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Radiolabeling of Glycine Oligomers with ^{99m}Tc Tricarbonyl Precursors for Heart Imaging Agent

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Abstract

To radiolabel glycine and glycine oligomers, we synthesized ^{99m}Tc tricarbonyl precursor with a low oxidation state (I). The ^{99m}Tc tricarbonyl glycine and glycine oligomers were prepared with high labeling yield(>95%). We evaluated the characteristics of ^{99m}Tc tricarbonyl glycine oligomer by carrying out *in-vitro* and *in-vivo* study. Nuclear imaging properties of ^{99m}Tc tricarbonyl-complexes with glycine oligomers in rabbits showed high radioconcentration in heart region. However, the results of biodistribution study with ^{99m}Tc tricarbonyl glycine trimer revealed low radioactivity in heart. From these results, we concluded that ^{99m}Tc tricarbonyl glycine trimer's activity in heart does not seems to be ideal enough to use as a diagonosis agent because of high radioconcentration in the blood.

Introduction

During the past two decades, major studies have been made in the diagnosis and treatment of heart disease. Advances in radionuclide techniques and computer applications have revolutionized the noninvasive evaluation of cardiovascular physiology and function. Radionuclide imaging is an excellent approach for assessing the cardiovascular function. Various attempts have been made to evaluate the heart function by synthesizing a suitable radiopharmaceutical which is useful in the diagnosis of organ. Nuclear cardiovascular examinations now request the sensitive detection and diagnosis of numerous cardiac abnormalities as well as the sequential determination of organ function. So far, nuclear cardiology has played a pivotal role in the establishment of the diagnosis of heart disease, in the assessment of disease

extent and the prediction of disease status of coronary artery disease.

Technetium-99m is an ideal radionuclide for diagnostic organ imaging because of its optimum γ-energy (140keV), short half-life (6hr), low cost and wide availability. ^{99m}Tc-sestamibi, a heart function diagnostic agent, is not ideal due to its low uptake and no redistribution. Thus attempt of the developing the new ^{99m}Tc labeled heart agent has been made.

Roser Alberto *et al.* synthesized ^{99m}Tc tricarbonyl complex as a precursor with a low oxidation state (I) for the biomolecules (1-5). They conclude that this new peptide labeling approach with $^{99m}Tc(CO)_3$ combines the highest possible specific activities with a minimal influence on the biologic properties of the peptide, including receptor affinity and metabolism and can be transferred to other peptides of choice (2). We established the preparation and radiolabeling method of ^{99m}Tc tricarbonyl precursor(6). Great effort has been made for the labeling of small peptides with ^{99m}Tc (7-11). Amino acid attracts considerable physiological interest because of their participation in many vital processes associated with the living system. Glycine is the simplelist amino acid.

This investigation have shown the preparation of ^{99m}Tc tricarbonyl glycine oligomers and evaluation of its the characteristics by carrying out *in-vitro* and *in-vivo* study.

Materials and Methods

Unless otherwise stated, all solvents and chemicals were of reagent grade. CO gas (99.5%) was obtained from the Daehan Gas Co. (Seoul, Korea) and prefiltered with oxygen trap. $^{99m}TcO_4^-$ was obtained by solvent extraction from $^{99}MoO_4^{2-}$ which was produced from the research reactor, HANARO, at KAERI. Glycine and glycine trimer were obtained from Sigma Chemical Co. (St. Louis, USA). Labeling yield was checked by HPLC (Waters, USA) coupled with μBondapak C-18 column (3.9× 300mm, Waters, USA). Mobile phase of HPLC was with gradient system based on 0.05M tetraethylammoniumphosphate (TEAP) buffer and 100% methanol.

Experimental animals were purchased from Bio Genobics, Inc. (Seoul, Korea). The animals were kept in polycarbonate cages for ICR mice and individual stainless steel cages for New Zealand White rabbits at 22±1℃ with relative humidity of 60± 10% and a 12hr light/dark cycle. After acclimation of approximately a week, the healthy animals were used for the experiments. The animals were allowed to free access of fresh tap water and laboratory animal chow.

