.

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