Preclinical pharmacological study on ^{125}I -IPPA*

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Abstract Myocardial uptake of ^{125}I-IPPA in rats showed a peak of 4.4% of injected dose per gram. The half elimination time of myocardium was 3.8 min and the maximal uptake of thyroid is only 0.005%ID/organ at 120 min. The initial half time of 2.7 min in rabbits was obtained from the elimination curve of radioactivity in blood. In vitro binding test for ^{125}I-IPPA to HSA showed rather constant level of activation during two hours. The second peak of extraction was observed in major organs of rats, in rabbits' elimination of radioactivity and in binding test for ^{125}I-IPPA to albumin in vivo. Toxicity trial was up to standard. The tolerance of a mouse to IPPA was 560 times as high as that of a person to IPPA. It demonstrated that ^{125}I-IPPA could be quickly extracted by myocardium, and its catabolites were excreted in the urine with almost no iodine loss. All the results were found to agree with the expectations based on the principal metabolic path of phenyl fatty acid.
Keywords ^{125}I-IPPA, Radioiodinated fatty acid, Animal study

1 Introduction

Because free fatty acid is the major source of energy for heart, radiolabeled fatty acids have been used to assess myocardial metabolism and its viability.[1] Although successful diagnosis of damaged myocardium with straight chain ω-iodoalkyl fatty acids has been reported, the clinical application of these straight chain fatty acids was thought to be limited by a background problem of releasing free iodine caused by β-oxidation of fatty acids by enzymes.[2,3,4]

To prevent deiodination, Machulla[4] developed 15-p-iodophenylpentadecanoic acid (IPPA), whose β-oxidation yields iodobenzoic acid as terminal catabolite, rather than iodine. Iodobenzoic acid was converted to hippuric acid after intrahepatic conjugation with glycine and rapidly cleared by the kidneys, thus reducing the background on the image. All these results mentioned above have been demonstrated in the isolated, perfusion and working rat heart by determining total heart activity and total activity distribution in the effluates including iodobenzoic acid, iodophenyl propionic acid and free acid. Therefore, IPPA has been proved to be a potential myocardial viability imaging agent for clinical applications.[5] In this paper, we try to support the concept of the in vivo metabolic pathway of IPPA reported previously with two animal studies: (1) in vivo and in vitro test for binding of IPPA to albumin, (2) biodistribution and elimination of radioactivity from major organs in rats.

2 Materials and methods

2.1 Instruments

PACKARD-COBRA γ-counter (made in USA), 80-2 centrifuge machine (made in Shanghai).

2.2 Reagents

^{125}I-IPPA was prepared by ourselves, its radiochemical purity was higher than 98%, Bovine Serum Albumin (BSA) and Human Serum Albumin (HSA) were from Shanghai Biochemical Co, and all were of biological grade.

2.3 Animals

Rats (Sprague-Dawley), mice (NIH) and rabbits (New zealand) were from the Center of Experimental Animals of East China.

2.4 Biodistribution in rats

^{125}I-IPPA (1.48×10^7Bq) was dissolved in 0.15 mL ethanol and suspended in freshly prepared BSA(4.0%, 10 mL). The resulted solution was incubated at 37°C for 1h before being used. ^{125}I-IPPA(0.2 mL, 1.85 MBq) was injected through a tail vein into rats (200~220 g,

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divided into 8 groups, 5 for each). The rats were killed at regular intervals (0.5, 1, 5, 10, 20, 30, 60, 120min) postinjection. The organs of interest (heart, liver, spleen, lung, kidney, thyroid, etc.) were dissected, weighed and prepared for counting, the uptake of each organ was expressed in fraction of injection dosage of per gram organ.

2.5 Blood disappearance in rabbits

^{125}I-IPPA (0.5 mL, 11.1 MBq) was injected through ear vein into rabbits (total 4, 2.5 kg for each). At regular intervals(1, 2, 5, 8, 10, 15, 20, 30, 45, 60, 90, 120 min) 20 μL blood was collected from another ear vein and prepared for counting. Blood radioactivity-time curve of ^{125}I-IPPA in rabbits was drawn out.

2.6 In vivo and in vitro tests for binding of ^{125}I-IPPA to albumin

In vivo: ^{125}I-IPPA (RCP>98%, 0.2 mL, approximately 0.185 MBq) was injected through tail vein into mice (18~22 g, divided into 7 groups, 5 for each). The mice were killed at regular intervals, 1 mL of blood was collected and centrifuged for 30 min(4000 r/min). Took out 50μL of the upper solution and added into 1 mL of phosphate buffer (0.01mol/L, pH7.40). After rotating, 1mL of trichloride acetic acid was added to stop the reaction. The solution and precipitate were separated and counted, respectively.

In vitro: ^{125}I-IPPA(RCP>98%, approximately 50,000 /min) was added into 1 mL of HSA (10%, pH7.40, 0.01 mol/L PB). The solution was incubated at 37°C for regular intervals, 1 ml of trichloride acetic acid was added. The solution and precipitate were separated and counted as described above.

2.7 Toxicity trial[7,8]

IPPA (not labeled, 0.2 mL, 0.5 g/L) was injected through tail vein into mice (total 5, 18g for each). After two day's routine feed, the mice were killed to be observed if there were anything disorder or dysfunction about all organs. The tolerance to IPPA was calculated by the ratio of received amount per kilogram mouse to received amount per kilogram person.