1. Synthesis of ^{99m}Tc tricarbonyl precursor

^{99m}Tc tricarbonyl precursor was prepared according to our previous report (6). Ten mililiter vial contains Na_2CO_3 (8mg, 0.076mmol) and $NaBH_4$ (10mg, 0.26mmol) was capped with rubber stopper and then flushed with CO gas (99.5%) at room temperature for 30 minutes. Six mililiter of saline containing up to 37GBq [$Na^{99m}TcO_4$] was added and then heated to 75℃ for 30 minutes under the bubbling of CO gas. For the safety reason, the syringe was inserted in the stopper during the processing. After rapid cooling down to a room temperature using ice bath, 0.6ml of phosphate buffer solution (1M, pH 7.4) was added to neutralize. The radiolabeling purity was checked by reverse phase high performance liquid chromatography (HPLC) as well as its stability (12).

2. Radiolabeling with ^{99m}Tc tricarbonyl precursor

Labeling was carried out by addition with 1ml of ^{99m}Tc tricarbonyl precursor to the 0.1ml of glycine, glycine timer and glycine pentomer solution (5mg/ml in saline) at room temperature. And then reaction vial was heated at 75℃ for 30 min followed by cooling. After cooling down at the room temperature, labeling yield was checked by HPLC(FR= 1ml/min, Gradient, TEAP/MeOH=1:1, pH 2.4).

3. Animal studies

Imaging study

Imaging studies were carried out using 6 week-old male New Zealand white rabbits (2,500 ~3,000g, n=6). Animals were anesthetized with ketamin and xylazine. Rabbits were placed in a posterior posture. The Diacam gamma camera (Simens, Germany) with low energy collimator was utilized. Energy gate and window width were set to 140keV and 10%, respectively. Rabbits were injected with 37MBq of test radiolabeled complexes per head (1.0mCi) through the left ear vein. The static images were obtained from Icon system (Simens, Germany).

Biodistribution study

Biodistribution studies were carried out using 6-week-old male SD rats (157.9± 12.0g, n=15, SPF grade). The mice were injected with 0.74±0.07MBq radiophar maceuticals per head (20±2μCi) through the tail vein. At each interval (2, 30 and 60 min post injection) 5 rats were sacrificed with ethylether. The liver, spleen, kidney, lung, heart, blood, small intestine and large intestine were excised, weighed and counted, along with the diluted standard injected radiopharmaceuticals in a well type

gamma counter (Canberra Industries Inc., CT, USA). The linear regression line for the standard curve of radioactivity (Bq) vs counts per min (cpm) in the range radioactivity of 10 350,000Bq showed high correlation coefficients (r) of 0.999. For the maintenance of detection efficiency, the standard injected dose radiophar maceutical was diluted by saline for the calculation in order to be under the 300,000Bq. Data were expressed as means±SD. Statistical analyses were performed by the Student ' s *t*-test. Differences were considered to be significant at $p<0.05$.

Results

1. Synthesis of ^{99m}Tc tricarbonyl precursor

^{99m}Tc tricarbonyl precursor was successfully prepared by applying the procedure described by Jang *et al.* (6). Radiolabeling yield was 98% which was high enough for the radiolabelling with ligand. And most importantly, the other additional purification step was not necessary.

2. Radiolabeling with^{99m}Tc tricarbonyl precursor

The radiolabeling results of glycine and glycine oligomers with ^{99m}Tc tricarbonyl precursor are summarized in Table 1. High labeling yield of 95% was obtained on glycne, glycine trimer and glycine pentomer. The typical chromatograms of ^{99m}Tc tricarbonyl precursor and ^{99m}Tc tricarbonyl glycine trimer are shown in Fig 1.

3. Animal studies

Fig 2 showed the static images of ^{99m}Tc tricarbonyl glycine and glycine trimer using male New Zealand white rabbits at 5, 15 and 30 min post injection(p.i.). The activity of ^{99m}Tc tricarbonyl glycine was found at 5 min p.i. in kidney and liver. And the activity of ^{99m}Tc tricarbonyl glycine in the kidney was starting to decrease and to increase in the bladder at 15 and 30 min p.i. Also it was found that the activity of heart was lower than liver ' s.

