^{125}I-interleukin-8 radiolabelling and in vivo distribution

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Abstract To study the radioiodinating condition of interleukin-8(IL-8) and observe its biodistribution in mice for understanding the possibility of its application in nuclear medicine, we labelled IL-8 with ^{125}I using Bolton-Hunter reagent, and the distributions in mice at 5 min, 30 min, 1h, 6h and 24h after injection of ^{125}I –IL-8 were measured. The blood clearance curve was obtained and fitted with the two-compartment model. The results showed that ^{125}I-IL-8 was obtained with a labeling efficiency of 12.2% ±6.5% and a radiochemical purity of 91.4%±6.5%. Its specific activity was 14.8 kBq/μg IL-8. A fast phase half – life $T_{1/2\alpha}$of 0.32 h and a slow phase half – life $T_{1/2\beta}$ of 8.01 h were calculated from the blood clearance curve. The uptakes of radioactivities in kidneys and lung had the peaks of 85.87%ID /g and 16.17%ID /g at 30 min after intravenous injection, respectively. The uptakes in liver and spleen were 12.05%ID /g and 8.97%ID /g as the maximum at 5 min after injection. The clearance in blood and other organs was fast. Except for kidneys and lung, ^{125}I –IL-8 was less than 1%ID/ g 24 h after administration. It is concluded that radioiodinated IL-8 is a promising radiopharmaceutical in nuclear medicine, especially for imaging infection. But to enhance the labeling efficiency of radioiodinated IL-8 and to decrease its in vivo deiodination are the subjects necessary to be further investigated.

Keywords Interleukin-8, ^{125}I, Radiolabeling, Biodistribution

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1 Introduction

Interleukin-8(IL-8) is expressed as a 99 amino acid protein by monocytes, endothelial cells, fibroblasts and many cell types of epithelial origin. Following cleavage of a signal peptide and further proteolytic processing at the amino terminus, IL-8 is secreted predominately as an 8.4 kD protein of 72 amino acid by monocytes or an 8.9 kD protein of 77 amino acid by endothelial cells and fibroblasts. Among members of the recently discovered family of chemotactic cytokines, IL-8 is a major neutrophil chemtactic and activating factor produced by various types of human cells.[1] It is a small protein belonging to the CXC subfamily of the chemokines, in which the first 2 cysteines are separated by 1 amino acid. Chemokines or chemotactic cytokines are involved in cell activation and recruitment of cells to the area of inflammation. The CXC chemokines play an important role in cell recruitment during acute inflammation.[2] IL-8 produced a marked neutrophilia after the initial leukopenia and as a chemoattractant it has been shown to induce a profound leukocytosis. IL-8 is also significant for cellular recruitment from bone marrow and for prevention from tissue damage as well as neutrophil infiltration in reperfusion injury of the heart.[3] The variances of IL-8 level were observed in many patients with diseases such as pneumonia, peritonitis, arthritis, sepsis and cigarette smokers.[4-7] In addition, small receptor-binding agents have been proposed to obtain high specific uptake at the site of infection or inflammation within several hours after injection. High concentration of IL-8 has been observed in various inflammatory conditions and it correlates with tissue neutrophils infiltration in diseases, such as adult respiratory distress syndrome and ulcerative colitis.[8] Neutrophils express 2 type of IL-8 receptors and bind to both receptor with high affinity. Therefore IL-8 may be targeted on inflammatory tissue, where massive infiltration of neutrophils is present.

We have studied the radiolabeling method of IL-8 with ^{125}I and in vivo distribution of ^{125}I-IL-8 in mice as a first step to understand its in vivo behavior

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and the possibility of using radiolabeled IL-8 for diagnosis and therapy of different diseases in nuclear medicine.

2 Experimental

2.1 Materials

Human recombinant IL-8 purified with HPLC was produced from School of Biological Technology, Soochow University. ^{125}I-NaI was delivered by China Institute of Atomic Energy. Bolton-Hunter reagent was purchased from Sigma Co. The other reagents were of analytical purity.

2.2 Radioiodination of IL-8

(1) Bolton-Hunter reagent (HPNS) was labeled with ^{125}I. 1.0 μg of HPNS was labeled with ^{125}I-NaI (3.7~18.5 MBq) by incubating it under gently shaking with 50 μg Chrolamin-T at room temperature. The reaction was terminated by adding $Na_2S_2O_5$ in 2% KI solution. The ^{125}I labeled HPNS was extracted with 2% dimethyl methanamide in benzol and the radiochemical purity of ^{125}I- HPNS in organic phase and the label efficiency were meaasured. The organic phase was evaporated by nitrogen flow.

