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Pharmacokinetic Alteration of ^{99m}Tc-MAG3 using Serum Protein Binding Displacement Method ☆

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ABSTRACT

Introduction: When a radiopharmaceutical is simultaneously administered with a medicine that has high affinity for the same plasma protein, the radiopharmaceutical is released at higher concentrations in blood, leading to enhanced transfer into target tissues. This is known as the serum protein binding displacement method. In this study, we investigated the pharmacokinetic alteration of technetium-99m-labeled mercaptoacetylglycylglycylglycine (^{99m}Tc-MAG3) using the serum protein binding displacement method.
Methods: Rat and human serum protein binding rates of ^{99m}Tc-MAG3 were measured by ultrafiltration with or without displacers of human serum albumin (HSA) binding sites I and II (200 μM and 400 μM loading). Male Wistar rats were injected with ^{99m}Tc-MAG3 (740 kBq/0.3 mL saline) via the tail vein, and biodistribution was assessed at 2, 5, 10 and 15 min. Dynamic whole-body images were obtained for ^{99m}Tc-MAG3 (11.1 MBq/0.3 mL saline)-injected rats, with or without HSA displacers.
Results: ^{99m}Tc-MAG3 strongly bound to HSA (87.37%±2.13%). Using HSA site I displacers, the free fraction of ^{99m}Tc-MAG3 increased significantly (1.20 to 1.47 times) when compared with controls. For biodistribution and imaging, rapid blood clearance was observed with bucolome (BCL) loading, which is an HSA site I displacer. With BCL loading, peak times for rat renograms were respectively shifted from 240 s to 110 s, and from 170 s to 120 s.
Conclusions: We found that ^{99m}Tc-MAG3 bound to the HSA binding site I. It was confirmed that pharmacokinetic distribution of ^{99m}Tc-MAG3 is altered by presence of BCL, which leads to increases in the free fraction of ^{99m}Tc-MAG3, and BCL produced rapid blood clearance and fast peak times on rat renograms. The serum protein binding displacement method using ^{99m}Tc-MAG3 and BCL, a safe displacer for humans, may be applicable to clinical study and lead to better diagnostic images with shorter waiting times and lower radiation doses for patients.

1. Introduction

Most radiopharmaceuticals have a weak or moderate affinity for albumin and acid glycoproteins, often depending on their lipophilicity [1]. When the affinity of the radiopharmaceuticals for blood components increases, the radiopharmaceuticals do not readily transfer into target tissue. Changes in the pharmacokinetics and distribution of radiopharmaceuticals would thus be expected to enhance the transfer into target tissues [2].

In the distribution of medicines for medical diagnosis or treatment, medicines move along with systemic blood circulation, and are transferred to the intravascular, interstitial and intracellular spaces by diffusion and transportation of unbound medicine (free fraction). The free fraction of medicines arriving at target tissues is considered to provide pharmacological activity [3–5]. Thus, the concentration of the pharmacological agent is typically determined by the level of protein binding.

When one medicine is simultaneously administered with a second medicine that has high affinity for the same plasma protein, the first medicine is released at higher concentrations and produces a stronger pharmacological action. This method is known as the serum protein

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binding displacement method. Our study group has already reported the use of the serum protein binding displacement method with ^{123}I-*N*-isopropyl-*p*-iodoamphetamine (^{123}I-IMP). We found that the pharmacokinetics of ^{123}I-IMP were markedly changed, and that the brain accumulation of ^{123}I-IMP was improved in a Japanese monkey study [6,7].

Technetium-99m-labeled mercaptoacetylglycylglycylglycine (^{99m}Tc-MAG3) is widely used in renal scintigraphy and has very high affinity (90% binding) for plasma protein at typical clinical doses [8]. However, the binding site of ^{99m}Tc-MAG3 has not been identified. In this study, we determined the binding site of the ^{99m}Tc-MAG3 and applied the protein binding displacement method in order to alter the pharmacokinetics of ^{99m}Tc-MAG3. In an *in vitro* study, the serum protein binding site of ^{99m}Tc-MAG3 was identified using ultrafiltration analysis with rat and human serum. Subsequently, the protein binding displacement method was applied to a rat study in order to explore the possibility of clinical application.

