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Synthesis of ^{99m}Tc-tricarbonyl Precursors for Labeling of Bioactive Molecules

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Abstract

To radiolabel bioactive molecules, we synthesized ^{99m}Tc-tricarbonyl precursor, $[^{99m}Tc(CO)_3(H_2O)_3]^+$ with a low oxidation state (I). The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared by low pressure carbonylation (1 atm of CO) of $[^{99m}TcO_4]^-$ in the presence of $NaBH_4$ resulting in higher than 98% of labeling yield and stability up to 8 hrs. We evaluated the characteristics of ^{99m}Tc-tricarbonyl labeled bioactive molecules by carrying out *in vitro* and *in vivo* study. Prepared $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was then reacted with some ligands of significance in modern diagnostic nuclear medicine and some amino acids. Labeling yields were checked by HPLC and found to be usually high, excluding ^{99m}Tc-tricarbonyl-MDP, -EDTMP and -mIBG. And the biodistribution properties of ^{99m}Tc-tricarbonyl complexes applied in rabbit showed different appearance comparing with that of the ^{99m}Tc-labeling by conventional means. From these results, we conclude that $[^{99m}Tc(CO)_3(H_2O)_3]^+$ is a potential precursor for development of radiopharmaceuticals, especially for labeling of biomolecules.

Key Words : ^{99m}Tc-tricarbonyl precursor, labeling property, bioactive molecules

1. Introduction

Technetium-99m is an ideal radionuclide for diagnostic organ imaging because of its optimum γ-energy (140keV), short half-life (6 hr), low cost and wide availability. Thus, many application based on ^{99m}Tc has been applied for nuclear medical purpose. Various tissue-specific technetium complexes are nowadays utilized for radio(immuno)diagnosis. Over the last 30 years, noninvasive radiographic methods have become a standard method for variety of diseases. Expansion

of radiographic method was enhanced from for anatomic diagnostics to for differentiation test of receptor distribution. This was attributed primarily to their favorable pharmacokinetics characterized by short retention time in the blood and high uptake in the target tissue. Great effort has been made for the labeling of small peptides with ^{99m}Tc (1-5). However, these approaches were of limited success. Direct labeling is unspecific and draws the loss of the peptide' s receptor affinities due to the possibility of reaction in the binding region. The alternative approach that uses bifuctional chelates, in which one functionality is designated for the attachment to the bioactive peptides and the other stabilizes the nuclides. A broad variety of bifunctional chelators mainly based on open chained tetradentate N, S systems such as N_2S_2 (diamine dimercaptide), N_3S_1 (triamine mercaptide). These kinds of ligand system have been successfully applied with $^{186/188}$Re/^{99m}Tc for the labeling of proteins and provided with high thermodynamic stability. However, disadvantages of synthesis, tendency to be oxidized and less availability of some of these ligands limit their general application. Almost all ^{99m}Tc radiopharmaceuticals are based in Tc(Ⅴ)oxo or octahed Tc(Ⅲ) cores. ^{99m}Tc agents with organometallic low-oxidation state are less common, partly because of the difficulty of controlling the reduction of the Tc(Ⅶ) to the Tc(Ⅰ) oxidation state. But, Roser Alberto *et al.* synthesized ^{99m}Tc-tricarbonyl complex as a precursor with a low oxidation state (Ⅰ) for the biomolecules (6-10). They presented that it was easily prepared as the organometallic aqua complex $[^{99m}Tc(H_2O)_3(CO)_3]^+$ directly from $[^{99m}TcO_4]^-$ in saline under 1 atm of CO which is stable at room temperature even though exposed to air (6). 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 (7).

Amino acid attracts considerable physiological interest because of their participation in many vital processes associated with the living system. One important property of this group compounds is its specificity toward the pancreas. The pancreas produces voluminous of secretory proteins and therefore, dictates the need for pancreatic uptake of amino acid to serve as sources for these proteins. Various attempts have been made to utilize this property of the pancreas for synthesizing a suitable radiopharmaceutical that could be used to image that organ. But they didn' t made suitable ligand. Another important property is its excretory pathway. It is known that urinary pathway is the main excretory route of amino acid in the body. Although several investigators observed the remarkable renal excretory properties while utilizing these ^{99m}Tc-amino acid complexes with different imaging objectives, no attempt was made to evaluate the potential of these compounds for renal function studies (11,12).