The results of the biodistribution study of ^{99m}Tc tricarbonyl glycine trimer in SD rats at 2, 30 and 60 min after injection are summerized at Table 2 as a percentage of injected dose per tissue weight (%ID/g). The %ID/g of labeled compound activity in kidney was the highest among all groups (7.03, 8.85 and 6.65%ID/g). In 2, 30 and 60 min groups, the %ID/g of labeled compound activity in heart were 1.00, 0.60 and 0.54 %ID/g, respectively. The %ID/g of blood of 2, 30 and 60 min groups were 1.76, 1.64 and 1.04%ID/g, respectively, which were higher than those of blood.

Discussions

The heart is a fist-sized muscular pump that has a remarkable capacity to work inceasingly for the 70 to 80 years of a human life. Damage to the myocardium caused mostly by ischemic heart disease limits the capacity of the left ventricle to pump the blood. And similar results are found in case of heart failure too. Various methods are available to address function of the heart and those are computed tomography(CT) and magnetic resonance image(MRI). In present, SPECT and PET are the commonly used techniques for cardiovascular imaging to determine the functional abnormality of myocardium at rest and stress stage. Nuclear cardiology studies use noninvasive techniques to assess myocardial blood flow, valuate the pumping function of the heart as well as visualize the size and location of a heart attack. Among the techniques of nuclear cardiology, myocardial perfusion imaging is the most widely used. For mycocardial perfusion imaging, a small amount of an imaging agent (^{201}Ti, ^{99m}Tc-sestamibi, ^{99m}Tc-tetrofosmin) is injected into the blood stream (13, 14). Myocardial perfusion studies can thus identify areas of heart muscle that have an inadequate blood supply as well as the areas of heart muscle that are scarred from a heart attack. To date many compounds have been developed for heart diagnostic ^{99m}Tc agent but only ^{99m}Tc-sestamibi is routinely used.

Our method for preparation of ^{99m}Tc tricarbonyl precursor was successfully established under moderate conditions. Glycine and glycine oligomers were labeled with ^{99m}Tc tricarbonyl precursor and its labeling yield was high (>95%). According to imaging study, it was found that ^{99m}Tc tricarbonyl glycine trimer was localized in the heart region with high radioactivity in rabbit. Also other region of radioactivity was found was a liver. Unfortunately, biodistribution study showed that heart localization was less than 1%ID/g which was not high enough to show myocardial uptake when it is compared ^{99m}Tc-sestamibi is 1%ID (13). Moreover, ^{99m}Tc-sestamibi is rapidly cleared from the blood with a biological half-life of 4.3 mins while ^{99m}Tc tricarbonyl glycine trimer has slow clearance manner (13). Since the activity of ^{99m}Tc tricarbonyl glycine trimer was much higher in the blood than heart, it does affect on heart imaging. According to image study, the result showed that the activity of ^{99m}Tc tricarbonyl glycine trimer in the kidney was decreased and that of bladder was increased as time was elapsed. On the contrary, the results of rat biodistribution study showed that the activity of ^{99m}Tc tricarbonyl glycine trimer in kidney was high at 30 mins p.i. and it was starting to decrease at 60 min p.i. This discrepancy might be due to difference in the disease animal species.

From the present study, the radioisotope labeling method without further purification procedure was established with ^{99m}Tc tricarbonyl precursor for glycine and glycine oligomers. Fact on the heart localization of ^{99m}Tc tricarbonyl labeled glycine oligomers may lead to the conclusive result, which is ^{99m}Tc tricarbonyl glycine trimer can be a good candidate for the myocardial imaging agent with slight modfication of glycine trimer, although it was not significant compared to other imaging agent. Study should be focused into the ligand structure modification to increase selective heart localization of labeled compound and reducing the blood circulation at the same time. Present study will be applied to the developing other imaging agent for the SPECT on heart function.