2. Materials and methods

2.1. Materials

Reagent-grade chemicals, HSA (essentially fatty acid-free albumin), AGP and human immunoglobulin G (IgG) were acquired from Sigma Chemical (St. Louis, MO). Valproic acid and oleic acid sodium salts were obtained from Tokyo Chemical Industry (Tokyo, Japan), and octanoic acid sodium salt was purchased from Katayama Chemical (Osaka, Japan). Bucolome (BCL), Paramidin (Grelan Pharmaceutical, Tokyo, Japan), potassium warfarin (Eisai, Tokyo, Japan), cefazolin sodium (Fujisawa Pharmaceutical, Osaka, Japan) and ibuprofen (Kaken Pharmaceutical Co., Ltd., Tokyo, Japan) were kind gifts. ^{99m}Tc-MAG3 (Daiichi Radioisotope Laboratories, Tokyo, Japan) was used as a radiopharmaceutical.

2.2. Measurement of protein binding rates of ^{99m}Tc-MAG3

Serum protein binding of ^{99m}Tc-MAG3 was evaluated by ultrafiltration (Ultracent-10; Tosoh, Tokyo, Japan) [9–11]. Mixtures (0.9 mL) of ^{99m}Tc-MAG3 (740 kBq in 20 μL saline) and serum (or HSA, AGP or IgG solution) were centrifuged at 3000 rpm for 10 min at room temperature (Model 5900; Kubota, Tokyo, Japan). Radioactivities (counts/20 μL) of the initial solutions and filtrates were measured using a well-type scintillation counter (ARC-360; Aloka, Tokyo, Japan), and their protein binding and free fraction rates were determined. With regard to bufferized solvent, 0.067 M phosphate buffer (adjusted to pH 7.4) was used. Human and rat sera were respectively adjusted at an albumin concentration of 500 μM and 375 μM with phosphate buffer. For purified human serum protein, HSA (740 μM, 4.9 g in 100 mL phosphate buffer), AGP (17 μM, 75 mg in 100 mL phosphate buffer) and IgG (62 μM, 1.0 g in 100 mL phosphate buffer) solutions were prepared at normal human serum concentrations. Effects of displacers of albumin binding were also examined in human and rat sera.

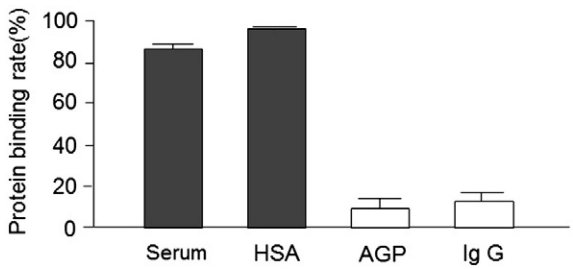

Fig. 1. Binding rates of ^{99m}Tc-MAG3 with human serum protein. ^{99m}Tc-MAG3 bound strongly to HSA.

Table 1
Displacement effects of inhibitors on ^{99m}Tc-MAG3 binding to plasma protein.

	Site-specific inhibitor	HSA		RSA	
		^{99m}Tc-MAG3 free fraction (%)		^{99m}Tc-MAG3 free fraction (%)	
Concentration		200 μM	400 μM	200 μM	400 μM
Site I	Control	10.20		24.75	
	BCL	12.23	13.74	32.76	43.85
	Valproic acid	11.98	13.02	28.48	29.30
	Warfarin	11.50	13.57	33.57	43.28
	Cefazolin	11.13	14.76	28.58	33.52
Site II	Ibuprofen	10.18	10.53	28.48	33.04
	Octanoic acid	9.60	9.86	N/A	N/A
	Oleic acid	8.74	9.44	N/A	N/A

N/A: not analyzed.

BCL, warfarin, valproic acid and cefazolin were used as displacers of HSA binding site I, while ibuprofen, octanoate and oleate were used as displacers of HSA binding site II. These were added to serum samples at the concentrations required for sufficient competitive inhibition (200 μM) and at double doses (400 μM) in order to confirm concentration dependence. Warfarin and octanoate were added to serum samples immediately prior to injection of radiotracer in order to avoid deactivation in serum samples. Binding rate to protein was calculated using the following formula:

$$100-(100\times[F]/[S])[\%]$$

where, [S] is total radioactivity of the mixture consisting of plasma protein and radiopharmaceutical, and [F] is radioactivity of the filtrate obtained by centrifuging the mixture.

2.3. Biodistribution studies of ^{99m}Tc-MAG3 in rats

Animal studies were approved by the Animal Care Committee at the University of Miyazaki, and were conducted in accordance with the international standards for animal welfare and institutional guidelines. To assess the competitive displacement of protein binding with BCL *in vivo*, five male Wistar rats (age, 6 weeks) were administered ^{99m}Tc-MAG3 (740 kBq) in 0.1 mL of saline by intravenous bolus injection via the tail vein. Rats were sacrificed at 2, 5, 10 and 15 min after injection of radiotracer by cervical dislocation under deep ether anesthesia, and blood and organs of interest were excised. After measuring organ weights, radioactivities were determined using a scintillation counter. Accumulation ratios (% dose/organ and % dose/g of tissue) were determined after decay correction. *In vivo* displacement studies on serum protein binding of radiopharmaceuticals were performed with BCL pretreatment (20 mg/kg body weight, i.v., 10 min before injection of radiotracer).

