Radioiodine labeled SP-4 as an imaging agent for atherosclerotic plaques

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Abstract The clinical prospect of radioiodinated SP-4 as an atherosclerotic plaque imaging agent was studied. The SP-4 was synthesized by a solid phase method and identified by an amino acid analysis after purification with HPLC. SP-4 was labeled with ^{131}I and ^{125}I by the Chloramine-T method and purified through Sephadex G-25 column. Tewlve New Zealand rabbits were divided into an atherosclerotic group ($n = 7$, AR) and a control group ($n = 5$, NR). All of the atherosclerotic rabbits were intravenous administrated with bovine serum albumin, then fed with high cholesterol and fat diet. ^{125}I-SP-4 was intravenous administrated to the rabbits of both groups. The biodistribution of ^{125}I-SP-4 in rabbits was investigated. The uptakes (%ID/g) in blood and thoracic aorta and abdominal aorta were calculated 4 hours postinjection. Macroautoradiography and microautoradiography were performed in 2 AR atherosclerotic abdominal aortas. The clearance of radioactivity from plasma was very rapid. ^{125}I-SP-4 was mainly excreted through kidneys. The radioactive uptakes of abdominal aorta and thoracic aorta of AR at 4 hours postinjection were significantly higher than that of NR. The films of macroautoradiography showed focal accumulation of the radioactivity in the areas of a newly formed edges of atherosclerotic plaques. On the slices of microautoradiography, the obvious radioactive accumulation could be found in the atherosclerotic plaques. Thus it was seen that the SP-4 remained its biological activity after radioiodination and was located at atherosclerotic lesions, it is potentially useful as an atherosclerotic plaque imaging agent.

Keywords Atherosclerotic plaque, Radioiodine labeled SP-4, Rabbit experiment

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1 INTRODUCTION

Atherosclerosis is the major cause of death on the world. Current imaging techniques for imaging atherosclerosis, such as angiography, ultrasound, computed tomography and nuclear magnetic resonance, are good at defining the extent of more advanced atherosclerotic plaques, but cannot detect the formation of atherosclerosis[1]. If we can find out a new method that can detect atherosclerosis in progress, then we may change the dietary of patient, use drug or other means to hold back the process of atherosclerosis in early stage.

There are many radiotracers based on several molecules and cells involved in atherosclerosis formation. At first, radiolabeled autologous platelets[2], monocytes[3] and lipoproteins[4] were developed. Then, ^{99m}Tc-ox-LDLs[5], ^{111}In-DTPA-human polyclonal IgG and ^{125}I-endothelin-1[6] were used. But these tracers are not suitable for imaging atherosclerosis,

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because of large molecules weight and low target-to-blood ratios. On the contrary, peptides are very small molecules, they clear from the circulation very rapidly. We used a radioiodine labeled apoB-based synthetic peptide SP-4 that contained amino acid resides 1000~1016 of apoB-100 to detect experimental atherosclerotic plaques in New Zealand white rabbits.

2 MATERIALS AND METHODS

2.1 Animal models

Studies were performed in 12 male New Zealand white rabbits ranging in weight from 1.5~2.0 kg. 7 rabbits were intravenous administrated bovine serum albumin (250 mg/ kg body weight), and then fed with cholesterol 1.0 g, lard 3.0 g every day for 2 months. 5 rabbits used as control.

2.2 Peptides synthesis and purification

The sequence of SP-4 was based on the report of Shih *et al*[7]: (Tyr)-Arg-Ala-Leu-Val-Asp-Thr-Leu-Lys-Phe-Val-Thr-Gln-Ala-Glu-Gly-Ala-Lys-$CONH_2$ (an amino-terminal tyrosine not present in the native sequence was added to facilitate radioniodination). SP-4 was synthesis by solid-phase method with PE 431 peptide synthesizer. Then purified to homogeneity by high-performance liquid chromatography (PE Co.) on a reverse-phase C18 column. The lyophilized peptide was store at $-20^{\circ}C$.