This investigation have shown the simple preparation of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ with carbon monoxide at atmospheric pressure in the presence of sodium borohydride. We also prepared ^{99m}Tc-tricarbonyl precursor labeled with commercial diagnostic ligands, some amino acids and its derivatives, and evaluated the characteristics of these tricarbonyl complexes by carrying out *in vitro* and *in vivo* study.

2. Materials and Methods

Unless otherwise stated, all solvents, amino acids and its derivatives and chemicals were of reagent grade. CO gas (99.5%) was obtained from the Daehan Gas Co. (Seoul, Korea) and prefiltered with oxygen trap. $[^{99m}TcO_4]^{2-}$ was obtained by solvent

extraction from $^{99}MoO_4]^{2-}$ which was produced from the research reactor, HANARO, at KAERI. *l*-Alanine, *l*-tyrosine *l*-cysteine methyl ester, glycine, glycine methyl ester, methionine, tryptophane, histidine, glutathion were obtained from Fluka (Buchs, Switzerland) or Sigma Chemical Co. (St. Louis, USA). Ethylcysteinate dimer (ECD), MIBI, MAG_3, hexamethylpropyleneamine oxime (HMPAO), diisopropyliminodiacetic acid (DISIDA), diethylenetriaminetetraacetic acid (DTPA), methylenediphosphonate (MDP), m-iodobenzylquanidine (mIBG) were obtained from Amersham (UK), Mallinckrodt Medical Inc. (St. Louis, USA), Dupont (USA) or KAERI (Korea) as commercially available labeling kits form. Labeling yield was cheched by HPLC (Waters, USA) coupled with μBondapak C-18 column (3.9×300 mm, Waters, USA). Mobile phase of HPLC was with gradient system based on 0.05 M tetraethylammoniumphosphate (TEAP) buffer and 100% methanol.

Experimental animals were purchased from Gyeryong Science, Inc. (Daejeon, Korea). Experimental animals were allowed free access to food and water.

2.1. Synthesis of $[^{99m}Tc(H_2O)_3(CO)_3]^+$

$[^{99m}Tc(H_2O)_3(CO)_3]^+$ was prepared by applying a modified procedure described by Alberto *et al.* (6). Ten ml vial containing Na_2CO_3 (8 mg, 0.076 mmol) and $NaBH_4$ (10 mg, 0.26 mmol) was capped with rubber stopper and then flushed with the stream of CO gas (99.5 %) at room temperature for 30 minutes. Six ml of saline containing up to 37 GBq $[Na^{99m}TcO_4]$ was added by a syringe and then heated to 75 ℃ for 30 minutes under the bubbling of CO gas. For the safety reason, the syringe was kept inserted in the stopper during the processing. After rapid cooling to room temperature at ice bath, 0.6 ml of phosphate buffer solution (1 M, pH 7.4) was added to neutralize. Quality control was performed by reversed phase high performance liquid chromatography (HPLC) and its stability was also checked by HPLC at every hour for consecutive 8 hrs (13).

2.2. Radiolabeling and Stability of $[^{99m}Tc(H_2O)_3(CO)_3]$-complexes

Labeling was performed by adding 1 ml of the prepared $[^{99m}Tc(H_2O)_3(CO)_3]^+$ into the commercial diagnostic cold vials or reacting 1 ml of the prepared $[^{99m}Tc(H_2O)_3(CO)_3]^+$ with the 0.2 ml of a amino acid solution (5 mg/ml in saline) at room temperature. Radiolabeling of some amino acids was required heating at 75 ℃ for 30 min. After cooling down to the room temperature, labeling yield was checked by HPLC.

2.3. Animal Studies

Imaging studies were done using 6 week-old male New Zealand White rabbits (2,500 ~3,000 g, n=20). 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 positioned. Energy gate and window width were set to 140 keV and 10%, respectively. Rabbits were injected with 37 MBq of test radiolabeled complexes per head (1.0 mCi) through the left ear vein. The static images were obtained from Icon system (Simens, Germany).

3. Results

3.1. Synthesis of $[^{99m}Tc(H_2O)_3(CO)_3]^+$

$[^{99m}Tc(H_2O)_3(CO)_3]^+$ was successfully prepared

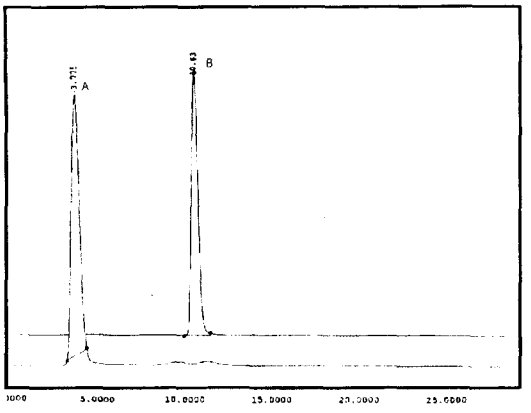


Fig 1. The Chromatogram of ^{99m}Tc-tricarbonyl Precursor (A) and Free $[^{99m}TcO_4]^-$ (B)
HPLC conditions: Mobile phase - gradient system based on 0.05 M TEAP buffer and 100% MeOH; Column - μBondapak C-18 column (3.9×300 mm); Flow rate - 1 ml/min

