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Research Article

Synthesis and biological characterization of ^{99m}Tc(I) tricarbonyl cysteine complex, a potential diagnostic for assessment of renal function

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Summary

Cysteine containing three functional groups, i.e. a carboxyl, sulfhydryl, and amino group, has been labelled with ^{99m}Tc(I) tricarbonyl precursor (**2**) in order to study the renal characteristics of the resulting ^{99m}Tc(I) tricarbonyl cysteine (**3**) versus the reference ^{99m}Tc-MAG$_3$ (**1**). The ^{99m}Tc(I) tricarbonyl cysteine (**3**) was prepared in good yields and characterized by HPLC, eletrophoresis, and eletrospray mass spectrometry. The plasma protein binding rate of ^{99m}Tc(I) tricarbonyl cysteine (**3**) was about 70 to 80%, and similar to that of ^{131}I-OIH (70%) but lower than that of ^{99m}Tc-MAG$_3$ (90%). The $T_{1/2}$ of ^{99m}Tc(I) tricarbonyl cysteine (4.30 ± 0.79 min) in the kidney was higher than the one of ^{99m}Tc-MAG$_3$ (2.43 ± 0.70 min). The static image of ^{99m}Tc(I) tricarbonyl cysteine at 5 min post injection showed high concentrated activity in the kidney. And in the 15, 30 min post injection images, most activity appeared in the bladder without a residue in other organs. The ^{99m}Tc(I) tricarbonyl cysteine exhibited biological behavior comparable to ^{99m}Tc-MAG$_3$ and it is expected to have diagnostic potential for imaging renal function in nuclear medicine. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: ^{99m}Tc(I) tricarbonyl complex; ^{99m}Tc(I) tricarbonyl cysteine; renal function diagnostic; renal agents; tubular secretion

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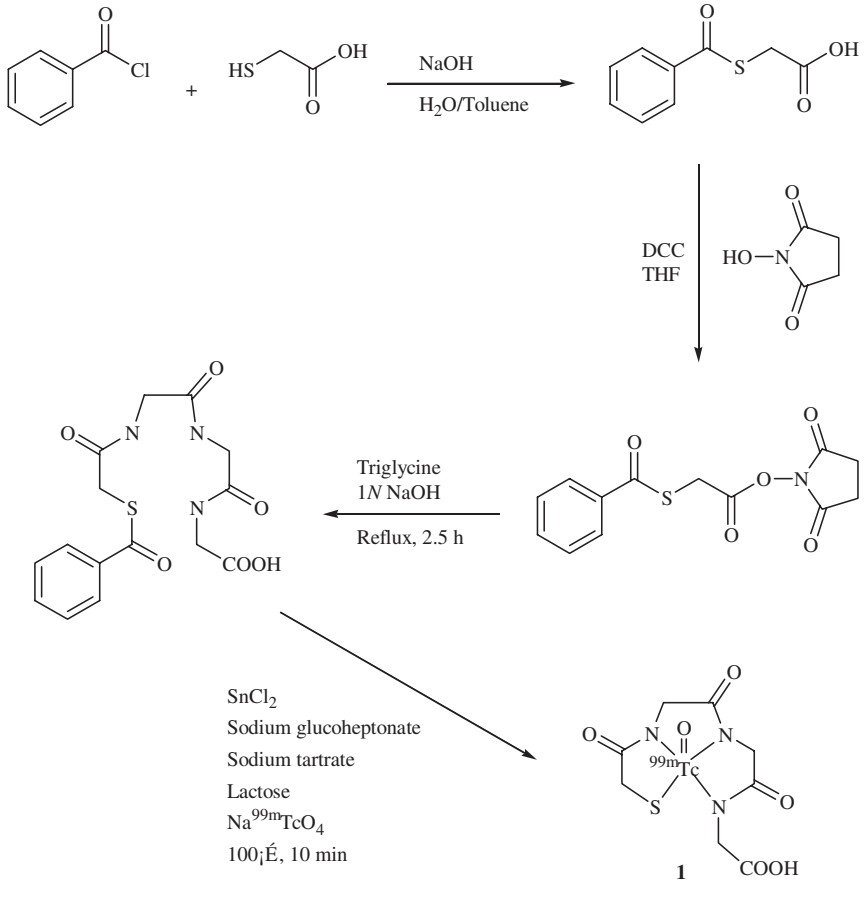
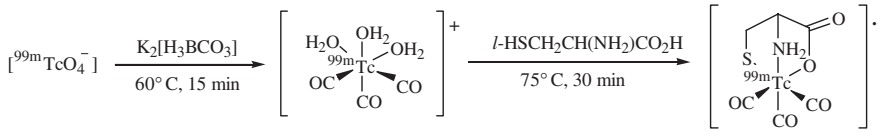
Introduction