2.3 Iodination of SP-4

Radioiodination of the SP-4 was performed according to the Chloramine-T method. For a single dose, 50μg SP-4 (1.0 mg/mL in 0.1 mol/L phosphate buffer, pH7.4)was combined with either 3.7~7.4 MBq $Na^{125}I$ or with 3.7~7.4 MBq $Na^{131}I$. Followed by addition of chloramine-T 15 μL (4.0mg/mL in 0.1 mol/L phosphate buffer, pH7.4). After 1 min of gentle agitation, the reaction was quenched by the addition of sodium metabisulfite 35μL (4.0 mg/mL in 0.1mol/L phosphate buffer, pH7.4).

2.4 Purification and radiochemistry purity test of radioiodine labeled SP-4

The labeled SP-4 was separated from unbound reactants by chromatography on a Sephadex G-25 column ($\phi 1 \times 50$ cm), first eluted with 1% bovine serum albumin, then eluted with 0.1 mol/L phosphate buffer (pH 7.4). Column fractions containing the iodinated peptide were collected. The radiochemistry purity was tested by ITLC-SG (Gelman Sciences Inc.) thin-layer chromatography, developed by acetone.

2.5 Ex-vivo stability test of radioiodine

Labeled SP-4. The purified radioiodine labeled SP-4 was put in room temperature

for 20 h, and radiochemistry purities were checked every 4 h.

2.6 Biodistribution of radioiodine labeled SP-4

3.7 MBq ^{125}I-SP-4 was injected to the marginal ear veins of normal New Zealand white rabbits. The animals were sacrificed in 2 min, 15 min, 1 h, 4 h and 24 h post injection. Samples of blood, heart, lung, liver, spleen, kidney, stomach, muscle and intestines were washed, their radioactivity were measured with a well type gamma counter. To correct for radioactive decay and permit calculation of the concentration of radioactivity in each organ as a fraction of the administered dose, aliquots of the injected doses (IDs) were counted simultaneously. The results were expressed as %ID/g.

2.7 Animal experiment

3.7 MBq ^{125}I-SP-4 was injected to the marginal ear veins of 5 normal New Zealand white rabbits (NR) and 5 atherosclerotic rabbits (AR). 4 h post injection the animals were sacrificed. Samples of blood, thoracic aorta and abdominal aorta were removed and washed. Their weights and radioactivity were measured, use the same methods described above. The results were expressed as %ID/g.

2.8 Aortic specimens

3.7 MBq ^{125}I-SP-4 was intravenous administrated into 2 atherosclerotic rabbits. The animals were sacrificed after 4 h, abdominal aorta were removed. The adventitia was stripped off, the aorta was opened along the ventral surface, pinned out, and the remaining aorta was washed with normal saline. The aorta was fixed for 2h in 10% formaldehyde. Some of the aortic specimens were freezing sliced and stained with Sudan III and hematoxylin.

2.9 Macroautoradiography and microautoradiography

Fixed aortas were washed with saline, excess moisture was removed, and the aortas were covered with a single layer of plastic wrap. They were then placed between two sheets of X-ray film (Fuji Co.) and stored in a dark bag at -10°C. after 1 week the films were taken out, developed and fixed.

Some of the aortic specimens were freezing sliced (the thickness was about 20μm); the slices were smeared with 0.5% collodion, and then smeared with atomic latex (Atomic-2, Department of isotope, China Institute of Atomic Energy) in a darkroom. The slices were stored in dark box at 4°C. After 2 weeks, the slices were taken out, developed and fixed.

2.10 Statistical methods

Statistical analysis was performed with the student's t-test. Significance was assumed at $p < 0.05$. The results were expressed as (means $\pm$ standard deviation).

3 RESULTS

3.1 Atherosclerotic rabbits models

After fed with high cholesterol dietary for 2 months, obvious atherosclerotic plaques could be seen on the aorta of the atherosclerotic rabbits. Photomicrographs of atherosclerotic plaques showed endothelial disruption or absence, and internal elastic membrane disruption, Smooth muscle cells and foam cells proliferated and infiltrated to intima, and extracellular lipid deposited.