by applying a modified the procedure described by Alberto *et al.* (7). Yield was higher than 98 %. Additional purification step was not required. The typical chromatograms of free $[^{99m}TcO_4]^-$ and ^{99m}Tc-tricarbonyl precursor are shown in Fig 1. The retention times of free $[^{99m}TcO_4]^-$ and ^{99m}Tc-tricarbonyl precursor were 10.6 min and 3.8 min, respectively, under the given condition. ^{99m}Tc-tricarbonyl precursor with high specific activity upto Ci could be prepared (data not shown). ^{99m}Tc-tricarbonyl precursor was stable upto 8 hrs at room temperature. With a view to medical applications, a convenient synthetic method of ^{99m}Tc tricarbonyl precursor was developed.

3.2. Radiolabeling of $[^{99m}Tc(H_2O)_3(CO)_3]^+$-Complexes

The radiolabeling results of various ligands of significance in modern diagnostic nuclear medicine and some amino acids and its derivatives with ^{99m}Tc-tricarbonyl precursor are summarized in Table 1, 2. High labeling yield with more than

Table 1. ^{99m}Tc Labeling Yield of Some Typical Ligands with ^{99m}Tc-tricarbonyl Precursor, Determined by HPLC

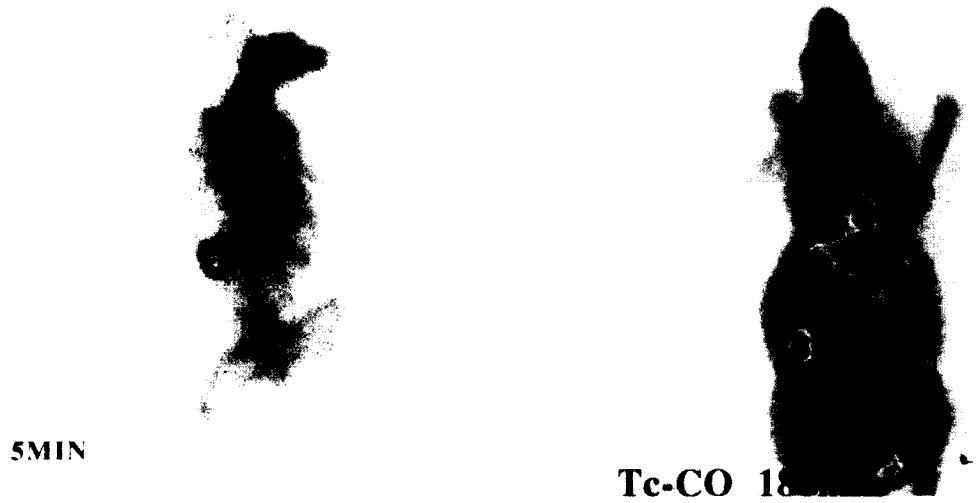
Compound	Labeling yield1	Retention time (min)[2]
DMSA	> 98%	19.3
ECD	> 95%	20.6
HMPAO	> 90%	17.7
MAG_3	> 90%	17.1
DISIDA	> 95%	21.1
DTPA	> 98%	13.2
MIBI	> 99%	20.3
MDP	No reaction	
EDTMP	No reaction	
mIBG	No reaction	

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 - μBondapak C-18 column (3.9×300 mm) Flow rate - 1 ml/min

Table 2. ^{99m}Tc Labeling Yield of Some Typical Amino Acids and Their Derivatives with ^{99m}Tc-tricarbonyl Precursor, Determined by HPLC

Compound	Labeling yield[1]	Retention time (min)[2]
l-Alanine	> 98%	11.3
l-Tyrosine	> 95%	16.9
l-Cysteine methyl ester	> 90%	12.2 ~ 20.4
Glycine	> 95%	6.7
Glycine methyl ester	> 99%	20.3
Methionine	> 98%	15.3
5 Methyl d,l-tryptopane	> 98%	19.9
Histidine	> 95%	17 ~ 22
Glutathion		< 40%
< 50%	13.0	18.0