(2) IL-8 conjugated with ^{125}I- HPNS: 10 μg of IL-8 in boron acid buffer at pH 8.0 was added to ^{125}I-HPNS at the temperature below 20 °C by shaking for 30 min. The reaction was ended by adding 10 μg of glycine.

(3) Purification of ^{125}I-IL-8: Heparin-Sepharose 4B and Sephadex G50 column were used for the separation of ^{125}I-IL-8 from free $^{125}I^-$ by using 0.25% gelatin in 20 mmol/L phosphate buffer of pH 7.5 as an eluant for comparing their purification efficiencies.

(4) The radioactivities of ^{125}I-IL-8 were collected, the labeling efficiency of ^{125}I was determined with trichloroacetic acid precipitation and radiochemical purity of ^{125}I-IL-8 were determined by paper chromatography with saline as a mobile phase.

2.3 Distribution of ^{125}I-IL-8 in mice

15 male mice of 18~20 g were random divided into 5 groups. The water containing 1% potassium iodinate was fed 24 h before administration to block the thyroid uptake. The mice injected by ^{125}I-IL-8 with the dose of 3.7 kBq/0.25 μg IL-8/0.3 mL from tail vein were sacrificed at 5 min, 30 min, 1 h, 6 h and 24 h after administration. The organs (tissues) of blood, heart, lung, liver, kidneys, bone and muscle were weighed and radioactivities were measured. Their uptakes were expressed as %ID/g. The time-activity curves were obtained from the measurement.

3 Results

^{125}I-IL-8 was obtained with a labeling efficiency of 12.2% ±6.5% and a radiochemical purity of 91.4%±6.5%. Its specific activity was 14.8 kBq/μg IL-8.

Boric acid buffer of pH 8.5, phosphate buffer (PB) of pH 7.5 and double distillate water were used for solving Chloramine-T(Ch-T). The labeling efficiency of ^{125}I-HPNS was only 28.7%±5.9% with double distillate water and it was 46.7%±4.1% and 57.7%±18.9% respectively with other two kinds of buffer. Two columns filled with heparin-Sepharose 4B or Sephadex G50 were used in the purification of ^{125}I-IL-8. The radiochemical purities of ^{125}I-IL-8 were 91.4%±6.5% and 89.6%±3.3%, respectively.

Iodogen and Ch-T methods were tried for radioiodination of IL-8. But they were unsuccessful in very low labeling efficiency of less than 10%. The chemical stracture of IL-8 is not very clear up to now. The low labeling efficiency with Iodogen and Ch-T maybe shows the lack of tyrosine group at the labeling site.

The distribution of ^{125}I-IL-8 in mice is shown in Table 1.

The radioactivities initially rose in kidneys and lung at 5 min and then dropped with the peaks of 85.87% ID/g and 16.17% ID/g at 30 min after intravenous injection, respectively. The radioactivity uptakes in liver and spleen were 12.05% ID/g and 8.97% ID/g as the maximum at 5 min after injection and decreased with the time. The radioactivity less than 1 % ID/g was measured after 24 h in both organs. The ^{125}I-IL-8 in muscle was always at low level during 24 h. In bone and heart ^{125}I-IL-8 level fell from 5.15% ID/g and 6.46% ID/g at 5 min to 0.27% ID/g and 0.42% ID/g at 6 h after injection. But they were slightly increased to 0.63% ID/g and 0.71% ID/g at 24 h after injection.

Blood clearance in mice is shown in Fig.1.

Table 1 Distribution of ^{125}I-IL-8 in mice(% ID/g) (n=3)

Organs	5 min	30 min	1 h	6 h	24h
Blood	17.37±1.87	4.69±0.52	3.06±0.64	0.56±0.11	0.12±0.04
Heart	5.15±0.42	1.90±0.54	1.29±0.31	0.27±0.09	0.63±0.05
Lung	13.86±1.63	16.17±0.81	11.47±0.76	1.01±0.18	1.53±0.07
Liver	12.05±3.07	8.66±1.32	7.32±0.69	1.11±0.27	0.16±0.02
Kidney	29.73±17.02	85.87±20.82	56.90±10.79	6.73±0.93	1.36±0.27
Spleen	8.97±1.21	8.47±2.05	5.66±0.65	0.59±0.07	0.52±0.11
Bone	6.46±0.63	4.79±0.58	3.19±1.05	0.42±0.05	0.71±0.09
Muscle	2.23±0.21	1.41±0.12	0.67±0.38	0.29±0.17	0.20±0.05