Various radiological methods are available to address anatomical questions about kidneys. However, to determine the differential function of each kidney, and to detect obstruction in urine flow, renography using radiotracers is the method of choice.[1] To date there has been found no compound that is completely extracted by kidneys, and only para-aminohippuric acid (PAH) comes close. Tubis *et al.* developed ortho-iodohippuric acid (OIH), a radiolabelled analogue of PAH for measurement of ERPF and imaging.[2] But, ^{131}I-labelled-OIH involves poor image and high radiation dose, especially in patients with urinary obstruction.[3] Because of these disadvantages, many new renal imaging agents labelled with ^{99m}Tc are being introduced. The introduction of ^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG$_3$, **1**, Scheme 1) into clinical use as a potential replacement of $^{123/131}$I-labelled-OIH has opened up a new era in the field of renal radiopharmaceuticals.[4] ^{99m}Tc-MAG$_3$ has played an unchallenged role as a radiopharmaceutical of choice for dynamic



Scheme 1. Preparation of ^{99m}Tc-MAG$_3$ (1)

kidney function studies. However, the wide acceptance of ^{99m}Tc-MAG$_3$ is mainly due to the excellent scintigraphic imaging qualities of ^{99m}Tc rather than its biological properties.[4–6] Since, compared to ^{131}I-OIH, ^{99m}Tc-MAG$_3$ displays lower extraction efficiency, a significantly higher protein binding, a smaller volume of distribution and consequently slower plasma clearance (about 60–65% of OIH clearance),[5–8] attempts have been made to develop a ^{99m}Tc-labelled renal agent with a more desirable biological behavior. As a result of these attempts, ^{99m}Tc(I) tricarbonyl cysteine (**3**, Scheme 2) has been prepared and evaluated in rabbits.



2 **3**

Scheme 2. Preparation of ^{99m}Tc(I) tricarbonyl cysteine complex (3)

Design of an improved ^{99m}Tc-labelled renal agent requires a systematic identification of the physiological properties which are directly associated with optimal tubular transport. For a long time, it was believed that the carbonylglycine moiety was required for recognition by tubular proteins.[9] In view of this finding, it was of interest to prepare cysteine labelled with ^{99m}Tc with low oxidation state. In general, ^{99m}Tc-radiopharmaceuticals are based on ^{99m}Tc(V) oxo or octahedral ^{99m}Tc(III) cores. ^{99m}Tc agents with low oxidation state are less common, because of the difficulty of controlling the reduction of the ^{99m}Tc(VII) to ^{99m}Tc(I). But, Alberto *et al.* synthesized the ^{99m}Tc(I) tricarbonyl complex as a precursor with low oxidation state for the ^{99m}Tc(I) radiolabelling of biomolecules.[10–15] They reported that it was easily prepared as the organometallic aqua complex $[^{99m}Tc(OH_2)_3(CO)_3]^+$ directly from $[^{99m}TcO_4]^-$ in saline which is stable at room temperature even though exposed to air (Scheme 2).[10,11] They concluded that this new peptide labelling approach with the ^{99m}Tc(I) tricarbonyl precursor combines the highest possible specific activities with a minimal influence on the biological properties of the peptide, including receptor affinity and metabolism, and can be transferred to other peptides of choice.[12] Since synthesis of MAG$_3$ (**1**) requires synthetic procedures (Scheme 1), the development of a new ^{99m}Tc renal imaging agent using a small molecule such as cysteine will be of interest.