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 - μBondapak C-18 column (3.9×300 mm) Flow rate - 1 ml/min



(A) (B)

Fig 2. Typical Image of Rabbit Administered ^{99m}Tc-tricarbonyl Precursor at 5 & 180 min After Injection
A: 5 min after injection; B: 180 min after injection

90% was shown on DMSA, ECD, MAG_3, DISIDA, DTPA, MIBI. But MDP, EDTMP, mIBG showed low labeling efficiency with ^{99m}Tc-tricarbonyl precursor. *l*-Alanine, *l*-tyrosine, *l*-cysteine methyl ester, glycine, glycine methyl ester, methionine, tryptopane, histidine were labeled very well with ^{99m}Tc-tricarbonyl precursor with higher than 90% yield except glutathion.

3.3. Animal Studies

The static image of ^{99m}Tc-tricarbonyl precursor in male New Zealand White rabbits at 5 and 180 min post injection are shown in Fig 2. At 5 min post injection image, high activity was found in bladder and kidney and a trace amount of ^{99m}Tc-tricarbonyl precursor seemed to be remained in liver and brain. However, a considerable radioactivity was noted in liver and kidney at 180 min post injection. ^{99m}Tc-tricarbonyl-ECD and -HMPAO showed lower activity in head region than those of the labeled with $[^{99m}TcO_4]^-$. Gamma image of ^{99m}Tc-tricarbonyl-DTPA was similar to that of ^{99m}Tc-DTPA, except slow excretion. Most of ligands labeled with ^{99m}Tc-tricarbonyl precursor exhibited different *in vivo* characteristics from those of the ligands labeled with $^{99m}TcO_4^-$ by conventional means (Fig 3).

Certain amino acids and its derivatives such as *l*-alanine, histidine, high radioactivity was uptaken into the liver and kidney region with slow excretion. No ^{99m}Tc-tricarbonyl complexes were absorbed in pancreas region.

3.4. Discussions

A modified Alberto' s method for preparation of ^{99m}Tc-tricarbonyl precursor was successfully established. The method was developed for the synthesis of ^{99m}Tc-tricarbonyl precursor under moderate conditions. The results of this study well explained and supported the data reported by Alberto *et al*. It is reported that the water and air stable organometallic aqua complex