3.2 Radioiodination of SP-4

The best SP-4/chT weight ratio was 1:1.20, if the weight ratio was more than or less than this value, the labeling rate would decrease. The reaction reached the best labeling rate in 20°C, pH7.4 and react for 1 min.

3.3 Purification results, radiochemistry purity and ex-vivo stability of radioiodine labeled SP-4

Fig.1 shows elute curve of chromatography on a Sephadex G-25 column ($\phi 1 \times 20$ cm). Fig.2 shows UV (280 nm) absorbance of eluted fractions. Results showed the first radioactive peak in Fig.1 in correspondence with the UV absorbance peak on Fig.2, which was the pure ^{125}I-SP-4. Thin-layer chromatography of ^{125}I-SP-4 on ITLC-SG showed an R_f of 0~0.1, the R_f of free ^{125}I was 0.9~1.0. The radiochemistry purity of purified ^{125}I-SP-4 was more than 95%. After store in room temperature for 20 h, the radiochemistry purity of purified ^{125}I-SP-4 was more than 90%.

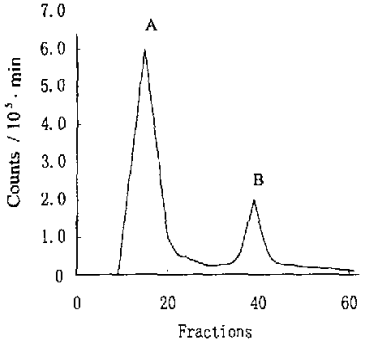

Fig.1 Sephadex G-25 column ($\phi 1 \times 20$ cm) chromatography of radioiodine product. Elute rate: 0.8 mL/min, 0.6 mL/fraction.

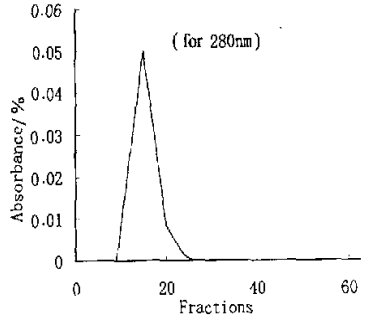

Fig.2 280 nm UV absorbance of eluted fractions

3.4 Biodistribution of radioiodine labeled SP-4 in normal New Zealand white rabbits

Table 1 summarized the biodistribution of ^{125}I-SP-4 in normal NZW rabbits. The

results showed kidney contained the highest concentration of SP-4.

Table 1 Distribution of ^{125}I-SP-4 in tissue of normal NZW rabbits (%ID/g)

Organ	2 min	15 min	1 h	4 h	24 h
Heart	0.2078±0.0633	0.1279±0.0071	0.1059±0.0162	0.0464±0.0131	0.0228±0.0042
Lung	0.4780±0.0832	0.3271±0.0718	0.1323±0.0229	0.0839±0.0073	0.0466±0.01020
Spleen	0.3552±0.0942	0.1709±0.0543	0.0729±0.0114	0.0240±0.0043	0.0221±0.0036
Liver	0.4445±0.0428	0.1790±0.0237	0.0805±0.059	0.0378±0.0060	0.0284±0.0030
Kidney	0.3241±0.0258	0.6327±0.0548	0.3384±0.0347	0.1346±0.0217	0.0542±0.0074
Stomach	0.0835±0.0071	0.1648±0.0130	0.2031±0.0136	0.0118±0.0171	0.0753±0.0091
Intestines	0.0898±0.0117	0.1027±0.0146	0.0734±0.0069	0.0548±0.0076	0.0281±0.0048
Muscle	0.0313±0.0037	0.0375±0.025	0.0257±0.0025	0.0130±0.0011	0.0050±0.0003
Blood	0.4725±0.0608	0.2113±0.0384	0.0683±0.0112	0.0388±0.0068	0.0185±0.0025

n=5, values=means±SD

3.5 Animal experiment

Table 2 shows the %ID/g of blood, thoracic aorta and abdominal aorta of AR and NR 4 h after injection of ^{125}I-SP-4. The results suggested ^{125}I-SP-4 accumulated in thoracic aorta and abdominal aorta of AR were significantly higher than that in NR. But ^{125}I-SP-4 in blood of AR and NR had no significant difference.

