Preclinical Evaluation of $^{99m}Tc(CO)_3$-Aspartic-*N*-Monoacetic Acid, a Renal Radiotracer with Pharmacokinetic Properties Comparable to ^{131}I-*o*-Iodohippurate

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In an ongoing effort to develop a renal tracer with pharmacokinetic properties comparable to *p*-aminohippurate and superior to those of both ^{99m}Tc-mercaptoacetyltriglycine and ^{131}I-*o*-iodohippurate (^{131}I-OIH), we evaluated a new renal tricarbonyl radiotracer based on the aspartic-*N*-monoacetic acid (ASMA) ligand, $^{99m}Tc(CO)_3$(ASMA). The ASMA ligand features 2 carboxyl groups and an amine function for the coordination of the $\{^{99m}Tc(CO)_3\}^+$ core as well as a dangling carboxylate to facilitate rapid renal clearance. **Methods:** *rac*-ASMA and L-ASMA were labeled with a ^{99m}Tc-tricarbonyl precursor, and radiochemical purity of the labeled products was determined by high-performance liquid chromatography. Using ^{131}I-OIH as an internal control, we evaluated biodistribution in normal rats with $^{99m}Tc(CO)_3$(ASMA) isomers and in rats with renal pedicle ligation with $^{99m}Tc(CO)_3$(*rac*-ASMA). Clearance studies were conducted in 4 additional rats. In vitro radiotracer stability was determined in phosphate-buffered saline, pH 7.4, and in challenge studies with cysteine and histidine. $^{99m}Tc(CO)_3$(ASMA) metabolites in urine were analyzed by high-performance liquid chromatography. **Results:** Both $^{99m}Tc(CO)_3$(ASMA) preparations had greater than 99% radiochemical purity and were stable in phosphate-buffered saline, pH 7.4, for 24 h. Challenge studies on both revealed no significant displacement of the ligand. In normal rats, the percentage injected dose in urine at 10 and 60 min for both preparations averaged, respectively, 103% and 106% that of ^{131}I-OIH. The renal clearances of $^{99m}Tc(CO)_3$(*rac*-ASMA) and ^{131}I-OIH were comparable ($P = 0.48$). The tracer was excreted unchanged in the urine, proving its in vivo stability. In pedicle-ligated rats, $^{99m}Tc(CO)_3$(*rac*-ASMA) had less excretion into the bowel ($P < 0.05$) than did ^{131}I-OIH and was better retained in the blood ($P < 0.05$). **Conclusion:** Both $^{99m}Tc(CO)_3$(ASMA) complexes have pharmacokinetic properties in rats comparable to or superior to those of ^{131}I-OIH, and human studies are warranted for their further evaluation.

Key Words: ^{99m}Tc; tricarbonyl; kidney; renal radiopharmaceuticals; ^{131}I-*ortho*-iodohippurate; $^{99m}Tc(CO)_3$(ASMA)

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Chronic kidney disease has emerged as a serious health problem worldwide. In the United States alone, an estimated 13% of the adult population has chronic kidney disease (*1*) and this number is increasing yearly because of the rising prevalence of diabetes, hypertension, obesity, and cardiovascular disease superimposed on an aging population (*2–5*). Moreover, the incidence of chronic kidney disease in children has also steadily increased during the past 2 decades (*6*). Early detection and diagnosis can lead to treatment that will reduce the risk of kidney failure.

The classification of chronic kidney disease is currently based on the estimated glomerular filtration rate using a 4-variable algorithm that attempts to compensate for the fact that serum creatinine may not become elevated until more than 50% of the renal function has been lost (*7*). Radionuclide imaging may detect unsuspected renal disease in patients with a normal serum creatinine level and continues to have an important role in evaluating suspected obstruction and renovascular hypertension and in monitoring renal function through measurements of glomerular filtration and effective renal plasma flow. The gold standard for the determination of effective renal plasma flow is *p*-aminohippurate. In practice, however, measurement of *p*-aminohippurate clearance requires a constant plasma infusion and time-consuming analysis, limiting its use in a clinical setting. ^{131}I-*o*-iodohippurate (^{131}I-OIH) is a radioactive standard used as an imaging agent and as a tracer to measure effective renal plasma flow, although its clearance is still only 85%–90% that of *p*-aminohippurate (*8*). Commercial production of ^{131}I-OIH has been discontinued in many countries because of the suboptimal characteristics of ^{131}I (maximum energy, 364 keV) and the fact that its β-emission can result in a high radiation dose, particularly to the kidneys and thyroid in patients with impaired renal function (*9*).