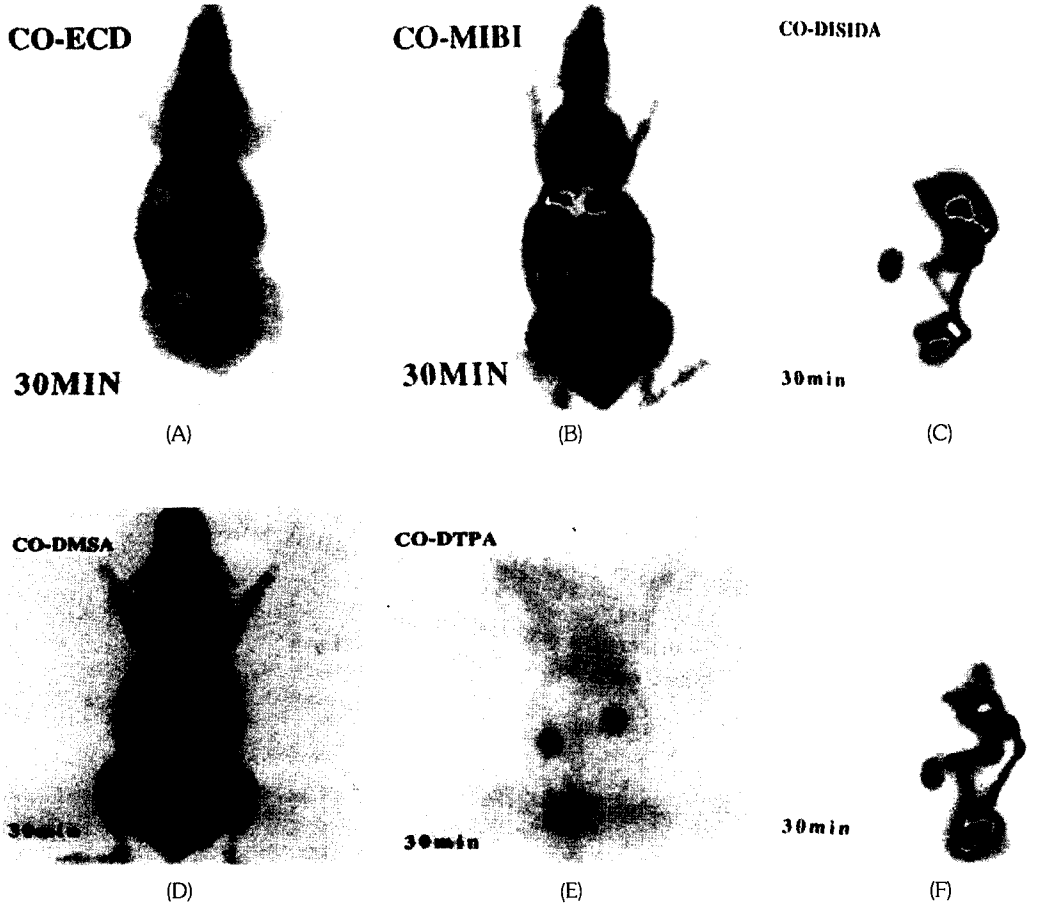


Fig 3. The Images of Rabbit Administered Typical ^{99m}Tc-ligands Labeled by Using ^{99m}Tc-tricarbonyl Precursor

A: ^{99m}Tc-tricarbonyl ECD, B: ^{99m}Tc-tricarbonyl MIBI,
C: ^{99m}Tc-tricarbonyl DISIDA, D: ^{99m}Tc-tricarbonyl DMSA,
E: ^{99m}Tc-tricarbonyl DTPA, F: ^{99m}Tc-tricarbonyl MAG_3

$[^{99m}Tc(H_2O)_3(CO)_3]^+$ with a oxidation state (I) is readily synthesized directly from $[^{99m}TcO_4]^-$ in saline without any high pressure applications(1). It has also been reported that Tc (I) oxidation state is particularly advantageous due to the kinetic inertness inherent in its low-spin d^6 configuration (14).

Alberto and his colleague used reagents for the synthesis of organometallic aqua complex such as $NaBH_4$, Na_2CO_3, and NaK tartrate in sealed vials with or without CO flushing under a heating condition after the addition of $Na^{99m}TcO_4$ dissolved in saline (2). We applied CO flushing during heating. Compared to the Alberto' s method, we obtained higher radiochemical purity of more than 98%. ^{99m}Tc-tricarbonyl precursor was labeled

efficiently with specific activity upto Ci/ml order. Stability of ^{99m}Tc-tricarbonyl precursor was checked for 8 hrs and it was found to be stable at room temperature. When we applied HPLC system for analysis of radiochemical purity, free $[^{99m}TcO_4]^-$ and ^{99m}Tc-tricarbonyl precursor were separated and showed a peak at 10 and 3.8 min, respectively. That is ^{99m}Tc-tricarbonyl precursor revealed increased hydrophilicity than $[^{99m}TcO_4]^-$. And retention times of ^{99m}Tc-tricarbonyl complexes were shifted right from 6.7 (glycine) to 21.1 min (DISIDA). Alberto suggested that two- or three-water molecules of ^{99m}Tc-tricarbonyl precursor substituted with coordination site of ligands. Upon the basis of our experience his suggestion is well agreeable. The data obtained in this study suggest that ligands containing two or three thiol, carboxyl, amine and amide groups are well labeled with ^{99m}Tc-tricarbonyl precursor while phosphoric acid is not.

In animal study, ^{99m}Tc-tricarbonyl precursor was localized with high activity in bladder and kidney and a few amount of ^{99m}Tc-tricarbonyl precursor in liver and brain at early stage. In image of rabbit at 3 hr post injection, residual activity in liver and kidney were revealed. This result was considered that a part of ^{99m}Tc-tricarbonyl precursor was remained or absorbed although some of them rapidly excreted.

^{99m}Tc-tricarbonyl-DMSA, -ECD, -MAG_3, -DISIDA, -DTPA and MIBI showed very high labeling yield (>90%). But they showed different characteristics in hydrophilicity and distribution pattern in animals comparing with those of conventionally labeled. The retention time of ^{99m}Tc-tricarbonyl labeled ligands were from 13.2 (DTPA) to 21.1 (glycine) min in afore described HPLC conditions. Amino acids were good binding ligands for ^{99m}Tc-tricarbonyl precursor. Unfortunately, no tricarbonyl amino acid was absorbed particularly in pancreas.

We concluded that the method adopted for labeling biomolecules with ^{99m}Tc tricarbonyl precursor was successful. It was simple and potent high yield labeling of new bioactive molecules excluding further purification procedure.

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