^{62}Cu-Labeling of Human Serum Albumin–Dithiosemicarbazone (HSA–DTS) Conjugate for Regional Plasma Volume Measurement: Application of New ^{62}Zn/^{62}Cu Generator System

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^{62}Cu-Labeling of human serum albumin–dithiosemicarbazone (HSA–DTS) conjugate was performed using a newly developed ^{62}Zn/^{62}Cu generator system. HSA–DTS was easily labeled with ^{62}Cu, by simple mixing with ^{62}Cu-generator eluate. *In vivo* blood clearance of ^{62}Cu-HSA–DTS was similar to ^{131}I-HSA, indicating the high applicability of ^{62}Cu-HSA–DTS as a method for plasma volume measurement. In a positron emission tomography study of a dog, a clear plasma pool image of the head region was obtained.

Keywords copper-62, generator; positron emitter; human serum albumin; human serum albumin–dithiosemicarbazone; plasma volume

Introduction

If human serum albumin (HSA) can be labeled with a generator produced positron emitting ultra-shortlived metal nuclide, it is certain to become a promising radiopharmaceutical for the non-invasive measurement of regional plasma volume, as follows: (1) labeling nuclide can be easily obtained by simple elution from the generator, namely it does not require an in-house cyclotron facility,[1] (2) use of ultra-shortlived radionuclide protects patients from unnecessery radiation, (3) a positron emitter provides quantitative detection of radioactivity with high resolution, in combination with positron emission CT (PET), (4) the labeling reaction based on complex formation may be completed quantitatively by simple mixing of the generator eluate and HSA, without further purification.

We recently developed a new ^{62}Zn/^{62}Cu generator system (^{62}Cu: $T_{1/2}=9.8$ min, positron emission=97%) to obtain ^{62}Cu under physiological conditions of neutral pH, isotonic ion strength, non-toxic elution ligand, and so on.[2] In this study, Cu labeling of HSA was performed using this new generator system. To achieve stable Cu-labeling, dithiosemicarbazone (DTS) containing bifunctional chelating agent[3] was pre-introduced to the HSA molecule as a Cu binding site.

In studies with either radioactive or stable Cu, Cu-labeling of HSA–DTS conjugate was quickly completed by mixing the Cu-eluate with an HSA–DTS conjugate solution; Cu selectively bound to the DTS-residue, but not directly to native Cu binding sites[4] of HSA. Using this ^{62}Cu-HSA–DTS, rabbit blood clearance as well as dog PET imaging were performed.

Materials and Methods

^{62}Zn was obtained as $ZnCl_2$ aqueous solution (pH 5.0) from Nihon Medi-Physics Co., Ltd. (Japan). *p*-Carboxyethylphenylglyoxal-*N*,*N'*-dimethyldithiosemicarbazone (CE-DTS) was synthesized.[5] Other reagents were of reagent grade.

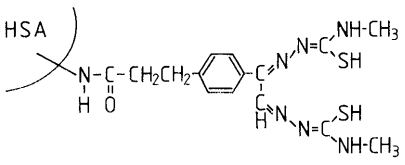

Fig. 1. Structure of HSA–DTS

Synthesis of HSA–DTS Conjugate HSA–DTS (Fig. 1) was synthesized by the slightly modified method previously reported.[5] Briefly, CE-DTS (5×10^{-5} mol) dissolved in 0.5 ml dimethylformamide (DMF) was cooled to -5 to -10 °C. Triethylamine (5×10^{-5} mol) and diphosphorylazide (5×10^{-5} mol) was added and stirred for 1 h at -5 to -10 °C. Then, 250 μl of the reaction mixture was added to 170 mg of HSA in 10 ml of borate buffer (pH 9.5, 0.01 M), stirred for 1 h at 0 °C, then dialyzed with acetate buffer (pH 5.0, 0.025 M). HSA–DTS was purified using a DEAE–Sepharose CL-6B column (2.8 × 8 cm), dialyzed with distilled water and lyophilized. Conjugation level (ratio of conjugated DTS to HSA) was determined spectrophotometrically.[5]

Preparation of ^{62}Zn/^{62}Cu Generator A ^{62}Zn/^{62}Cu generator was prepared, using 3.7—12.1 MBq of ^{62}Zn.[2]

Cu-HSA–DTS Binding Studies Using Stable Cu HSA–DTS (2—20 mg) was dissolved in 2 ml of acetate buffer (pH=6.0, ion strength=0.1). Cu binding was performed by simple mixing of HSA–DTS solution with 2 ml of Cu solution (Cu, 15.6 μM, in 200 mM glycine solution). The mixture was put into a centrifuge-type ultrafiltration apparatus (UNICEP ULTRA-CENT-30, Toyo Soda Co., Ltd., Japan) and then it was centrifuged at 3000 rpm for 10 min. The filtrate was collected and the Cu concentration was determined by atomic absorption spectrophotometry (AA-301, Shimadzu Co., Ltd., Japan). The percentage of Cu binding to HSA–DTS was calculated as labeling efficiency. Direct Cu binding to non-conjugated HSA was also studied for reference.