2.4. Scintigraphic studies of ^{99m}Tc-MAG3 in rats

Wistar rats weighing 400 g were administered ^{99m}Tc-MAG3 (11.1 MBq) in 0.3 mL of saline via the tail vein. Dynamic whole-body images (10 s/frame for 20 min) were obtained with a scintillation camera (Prism 3000; Picker International, Cleveland, OH). Time–activity curves were obtained from the main tissue region using a supercomputer (Odyssey; Picker International), and regions of interest (ROIs) in the kidneys were examined on renograms. In the same rats, scintigraphic studies with displacer treatment were also carried out. BCL (20 mg/kg body weight) was loaded by intravenous infusion for 10 min using a syringe pump (A-99; Razel Scientific Instruments, Stamford, CT) at 5–15 min before ^{99m}Tc-MAG3 injection.

Table 2
Biodistribution of ^{99m}Tc-MAG3 in rats at 10 min after administration with or without BCL (20 mg/kg) loading.

Organ	Control group(% dose/g tissue)	BCL-loading group(% dose/g tissue)
Blood	0.317±0.073	0.047±0.044*
Brain	0.010±0.001	0.001±0.001*
Spleen	0.052±0.008	0.009±0.008*
Pancreas	0.046±0.001	0.006±0.007*
Stomach	0.024±0.024	0.040±0.036*
Liver	0.151±0.001	0.033±0.026*
Kidney	6.191±0.187	0.651±0.324*
Heart	0.101±0.016	0.014±0.010*
Lung	0.195±0.030	0.041±0.037*

* : $P<.01$ vs. controls.

2.5. Statistical analysis

All results for *in vitro* assays and rat studies represent the average of least three experiments and are expressed as means±SD except protein binding rate. Data were analyzed by ANOVA *t*-test, and $P<.05$ was considered to indicate significance.

3. Results

As shown in Fig. 1, strong binding of ^{99m}Tc-MAG3 with HSA was observed. Binding levels of ^{99m}Tc-MAG3 with HSA were almost equal to those of serum. ^{99m}Tc-MAG3 scarcely binds with other proteins, such as AGP and IgG. The effects of several displacers on serum protein binding of ^{99m}Tc-MAG3 were then examined (Table 1). In the presence of HSA site I displacers, such as BCL, warfarin, valproic acid and cefazolin, the free fractions of ^{99m}Tc-MAG3 were significantly elevated (1.20 to 1.47 times), as compared to controls. Displacement rates increased markedly with concentration of HSA and RSA. On the other hand, there were no significant differences in the presence of ibuprofen, octanoate or oleate. Therefore, ^{99m}Tc-MAG3 is considered to combine specifically with binding site I on HSA.

In the rat serum study, BCL and warfarin significantly interacted with ^{99m}Tc-MAG3 binding to serum. BCL was found to be a better displacer than warfarin for clinical application, thereby allowing its blood concentrations to be maintained at high levels and maximizing its protein binding displacement effect. Table 2 shows the effects of BCL treatment (20 mg/kg body weight) on ^{99m}Tc-MAG3 distribution at 10 min after tracer injection. Accumulation with BCL loading was significantly lower in all organs. In particular, accumulation in the blood was about 15%, and that in the kidney was 11% of control levels. Thus, the presence of BCL, which is a displacer of binding site I can alter the pharmacokinetics of ^{99m}Tc-MAG3. ^{99m}Tc-MAG3 distribution at various time intervals with and without BCL is shown in Fig. 2. The dose of BCL was 20 mg/kg body weight, and this is a therapeutic dose in humans. Without BCL loading, moderate blood clearance was observed, and peak time of accumulation in kidney was seen at 5 min after ^{99m}Tc-MAG3 injection. On the other hand, with BCL loading, rapid blood clearance was observed. Peak time of accumulation in kidney shifted to within 2 min after tracer injection. Subsequently, radioactivity cleared rapidly from kidneys. Based on these results, BCL treatment increased free ^{99m}Tc-MAG3 *in vivo*, and rates of kidney accumulation and blood clearance increased as a result. Indeed, the free fraction of ^{99m}Tc-MAG3 was substantially higher than in the control group on ultrafiltration analysis of rat serum in the BCL-treated group after ^{99m}Tc-MAG3 injection.