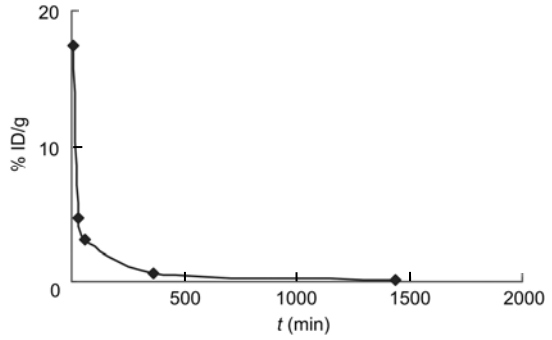

Fig.1 Blood clearance of ^{125}I-IL-8 in mice.

According to the blood clearance curve a fast phase half–life $T_{1/2\alpha}$ of 0.32 h and a slow phase half–life $T_{1/2\beta}$ of 8.01 h were obtained by making least square fit with the two-compartment model.

A urine sample 6 h after injection was obtained and precipitated with trichloroacetic acid to analyse the residual ^{125}I-IL-8 in urine.

4 Discussion

The labeling efficiency of HPNS with ^{125}I is somewhat lower than the value in the Reference.[9] Keeping ^{125}I in the reduced state is an important factor to label IL-8. We failed in radiolabeling with old ^{125}I-NaI that had stored for two months. May be the pretreatment of ^{125}I-NaI is necessary for completely reducing ^{125}I to −1 state in order to enhance the labeling efficiency.[10]

Labeling efficiency of ^{125}I-IL-8 is influenced mainly by labeling efficiency of ^{125}I- HPNS. Iodine ions with −1 state is oxidized to iodine atom by chloros decomposed from Ch-T. The neutral medium is more feasible for the release of chloros and double distillate water with slight acidity is not very suited. Boric acid buffer is suited for chloros, but the alkalescence will bring hydrolysis to HPNS. Buffer pH affects the labeling efficiency of ^{125}I- HPNS. So phosphate buffer (PB) of pH7.5 was used in the radioiodination.

There is no significant difference in radiochemical purities 91.4%±6.5% and 89.6%±3.3% of ^{125}I-IL-8 with two columns. Comparatively, ^{125}I-IL-8 is in the first radioactivitve peak for Sephadex G50 and in the second peak behind free ^{125}I peak for heparin-Sepharose 4B. We took Sephadex G50 for easy elution.

It is necessary to add some protein for storing ^{125}I-IL-8. ^{125}I-IL-8 keeps the radiochemical purity of 90.2%±4.5% during 15 d at 4 °C with bovine serum albumen and radiochemical purity has decreased to 45.0%±0.5% after 3 d without BSA in the same storage condition.

Conny[9] provided in their experiments the high uptake of ^{123}I- IL-8 in the abscess of rabbits and the imaging characteristics for infection were clearly superior to any of the receptor-binding agents that they had ever tested. Moreover, the higher uptake in abscess and faster background clearance of ^{123}I-IL-8 labeled with HPNS were found than those labeled with Iodogen. The abscess to background ratios of iodinated IL-8 were even 2 times higher than the ratios of ^{99m}Tc labeled chemotactic peptides. In our experiment the fast clearance of radioactivities from blood and kidneys of normal mice shows the feasibil-

ity for reducing the background if radioiodinated IL-8 is used as an imaging agent and proves that ^{125}I-IL-8 is normally metabolized by granulocytes and kidneys.

The correspondence between metabolism time kinetics of ^{125}I-IL-8 in blood, bone, lung, spleen and that of hematopoietic stem-cells indicates that IL-8 plays an important role to stimulate hematopoietic stem-cells. ^{125}I-IL-8 in lung with peak value at 30 min after administration illuminated the granulocytes accumulation in lung and thymine, corresponding to the results of Bohnet.[11]

The paper chromatography of the urine sample showed that about 90% of radioiodine was free ^{125}I. It indicated most of ^{125}I-IL-8 was deiodinated.

Combing our experiment and the results in References, we can conclude that radioiodinated IL-8 is a promising radiopharmaceutical in nuclear medicine, especially for imaging infection. ^{125}I-IL-8 is also possible for therapy since ^{125}I emits Auger electrons. However, there are two problems, i.e. to enhance the labeling efficiency of radioiodinated IL-8 and to decrease its in vivo deiodination, have to be solved for ^{125}I-IL-8 before its application in nuclear medicine.

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