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Table 1 ^{99m}Tc labeling yield of glycine oligomers with ^{99m}Tc tricarbonyl precursor, determined by HPLC

Compound	Labeling yield[1]	Retention time (min)[2]
Glycine	> 98%	11.3
Glycine3	> 95%	13.1
Glycine5	< 95%	14.2

1: Reaction conditions of ^{99m}Tc tricarbonyl complex: 5mg/0.2 ml of ligand solution was reacted with 1 ml ^{99m}Tc tricarbonyl precursor , then heated at 75 ℃ or at room temperature for 30 min.

2: HPLC conditions:

Mobile phase - gradient system based on 0.05 M TEAP buffer and 100% MeOH

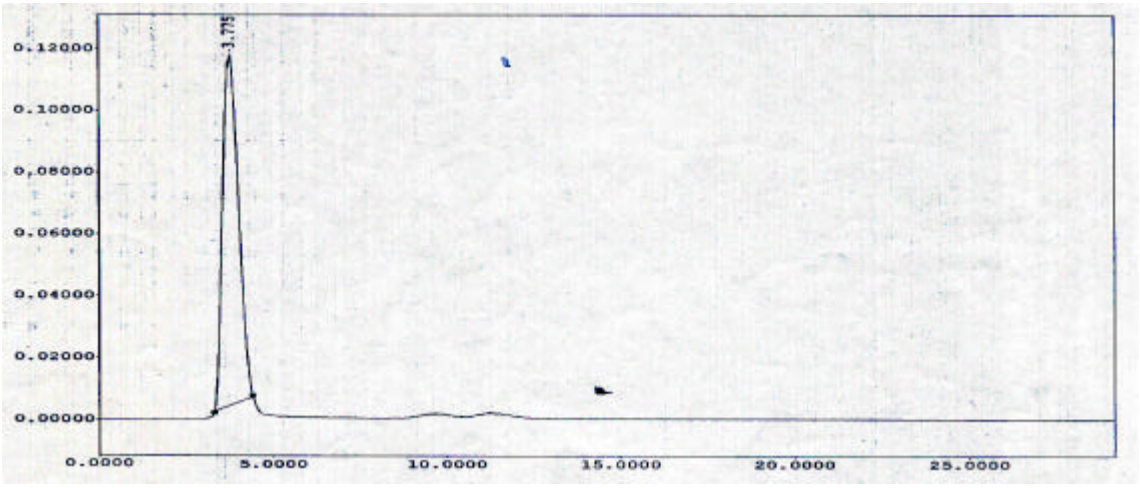
Column - μ Bondapak C-18 column (3.9×300 mm)

Flow rate - 1 ml/min

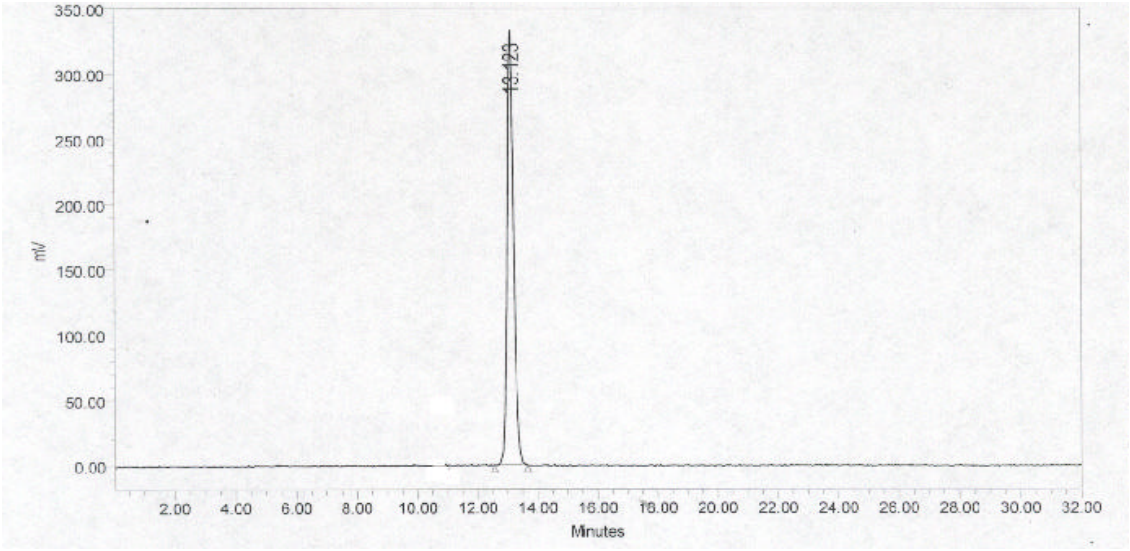
Table 2. Biodistribution of ^{99m}Tc tricarbonyl glycine3 in SD rats at 2, 10 and 30 min after 1.48 MBq/0.2ml intravenously administration