On ^{99m}Tc-MAG3 scintigraphy, time–activity curves peaked at an early time point after ^{99m}Tc-MAG3 injection, and then decreased gradually (Fig. 3). Table 3 shows the peak time and up-slope on the renograms of ^{99m}Tc-MAG3 in rats. With BCL loading, the renogram pattern changed; in BCL-loaded rats, peak times shifted to 110 s and 120 s from 240 s and 170 s in controls, respectively.

4. Discussion

In the present study, we applied the serum protein binding displacement method reported in our previous study to ^{99m}Tc-MAG3 scintigraphy. ^{99m}Tc-MAG3 is a radiopharmaceutical for *in vivo* imaging and is widely used for the purpose of diagnosing renal diseases [12–17], as it accumulates in the kidneys. We believed that it would be very useful to regulate the pharmacokinetics and biodistribution of this radiopharmaceutical.

Generally speaking, competitive inhibition between the radiopharmaceutical and displacer for plasma protein binding requires a displacer concentration of 1/3 that of protein concentration to obtain a sufficient effect. For this reason, we set displacer concentration at 200 μM and 400 μM in order to confirm concentration dependence. In the *in vitro* study, the free fraction of ^{99m}Tc-MAG3 was significantly higher than in the control sample after administration of a site I-specific agent (e.g., BCL, valproic acid, warfarin or cefazolin). On the other hand, site-II specific agents (e.g., ibuprofen, octanoic acid or sodium oleate) did not increase the free fraction of ^{99m}Tc-MAG3. Similarly, in rat serum, the free fraction of ^{99m}Tc-MAG3 increased with administration of site I-specific agents. With the rise in displacement rates with increasing concentrations of HSA and RSA, a clear concentration dependence of displacement rates was confirmed. For this reason, the binding site of ^{99m}Tc-MAG3 is considered to be site I on HSA, and we selected BCL as a site I-specific agent. BCL is a non-steroidal anti-inflammatory medicine that can be safely administered in large doses (600–1200 mg/day). It has been reported that BCL does not affect urinary enzyme activities and that nephrotoxicity by this agent is very low [18–21]. In addition, warfarin–BCL combination therapy has been used to treat patients with nephritic syndrome [22].

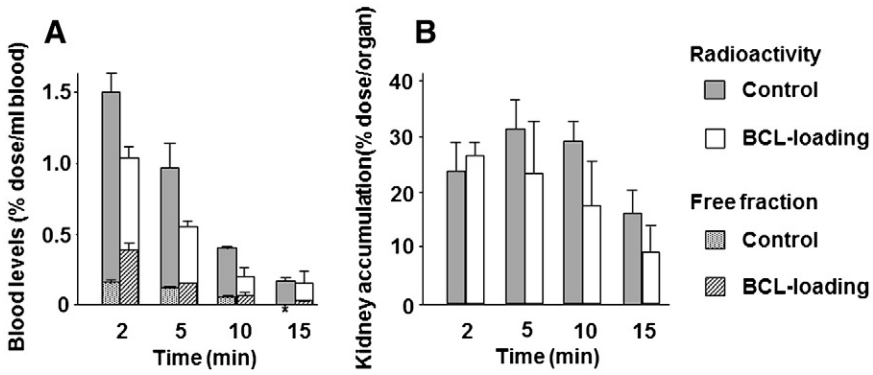

Fig. 2. Biodistribution of ^{99m}Tc-MAG3 (740 kBq) with/without BCL in rats. BCL (20 mg/kg) was loaded 10 min before injection of ^{99m}Tc-MAG3. With BCL loading, blood levels were lower (A), and peak time for kidney accumulation was more rapid (B) than in the control group. *; not detected.