^{62}Cu-HSA–DTS Labeling Studies ^{62}Cu labeling of HSA–DTS was performed by the simple mixing of 2 ml of HSA–DTS solution (5 mg/ml, in acetate buffer (pH 6.0)) and 2 ml of ^{62}Cu-generator eluate (Cu, carrier free, in 200 mM glycine solution). ^{62}Cu-Labeled solution was ultrafiltrated by a similar method as in the non-radioactive Cu studies. The filtrate (100 μl) was collected and radioactivity measured with a well-type scintillation counter (ARC-301, Aloka Co., Ltd., Japan). As a reference, direct ^{62}Cu-labeling of HSA was also performed.

***In Vivo* Rabbit Blood Clearance Studies** Male Japanese white rabbits (4.0 kg body weight) were injected with a mixture of ^{62}Cu-HSA–DTS and ^{131}I-HSA (2.3 ml, containing ^{62}Cu, 22.2—29.6 MBq; ^{131}I, 0.185 MBq) through an ear vein; blood was subsequently collected from another ear vein at various time intervals. Just after blood sampling, apparent ^{62}Cu radioactivity, that is the sum of ^{62}Cu radioactivity [RA/Cu] and ^{131}I radioactivity detected in the ^{62}Cu-range [RA/I in Cu], was measured quickly. After ^{62}Cu had decayed out (*i.e.* after 24 h), ^{131}I radioactivity [RA/I] was measured. At the same time, ^{131}I radioactivity detected in the ^{62}Cu-range was measured and [RA/I in Cu] was calculated by decay correction. From these data, [RA/Cu] was obtained. As references, blood clearance studies were also performed of ^{62}Cu-glycine (generator eluate) and ^{62}Cu-CE-DTS.

PET Study of ^{62}Cu-HSA–DTS in Dog A male mongrel dog (21 kg body weight) was anesthesized with sodium pentobarbital. ^{62}Cu-HSA–DTS (37 MBq, 2 ml) was injected intravenously and PET imaging was then performed at the OM line of the head, using Positologica III.[6]

Results and Discussion

Preparation of HSA–DTS CE-DTS was originally

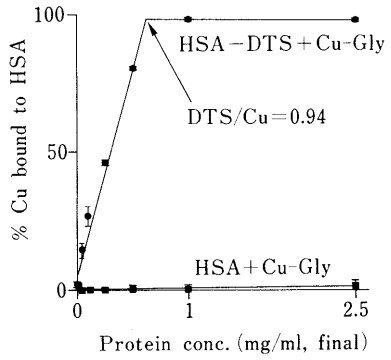

Fig. 2. HSA–DTS Concentration Effect on Stable Cu-Binding Efficiency
Data is average (1 S.D.) of 5 experiments.

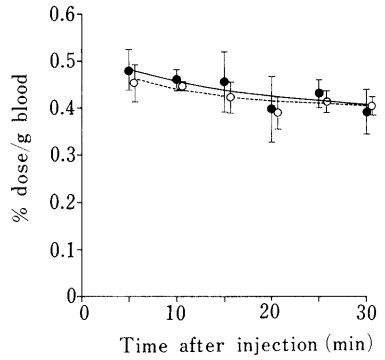

Fig. 3. Blood Clearance of ^{62}Cu-HSA–DTS and ^{131}I-HSA in Rabbits
Data is average (1 S.D.) of 3 animals. ●, ^{62}Cu-HSA–DTS; ○, ^{131}I-HSA.

developed as a bifunctional chelating agent for stable ^{99m}Tc-labeling of proteins in our laboratory.[5] This chelate structure was originally reported for Cu-complex formation[7]; thus we used CE-DTS as a Cu-complexing ligand. Under the reaction conditions described above, HSA–DTS conjugate having a DTS/HSA conjugation level of 0.842 was obtained.

Cu-Binding Efficiency Studies Using Stable Cu The effect of HSA–DTS concentration on Cu-labeling efficiency was studied using stable Cu-glycine solution (Fig. 2). At a low HSA–DTS concentration range (DTS/Cu < 1), Cu-labeling efficiency was in direct proportion to the DTS/Cu ratio, indicating the quantitative Cu-binding to DTS residue. Thus, at a final HSA–DTS concentration greater than 1 mg/ml (DTS/Cu > 1), Cu-labeling was completed quantitatively.