3 Results and discussions

3.1 The biodistribution data in rats (see Table 1) show that ^{125}I-IPPA had rapid and pronounced myocardial uptake in heart muscle. The maximal myocardial accumulation of 4.4%ID/g and initial half time $T_{1/2}$ of 3.8min in myocardium were achieved, which was in accordance with literatures[9,10,11]. The low thyroid uptakes (0.001%ID at 1min, and 0.005%ID at 120 min) demonstrated that ^{125}I-IPPA was stable in vivo with almost no released radioiodide observed. The second peak of extraction was observed in liver, lung, and kidney because ^{125}I-IPPA behaved metabolically and its catabolite (p-iodo-benzoic acid) rapidly entered blood circulation and removed from the blood as iodo-hippuric after intrahepatic conjugation with glycine and excreted in the urine. Other studies[12,13,14] including investigations in isolated rat hearts also have not demonstrated rapid loss of iodide, and the point during β-oxidation when its catabolite (p-iodo-benzoic acid) is produced remains to be determined, so in this experiment we found the second peak of every organ was different from each other. All the results we obtained well supported the concept that IPPA could be extracted by myocardium and metabolized to yield p-iodo-benzoic acid.

Table 1 Biodistribution of ^{125}I-IPPA in rats (n=5, $x \pm s$, I D/%g^{-1})

	Heart	Liver	Spleen	Lung	Kindney	Intestine	Thyroid
30″	3.33±0.59	4.01±0.57	1.41±0.21	2.99±0.57	1.03±0.14	0.51±0.08	0.003±0.001
1′	4.38±0.27	4.66±0.85	1.16±0.21	2.87±0.39	1.09±0.17	0.61±0.09	0.01±0.003
5′	2.47±0.41	3.45±0.60	0.85±0.17	1.52±0.39	1.00±0.25	0.56±0.08	0.003±0.02
10′	1.66±0.18	1.81±0.32	0.55±0.12	0.86±0.09	1.40±0.13	0.23±0.06	0.03±0.001
20′	1.25±0.48	1.65±0.24	0.51±0.16	0.56±0.20	1.62±0.45	0.58±0.19	0.004±0.002
30′	1.13±0.21	2.77±0.52	0.75±0.19	1.53±0.19	1.09±0.14	0.59±0.03	0.004±0.001
60′	1.10±0.41	2.57±0.62	0.64±0.11	1.44±0.21	1.47±0.46	0.68±0.08	0.005±0.002
120′	0.67±0.12	1.51±0.19	0.46±0.10	1.17±0.22	1.20±0.19	0.45±0.08	0.005±0.001

3.2 Blood radioactivity-time curve in rabbits was shown in Fig.1 Early rapid clearance of ^{125}I-IPPA from blood can be obviously observed. $T_{1/2}$ is 2.7 min, which is in accordance with the half elimination time of fatty acid from blood.[15] The catabolite resulted in the second peak in Fig.1. So the latter slow clearance of radioactivity from blood should be assigned to ^{125}I-IPPA and its metabolites.

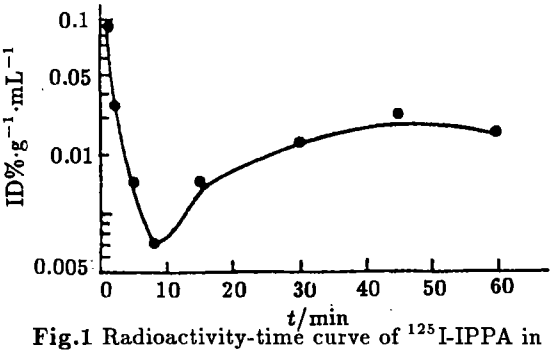

Fig.1 Radioactivity-time curve of ^{125}I-IPPA in rabbits

3.3 In vivo and in vitro the result of binding tests for ^{125}I-IPPA to albumin is given in Table 2. In vivo test showed that the binding to albumin was high, and higher myocardium extraction of IPPA may result from higher binding affinities to HSA, which was in accordance with the experiments on comparison of IHDA and IPPA metabolism and kinetics in the isolated rat hearts reported[15] by Timothy. Meanwhile, the catabolite (^{125}I-benzoic acid) could also bind to albumin, it showed a significant increase level of binding yield at about 20 min, which was the co-action of ^{125}I-IPPA and its catabolites to albumin. In vitro test exhibited high and stable binding, and its average binding yield was 97%.

Table 2 Binding yield (BY) of ^{125}I-IPPA to HSA in vitro and vivo (n=5, $x \pm s$%)

BY	1 min	5 min	10 min	20 min	30 min	60 min	120 min
In vivo	98.6±0.65	70.5±4.36	58.3±4.67	67.5±4.5	71.5±5.35	69.9±9.51	59.9±5.27
In vitro	97.5±0.25	97.0±0.30	96.8±0.71	97.2±0.32	97.4±0.54	97.4±0.34	97.2±0.52

3.4 Toxicity trial was up to standard. The mice were growing normally for 2 d postinjection. No death or adverse reaction were observed when dissected. If a person's weight was assumed to be 50 kg, once received IPPA was assumed to be 0.5 mg, then the tolerance of a mouse to IPPA was 560 times as high as that of a person to IPPA.

4 Conclusions

^{125}I-IPPA is readily taken up in heart and cleared from blood. It has low toxicity and high safety. It will be a promising myocardial viability imaging agent in our country along with ^{123}I's having been put into clinical uses.

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