Cysteine contains three functional groups such as carboxyl, sulfhydryl, and amino group that are well known to be an efficient chelating moiety for ^{99m}Tc labelling. Most of ^{99m}Tc-cysteine complexes are synthesized under various reaction conditions as renal scintigraphic agents.[16–19] However, the ^{99m}Tc(I)

tricarbonyl cysteine as a renal scintigraphic agent has not been reported for present time.

In this paper, we describe the synthesis of ^{99m}Tc(I) tricarbonyl cysteine (**3**) and evaluation of its biological characteristics as a potential diagnostic radiopharmaceutical for renal function.

Results and discussion

^{99m}Tc(I) tricarbonyl precursor **2**, $[^{99m}Tc(OH_2)_3(CO)_3]^+$, was successfully prepared with 98% radiolabelling using a modification of the procedure described by Alberto *et al.* (Scheme 2).[10–12] The ^{99m}Tc(I) tricarbonyl cysteine (**3**) was prepared by reacting 1 ml of **2** with 0.1 ml of *l*-cysteine solution (10 mM in saline) for 30 min at a reaction temperature of 75°C (Scheme 2). The HPLC chromatograms of $^{99m}TcO_4^-$, ^{99m}Tc(I) tricarbonyl precursor **2** and ^{99m}Tc(I) tricarbonyl cysteine (**3**) showed that retention times of those species are 8.3, 4.8, and 16.5 min, respectively. The radiolabelling yield of **3** in the reaction mixture was determined by HPLC analysis and found to be 92%. The HPLC chromatogram of **3** in the reaction mixture is shown in Figure 1. The complex is stable (> 90%) for approximately 3 h. After this time, decomposition of the complex was observed. Paper electrophoresis investigations in aqueous solution confirmed the anionic charge of **3** in neutral solution. To get mass spectral data, $^{99/99m}$Tc(I) tricarbonyl cysteine was prepared by reacting 1 ml of $^{99/99m}$Tc(I) tricarbonyl precursor with 0.1 ml of *l*-cysteine solution (10 mM in saline). The complexes revealed the same retention times on HPLC column ($[^{99/99m}Tc(OH_2)_3(CO)_3]^+$:4.9 min; $^{99/99m}$Tc(I) tricarbonyl cysteine: 16.7 min) as the corresponding complexes **2** and **3**. Analysis of the mass spectrum of $^{99/99m}$Tc(I) tricarbonyl cysteine complex showed the corresponding molecular weight.

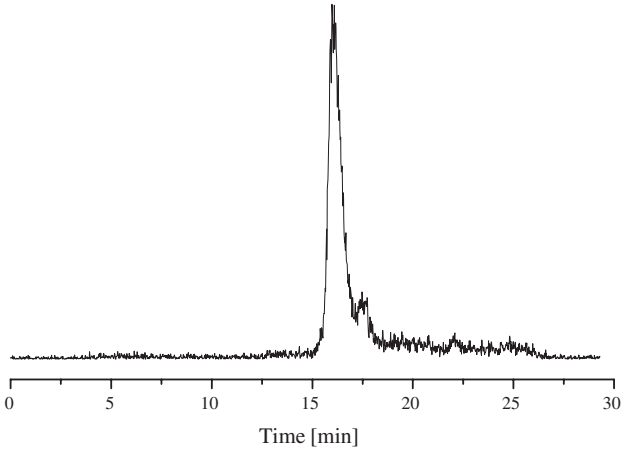


Figure 1. HPLC chromatogram of complex 3

Table 1. Plasma protein binding rate of ^{99m}Tc(I) tricarbonyl cysteine (3) in rabbits

	5 min	60 min
^{99m}Tc(I) tricarbonyl cysteine	69.20 ± 1.74	79.01 ± 0.27

Values are expressed as mean ± SD of plasma protein binding percentage for three rabbits.