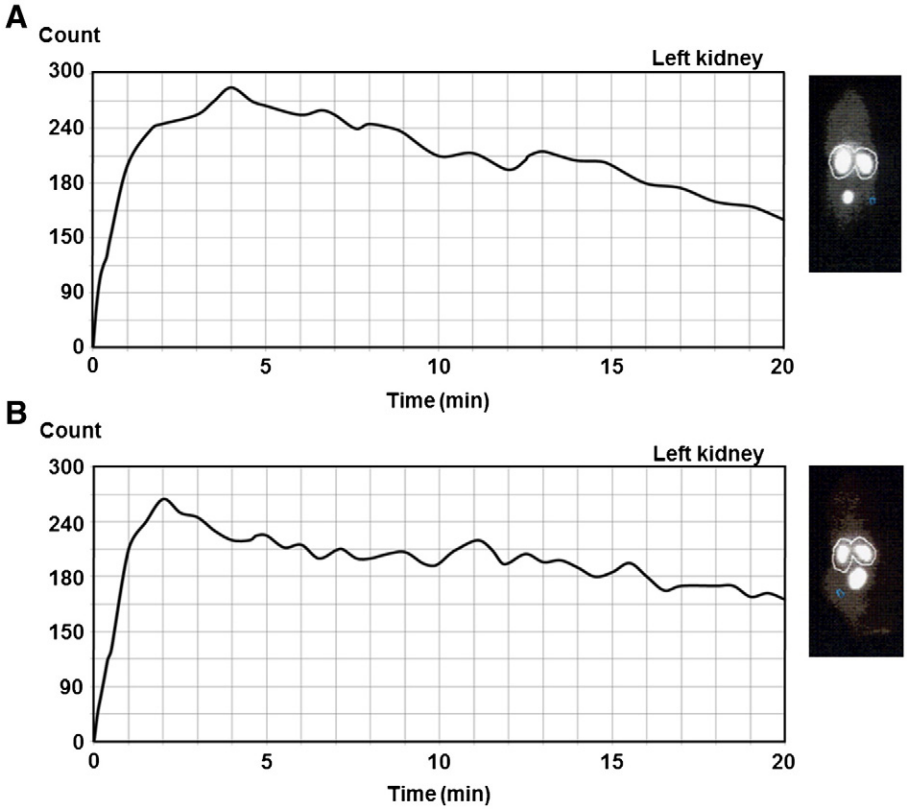

Fig. 3. Renograms of ^{99m}Tc-MAG3 scintigraphy in control rat (A), and in BCL-treated rat (B). BCL (20 mg/kg) was loaded at 10 min before injection of ^{99m}Tc-MAG3. Peak time of the renogram shifted to 110 s (B) from 240 s (A).

From the results of the biodistribution study in BCL-treated rats, blood clearance was clearly accelerated in the BCL-treated group, and the free fraction of ^{99m}Tc-MAG3 *in vivo* was markedly higher in the test group. In the BCL-treated group, accumulation of ^{99m}Tc-MAG3 in the kidney increased rapidly to a peak value after administration, and then decreased and was eliminated quickly, in contrast to the control group without BCL treatment. ^{99m}Tc-MAG3 cleared rapidly from the kidney, which is the target organ of this radiotracer after BCL treatment. Therefore, BCL induced the rapid elimination of radioactivity, and enhanced the clearance from the blood and organs.

On *in vivo* whole body dynamic imaging of rats, we confirmed that the radioactivity increased gradually at the initial stages after administration until the peak time of 240 s in the control group; however, in the BCL-treated group, radioactivity rose quickly and the peak time was shortened to 120 s. Renal function is typically analyzed by determining the peak time in renograms, and the slope of the straight line in linear regression. By inhibiting the binding ability of ^{99m}Tc-MAG3 to site I on HSA, renograms were ideally approximated to simple curves by inhibiting the binding ability of ^{99m}Tc-MAG3 to site I on HSA using BCL. These results suggest that the pharmacokinetics of ^{99m}Tc-MAG3 and subsequent imaging can be altered in clinical settings. The free fraction rate was equal to the *in vitro* human serum data. Therefore, in the rat study, rapid accumulation in the kidney and accelerated clearance were achieved due to the increase in free ^{99m}Tc-MAG3 by BCL loading *in vivo*.

As described above, the protein binding displacement method could be readily applied to human studies under similar conditions using BCL. Displacement of radiopharmaceutical binding to serum proteins may lead to better diagnostic images with shorter waiting times and lower radiation doses for patients.

Table 3
Peak times and up-slopes of renograms in rats.

	Peak time (s)	Up-slope (counts/s)
Rat 1		
Control	240	1.166
BCL-treated	110	2.208
Rat 2		
Control	170	0.941
BCL-treated	120	2.000

5. Conclusion

We confirmed that ^{99m}Tc-MAG3 binds to HSA binding site I, and applied the serum protein binding displacement method using co-administration of BCL with binding affinity for HSA site I in a rat study. Therefore, the pharmacokinetics of ^{99m}Tc-MAG3 were altered by BCL, and BCL produced rapid blood clearance and peak times on rat renograms. There is a good possibility that the serum protein binding displacement method of ^{99m}Tc-MAG3 and BCL, a safe displacer for humans, can be applied to clinical study.

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