Table 2 Distribution of ^{125}I-SP-4 in blood, thoracic aorta and abdominal aorta of AR and NR 4 h after injection. (%ID/g)

	Blood	Thoracic aorta	Abdominal aorta
Atherosclerotic rabbits(AR)	0.0378±0.0028	0.0544±0.0026	0.0882±0.0101
Normal rabbits (NR)	0.0371±0.0033(0.39$^{(1)}$)	0.0220±0.0021 (22.36$^{(2)}$)	0.0276±0.0044 (12.23$^{(2)}$)

The values in the parentheses are t values. In comparison with AR, $^{(1)}p > 0.05$, $^{(2)}p < 0.01$. n=5, values=means±SD

3.6 Results of autoradiography

The films of macroautoradiography showed focal accumulation of radioactivity in the areas of newly formed edges of atherosclerotic plaques. On the slices of microautoradiography, the obviously radioactive accumulation could be found in the atherosclerotic plaques, especially accumulated in the areas of proliferated foam cells.

4 DISCUSSION

Since pathological arterial wall accumulation of cholesterol carried by low density lipoproteins (LDLs) is characteristic of atherosclerosis, some researchers tested radiolabeled LDL for its ability to detect atherosclerosis[4]. However, the effectiveness of LDL as an imaging agent was sometimes decreased by low target-to-blood ratio and relatively long half-life of LDL in human plasma (2~3 d) compared with the short physical half-life of ^{99m}Tc (6 h). Lipoproteins are large molecules and consequently clear from circulation very slowly.

By contrast, peptides are very small molecules (normally 10~20 amino acids) and clear from the circulation very rapidly. Specific cellular uptake of LDL by fibroblasts and hepatocytes involves the classic LDL receptor that recognizes a particular portion of apo-B. This LDL receptor binding domain, however, is not involved in focal accumulation of LDL by monocyte macrophages in atheroma because homozygous familial hypercholesterolemic patients, with absent LDL receptors, also develop atherosclerotic lesions. The apoB based synthetic peptide-4 (SP-4) contained amino acid residues 1000~1016 of apoB-100. An amino-terminal tyrosine not present in the native sequence was added to facilitate radioiodination. The classic LDL binding domains are not included in this peptide, by this way we can avoid the nonspecific binding of SP-4 in liver and other organs.

In our study, we found the best radioiodine labeling temperature was 20 ℃, and pH 7.4, the reaction last for 1 min. The best SP-4/chT weight ratio was 1:1.20, if the weight ratio was more than or less than this value, the labeling rate will decrease.

The results of biodistribution test suggested radioiodinated SP-4 clear from circulation very quickly; there was only 21.3% of the injected dose in plasma in 15 min postinjection, and 3.88% of the injected dose in 4h. SP-4 was mainly excreted from kidneys. The radioactivity accumulation in the stomach was increased with time; this result suggested there was deiodination in the metabolism of radioiodinated SP-4.

The results of Shih *et al*[7] showed that focal accumulation of apoB SP-4 was still intense 24 h after injection, at a time when there was <1% of the injected dose remaining in plasma. The prolonged persistence of the radioiodinated peptide in arterial lesions suggests strongly that it was protected from catabolism by cellular lysosomal enzymes and thus not intracellular, but bound to some component of extracellular matrix.

The clear focal radioactive accumulations on the atherosclerotic lesions on macroautoradiographys showed: SP-4 kept its biological activity after radioiodination. The microautoradiography results revealed SP-4 accumulated on foam cells in the atherosclerotic lesions. Thus radioiodinated SP-4 specifically localizes in atherosclerotic lesions and appears to bind to foam cells.

5 CONCLUSION

SP-4 kept its biological activity after radioiodination and clearly accumulated at atherosclerotic lesions. The results of our study suggest radioiodine SP-4 is a potentially useful imaging agent for detection of atherosclerotic plaques.

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