Table 2. T_{max} and $T_{1/2}$ of ^{99m}Tc(I) tricarbonyl cysteine (3) and ^{99m}Tc-MAG$_3$ (1) renography in rabbits with or without probenecid pretreatment

	Control (min)		Probenecid (min)	
	T_{max}	$T_{1/2}$	T_{max}	$T_{1/2}$
^{99m}Tc(I) tricarbonyl cysteine	2.33 ± 0.56	4.30 ± 0.79	2.30 ± 0.17	17.03 ± 2.47**
^{99m}Tc-MAG$_3$	1.76 ± 0.66	2.57 ± 0.72	2.37 ± 0.71	3.57 ± 0.39*

Values are expressed as mean ± SD (min) for three rabbits. Probenecid dose was 20 mg/kg, and it was given 10 min prior to injection of test radiopharmaceutical **3** and **1**. Statistically different from probenecid non-treated control group (*:$0.01 < p < 0.05$, **$p < 0.001$).

The binding rates of **3** with plasma protein in rabbits at 5 and 60 min post injection are summarized in Table 1. The plasma protein binding rate of **3** was about 70 to 80%, and similar to that of ^{131}I-OIH (70%) but lower than that of ^{99m}Tc-MAG$_3$ (90%).[20]

The renograms of **3** with or without probenecid pretreatment are summarized in Table 2. The T_{max} of **3** in the kidney without probenecid pretreatment was 2.33 ± 0.56 min and the elapsed time of the T_{max} to $T_{1/2}$ was 4.30 ± 0.79 min. The renogram of **3** in rabbits showed that the activity of the kidney was rapidly reduced within 30 min post injection to the background level (Figure 1), which indicates that **3** was rapidly excreted via the kidney and radioactivity in kidney reached the background level within 30 min post injection. The $T_{1/2}$ of ^{99m}Tc tricarbonyl cysteine (4.30 ± 0.79 min) was higher than the one of ^{99m}Tc-MAG$_3$ (2.43 ± 0.70 min) but lower than that of ^{99m}Tc-ECD (7.00 ± 1.35 min) and ^{99m}Tc-EC (4.83 ± 0.67 min) which contain cysteine moiety[21] (Figure 2).

The static image of **3** at 5 min post injection showed high concentrated activity in the kidney. And in the 15, 30 min post injection images, most activity appeared in the bladder without a residue in other organs (Figure 3).

The excretion mechanism of **3** was confirmed by the probenecid blocking study. The T_{max} of **3** in the kidney with probenecid pretreatment was 2.30 ± 0.17 min and the elapsed time of T_{max} to $T_{1/2}$ was 17.03 ± 2.47 min. The T_{max} of **3** in probenecid-pretreated rabbits was similar to that of untreated ones. However, the $T_{1/2}$ of **3** in rabbits pretreated with probenecid was increased four-fold (409%) in comparison with that of untreated ones, which means the excretion of **3** was extremely affected by probenecid.