Time p.i. (min)	Blood	Heart	Liver	Lung	Spleen	Lung
2	1.76±0.25	1.00±0.19	0.57±0.04	1.08±0.11	0.53±0.03	7.03±0.26
30	1.64±0.83	0.60±0.04	0.80±0.03	0.81±0.14	0.38±0.09	8.85±0.80
60	1.04±0.37	0.54±0.06	0.65±0.03	0.78±0.04	0.29±0.07	6.65±1.46

*Data were expressed as means±SD (n=5) %ID/g.



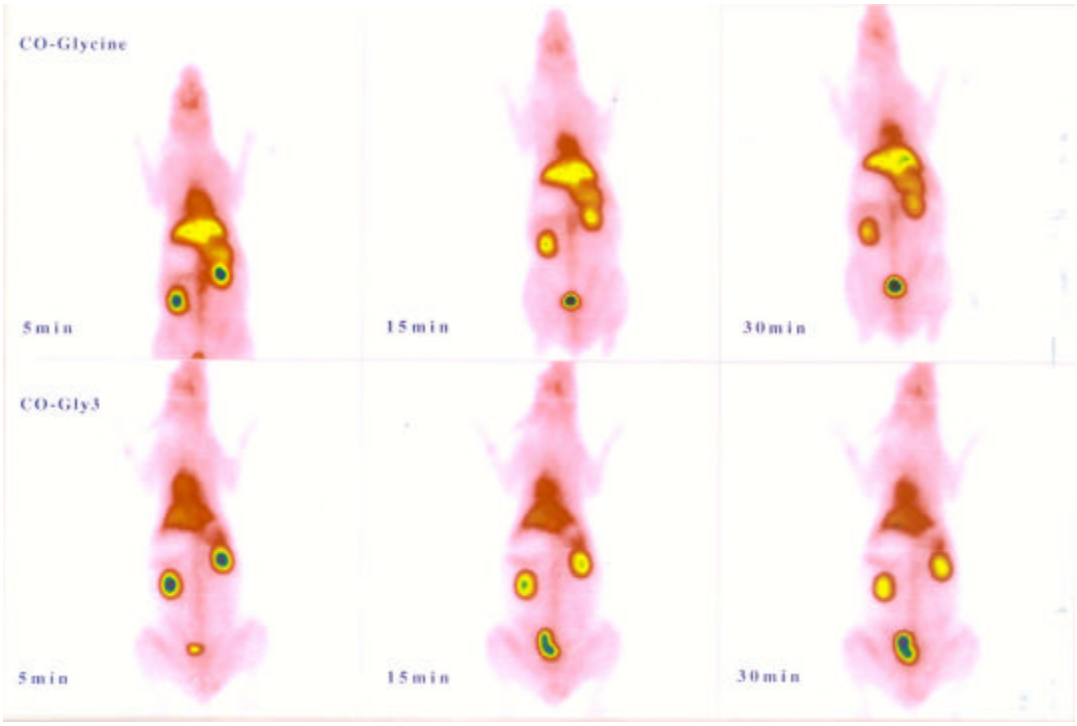
(A)



(B)

Fig 1. The chromatogram of ^{99m}Tc tricarbonyl precursor (A) and ^{99m}Tc tricarbonyl glycine trimer (B).

HPLC conditions: Mobile phase - gradient system based on 0.05 M TEAP buffer and 100% MeOH; Column - μ Bondapak C-18 column (3.9×300 mm); Flow rate - 1 ml/min



(A) (B) (C)

Fig 2. The images of rabbit administered ^{99m}Tc tricarbonyl glycine and ^{99m}Tc tricarbonyl glycine trimer.