Cu potentially binds to the peptide chain of HSA.[4] However, under the present labeling conditions, very high concentrations of glycine were present, and HSA showed little Cu binding. Without glycine, Cu directly bond to HSA (bound % = 85.3%, final HSA concentration = 2.5 mg/ml, data not shown). These results suggested that glycine at high concentrations could compete with native Cu binding sites of HSA, but not with DTS residue. Thus, glycine acted not only as a Cu-selective elution ligand in the ^{62}Zn/^{62}Cu generator, but as a competing ligand with the native Cu binding sites of HSA. Cu, directly labeled with HSA, disappears very rapidly, from the plasma[8] (also shown below). Thus, a high concentration of glycine in the eluate is essential for stable HSA labeling with ^{62}Cu.

^{62}Cu-Labeling Efficiency Studies Since carrier free ^{62}Cu solution was applied to the labeling reaction, lower HSA–DTS concentration than that used in this study was theoretically sufficient for labeling. In this study, however, 5 mg/ml of HSA–DTS solution was used as a "labeling kit" to avoid non-specific protein binding to the vials and/or syringes. Under these conditions, ^{62}Cu labeling was completed by simple mixing with a ^{62}Cu genertator eluate (labeling efficiency: 97.9 ± 1.2%, average of 3 experiments ± 1 S.D.). On the contrary, ^{62}Cu showed little direct binding to native HSA unconjugated with CE-DTS in the presence of glycine (100 mM) (9.4 ± 0.7%, average of 3 experiments ± 1 S.D.).

***In Vivo* Rabbit Blood Clearance Studies** Figure 3 shows the blood clearance of ^{62}Cu-HSA–DTS and ^{131}I-HSA in rabbits. ^{62}Cu-HSA–DTS showed a stability in the blood similar to ^{131}I-HSA, a well established radiopharmaceutical for plasma volume measurement. On the other hand, ^{62}Cu-glycine eluate and ^{62}Cu-CE-DTS were eliminated from the blood very quickly (^{62}Cu-HSA–DTS, 0.394% dose/g; ^{62}Cu-glycine, 0.086% dose/g; ^{62}Cu-CE-DTS, 0.088% dose/g; 10 min post injection). Thus, it was concluded that ^{62}Cu-HSA–DTS exhibits a high degree of stability *in vivo*, which may be useful for regional plasma volume measurement in positron nuclear medicine methodology.

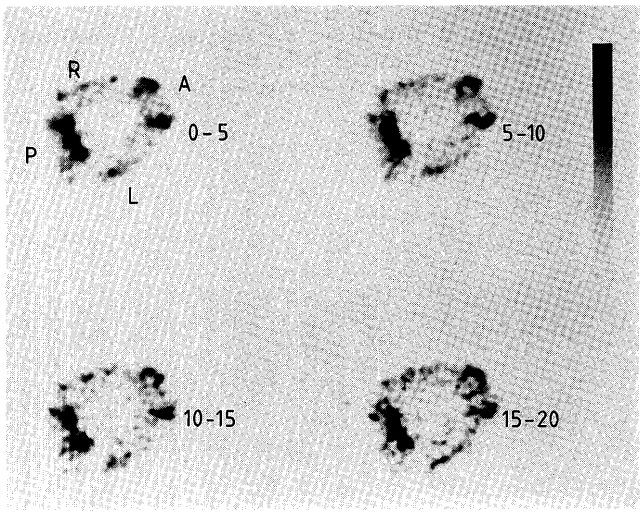

Fig. 4. PET Images of ^{62}Cu-HSA–DTS in Dog

PET Study in Dog Figure 4 shows the PET images of ^{62}Cu-HSA–DTS in dog at the OM line of the head. Clear plasma pool images of the head region were visualized. During the experiment, ^{62}Cu-radioactivity distribution was unchanged, indicating the stability of ^{62}Cu-HSA–DTS *in vivo*.

Conclusion

^{62}Cu-Labeling of HSA–DTS was accomplished by the simple mixing of generator eluate and HSA–DTS solution. Moreover, ^{62}Cu, mixed as a glycine complex, was selectively bound to the DTS residue in HSA–DTS, but not directly to the peptide chain of HSA. As a result, extremely stable ^{62}Cu-labeling of HSA–DTS was easily achieved. Aslo, in the PET study clear plasma pool images were obtained of the dog head. ^{62}Cu-HSA–DTS will provide us with more

reasonable data on regional plasma volume measurement than the ^{11}CO-red blood cell method, because the labeled HSA method is not affected by differences in regional hematocrit values.[9]

These results may facilitate the application of ^{62}Zn/^{62}Cu generator not only to DTS-containing compounds,[7,10,11,12] but also to various other radiopharmaceuticals with high Cu binding abilities.

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