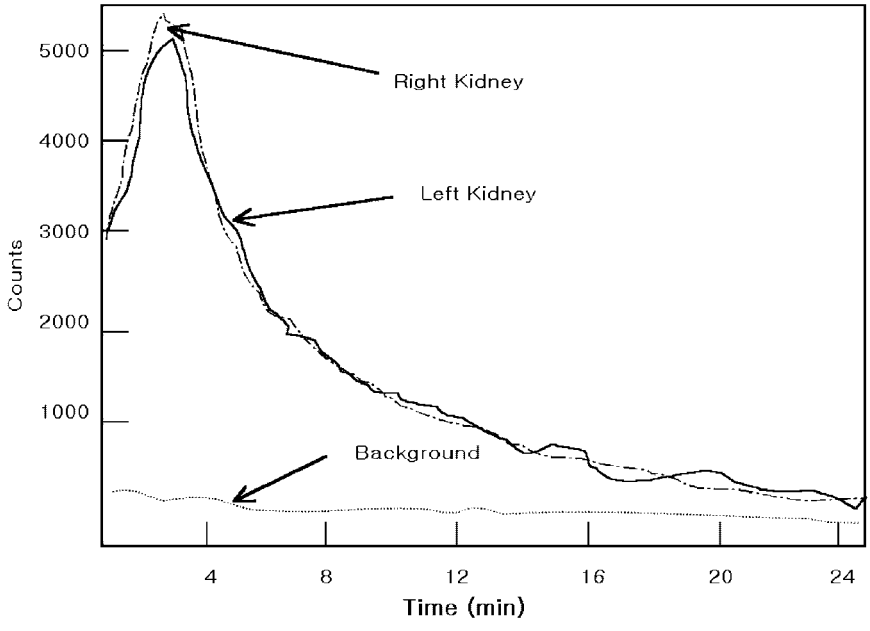


Figure 2. The renogram of ^{99m}Tc tricarbonyl cysteine (3) in New Zealand white rabbit for 24 min after injection

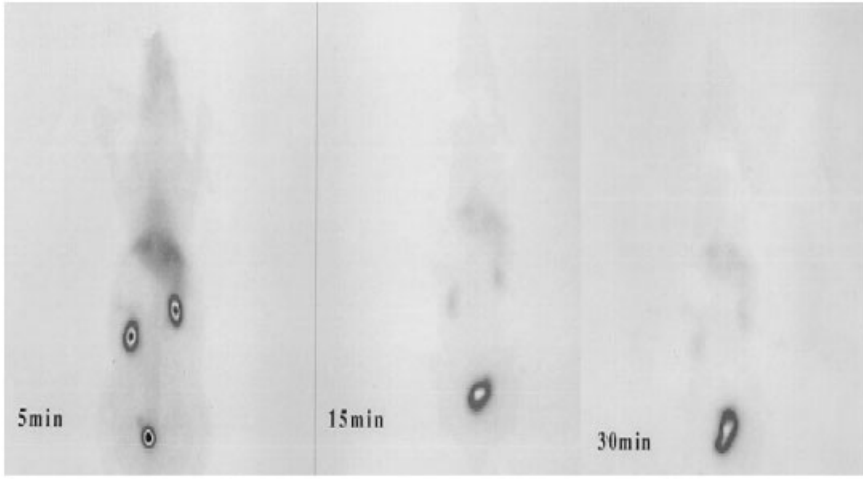


Figure 3. The images of a rabbit administered ^{99m}Tc-tricarbonyl cysteine (3) at 5, 15, and 30 min after injection

A probenecid blocking study of the ^{99m}Tc-MAG$_3$ (**1**) was additionally performed for comparison, and the results are summarized in Table 2. The T_{max} of ^{99m}Tc-MAG$_3$ (**1**) in rabbits ($n = 3$, each) with or without probenecid pretreatment was similar, but the elapsed time of T_{max} to $T_{1/2}$ in rabbits with

probenecid pretreatment was 4.30 ± 0.79 min, which is different from 2.57 ± 0.71 min with no probenecid pretreatment ($0.01 < p < 0.05$). The $T_{1/2}$ of **1** in rabbits pretreated with probenecid was increased to 145% of untreated ones. This study proved the excretion of **3** was more extremely affected by probenecid than **1**. Thus, we concluded that ^{99m}Tc(I) tricarbonyl cysteine (**3**) was principally excreted by active tubular transport, probably by the same carrier proteins that are responsible for the renal excretion of ^{99m}Tc-MAG_3 (**1**).[1,20]

In this study, it is shown that **3** exhibits desirable properties as a renal function diagnostic agent in terms of its rapid renal excretion and non-accumulation in other organs except the kidney. Furthermore, ^{99m}Tc tricarbonyl cysteine complex is considered to be excreted principally by the active tubular secretion through which commercially available ^{131}I-OIH and ^{99m}Tc-MAG_3 are secreted.

Experimental

Unless otherwise stated, all chemicals were of reagent grade. CO gas (99.5%) was obtained from Daehan Gas Co. (Seoul, Korea) and pre-filtered with an oxygen trap. $Na^{99m}TcO_4$ was obtained by solvent extraction from $^{99}MoO_3$, which was produced by irradiation of $^{98}MoO_3$ using the high-flux advanced neutron application reactor (HANARO) of the Korea Atomic Energy Research Institute (KAERI) or from a commercial ^{99}Mo/^{99m}Tc generator (Mallinckrodt). *l*-Cysteine and probenecid were purchased from Sigma Chemical Co. (St. Louis, USA). MAG_3 labelling kit was obtained from Mallinckrodt Medical, Inc. (St. Louis, USA). Potassium boranocarbonate was synthesized by using the procedure described by Alberto *et al.*[10] In this study, labelling yield was checked by high performance liquid chromatography (HPLC) equipped with a reversed phase μBondapak C-18 column (3.9×300 mm, Waters, USA) or Jupiter 5μ C-18 300A column (4.6×250 mm, Phenomenex, UK), applying a gradient system with 0.05M tetraethylammoniumphosphate (TEAP) buffer and 100% methanol. Anionic ^{99m}Tc species were characterized by paper electrophoresis (400 V, 20 V/cm, 45 min, paper chromatography (Whatman International Ltd, Maidstone, England), 0.1M phosphate buffer (50/50 v/v, pH 7.4)). All solvents for the mobile phase were of HPLC grade and pre-filtered with 0.2 μm pore size bottle filter. Experimental animals were purchased from Bio Genomics, Inc. (Seoul, Korea) that has technical cooperation with Charles River, experimental animals, and they were allowed free access to food and water. The orbiter gamma camera (Simens, Germany) with a low energy collimator was positioned. The energy gate was set to 140 keV and the window width to 10%. Dynamic image acquisition was performed for 30 min according to the dynamic procedure of the Microdelta System (Simens, Germany). The region

of interest (ROI) was drawn on the right and left kidney using 1–3 min composition image and the time-activity curve was obtained.

*Preparation of ^{99m}Tc tricarbonyl precursor, $[^{99m}Tc(OH_2)_3(CO)_3]^+$ (**2**)*

The ^{99m}Tc tricarbonyl precursor **2** was prepared using a modification of the procedure described by Alberto *et al.* (Scheme 2).[10,11]

Method A. A 10 ml-vial containing potassium boranocarbonate (5.9 mg, 0.043 mmol), sodium tetraborate (2.85 mg, 0.007 mmol), potassium sodium tartrate (10.4 mg, 0.037 mmol) and sodium carbonate (7.15 mg, 0.067 mmol) was capped with a rubber stopper and lyophilized. One ml of sodium pertechnetate ($Na^{99m}TcO_4$) with up to 3.7 GBq was added into the vial by a syringe and then heated to 90°C in a boiling water bath for 20 min. After rapid cooling down to room temperature, 0.15 ml of 0.1N HCl and 1.0 ml of phosphate buffered saline (0.05M phosphate buffer, pH 7.4) were added to neutralize **2**. Yield: >98% determined by means of HPLC and TLC.

Method B. A 10 ml vial containing Na_2CO_3 (8 mg, 0.076 mmol) and $NaBH_4$ (10 mg, 0.26 mmol) or tetrahydroborate exchange resin[22] (10–20 mg) was capped with a rubber stopper and then flushed with a stream of CO gas (99.5%) at room temperature for 30 min. Six ml of sodium pertechnetate ($Na^{99m}TcO_4$) with up to 3.7 GBq was added by a syringe and then heated to 75°C for 30 min under the bubbling of CO gas. After rapid cooling down to room temperature, 0.6 ml of phosphate buffered saline (0.05M phosphate buffer, pH 7.4) was added to neutralize **2**. Yield: >98% determined by means of HPLC and TLC.

*Preparation of $^{99m}Tc(I)$ tricarbonyl cysteine complex (**3**)*

One ml of ^{99m}Tc(I) tricarbonyl precursor **2** was added into 0.1 ml of *l*-cysteine solution (0.010M in saline) and the reaction mixture was heated 75°C for 30 min. After cooling it to room temperature, ^{99m}Tc(I) tricarbonyl cysteine (**3**) was characterized. Analytical data for yield: 92% ($R_t = 16.7$ min) determined by means of HPLC; stability: >90% for 3 h determined by means of HPLC; charge: a negative charge of the complex determined by means of paper eletrophoresis.

Preparation of $^{99/99m}Tc(I)$ tricarbonyl cysteine complex for mass spectrometry

$^{99/99m}$Tc(I) tricarbonyl precursor was prepared using method A except for 20 µl of 0.05M sodium pertechnetate ($Na^{99}TcO_4$)(1×10^{-7} mol) and a solution of 0.5 ml of sodium pertechnate ($Na^{99m}TcO_4$)(120 MBq) in 1.5 ml of physiological saline instead of 6 ml of sodium pertechnate ($Na^{99m}TcO_4$) with up to 3.7 GBq. Preparation of $^{99/99m}$Tc(I) tricarbonyl cysteine was prepared as

described previously except for $^{99/99m}$Tc(I) tricarbonyl precursor instead of ^{99m}Tc(I) tricarbonyl precursor. Analytical data for MS(ES): m/z 301.97 [M], 301.05 [M-1], 101.91, 103.08.

Plasma protein binding test

The extent of plasma protein binding was measured in triplicate. Three male New Zealand white rabbits (3045.8 $\pm$ 458.4 g) were injected with 3.7 MBq of **3** per head through the left ear vein. Blood samples were collected at 5 and 60 min after administration. The fresh heparinized whole blood was centrifuged using a swing-out rotor at 4120 g for 10 min. The count rates of plasma was measured in a well-type NaI(Tl) scintillation detector. For the determination of plasma protein binding, the plasma protein was precipitated by a method using trichloroacetic acid.[23] The plasma protein is precipitated by adding 0.2 ml of 50% trichloroacetic acid to 1 ml of plasma. The supernatant and the precipitate were separated by centrifugation at 13 000 g for 10 min. The activities of both phases were measured separately in a well-type NaI(Tl) scintillation detector.

The percentage of protein binding was determined by the following equation:

$$\text{Plasma protein bound (\%)} = \frac{\text{Plasma (cpm)} - \text{supernatant (cpm)}}{\text{Plasma (cpm)}} \times 100$$

Dynamic imaging studies and probenecid blocking studies

Dynamic imaging studies were performed with 6-week-old male New Zealand white rabbits (1987.4 $\pm$ 45.7 g, $n = 3$) which were anesthetized with 25 mg/kg of ketamine (Ketamine 50 inj., Yuhan Co., Korea) and 6 mg/kg of xylazine (Rompun inj., Bayer-Korea, Korea). The effect of probenecid was studied by the injection of probenecid (20 mg/kg) into the right ear vein of rabbits (2004.6 $\pm$ 72.3 g, $n = 3$) at 10 min prior to the injection of either **3** or **1**. The rabbits were placed in a posterior position and injected with 37 MBq of test complex per head (37 MBq, 1.0 mCi) via the left ear vein. In addition, the static images of 3 were obtained at 5, 10, 30 min post injection with a microdot imager (Simens, Germany). Data were expressed as mean $\pm$ SD. Statistical analyses were performed by the Student's t-test. Differences were considered to be significant at $p < 0.05$.

Conclusion

A novel radiosynthesis of ^{99m}Tc(I) tricarbonyl cysteine (**3**) for studying dynamic kidney function has been developed. Biological evaluation of **3** showed an excellent renal excretion property. The ^{99m}Tc(I) tricarbonyl cysteine is expected as a potential renal function diagnostic. Currently, we

are in the process of using this complex **3** for further comparison with ^{99m}Tc-MAG_3 (**1**) and evaluation in higher mammals before utilization for clinical use, the results of which will be reported in due course.

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