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Simple and rapid labeling methods with the ^{99m}Tc isotope have been developed for clinical applications (*10,11*) because of the highly favorable physical characteristics of ^{99m}Tc (half-life, 6 h; maximum energy, 140 keV) and its easy availability and low cost. Although several ^{99m}Tc complexes have been tested as potential alternatives for ^{131}I-OIH (*12–21*), ^{99m}Tc-mercaptoacetyltriglycine was shown to have favorable properties, has become commercially available, and is the most widely used ^{99m}Tc renal tracer in the United States (*22,23*). Nevertheless, ^{99m}Tc-mercaptoacetyltriglycine is not an ideal replacement for ^{131}I-OIH because its clearance is only 50%–60% that of ^{131}I-OIH and it does not provide a direct measurement of effective renal plasma flow. Our recent studies showed that a new ^{99m}Tc renal agent based on the nitrilotriacetic acid (NTA) ligand (Fig. 1), ^{99m}Tc(CO)$_3$(NTA), had pharmacokinetics essentially identical to those that ^{131}I-OIH had in rats (*24*) and subjects with normal renal function (*21*), suggesting that an anionic renal tracer with a $\{^{99m}Tc(CO)_3\}^+$ core and an aminopolycarboxylate chelate is capable of providing a measurement of effective renal plasma flow in humans equivalent to that of ^{131}I-OIH. However, the promising biologic properties of ^{99m}Tc(CO)$_3$(NTA) still need to be confirmed in patients with impaired renal function. Even if ^{99m}Tc(CO)$_3$(NTA) proves to be equivalent to ^{131}I-OIH in patients with renal failure, ^{99m}Tc(CO)$_3$(NTA) clearance is still likely to be inferior to that of *p*-aminohippurate because the clearance of ^{131}I-OIH is only 84% that of *p*-aminohippurate (*25*).

Our goal was to assess a new ^{99m}Tc(CO)$_3$(NTA) isomer, ^{99m}Tc(CO)$_3$(aspartic-*N*-monoacetic acid) (ASMA; Fig. 1), in an animal model to determine whether a change in ligand design could lead to a renal tracer with improved pharmacokinetic properties.

MATERIALS AND METHODS

General

The ASMA ligand was synthesized as previously described as a racemic mixture of D- and L-isomers (*rac*-ASMA) (*26*) and as the enantiomerically pure L-isomer (L-ASMA) (*27*). ^{99m}Tc-pertechnetate ($Na^{99m}TcO_4$) was eluted from a ^{99}Mo/^{99m}Tc generator (Lantheus Medical Imaging) with 0.9% saline. IsoLink vials were obtained as a gift from Covidien, and $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared according to the manufacturer's insert. High-performance liquid chromatography (HPLC) of the ^{99m}Tc tracers was performed on a System Gold Nouveau apparatus (Beckman) equipped with a model 170 radiometric detector, a model 166 ultraviolet–visible light detector, and a C18 RP Ultrasphere octyldecyl silane column (5-μm, 4.6 × 250 mm; Beckman). The flow rate of the mobile phase was 1 mL/min. The mobile phase consisted of aqueous 0.05 M triethylammonium phosphate buffer, pH 2.5 (solvent A), and methanol (solvent B), and the gradient method used was the same as reported previously (*28*). Tissue and organ radioactivity was measured using an automated 2480 Wizard 2 γ-counter (Perkin Elmer) with a 7.62-cm (3-in) NaI(Tl) detector. All animal experiments followed the principles of laboratory animal care and were approved by the Institutional Animal Care and Use Committee of Emory University. Re(CO)$_3$(ASMA) was used as a nonradioactive analog of ^{99m}Tc(CO)$_3$(ASMA), and its synthesis and characterization will be published elsewhere. ^{99m}Tc(CO)$_3$(ASMA) is used as a general reference to the complex, which may be a mixture of forms, whereas a specific form is designated as, for example, ^{99m}Tc(CO)$_3$(L-ASMA).

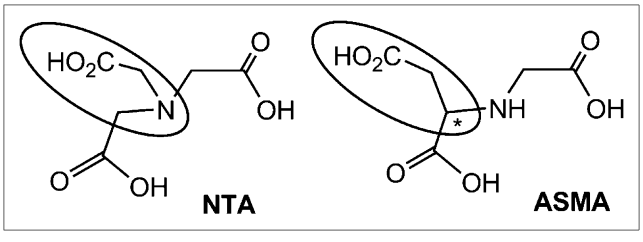

FIGURE 1. Structure of NTA and ASMA ligands. Ellipse encloses dangling carboxyl group, and asterisk indicates asymmetric carbon.

^{99m}Tc Radiolabeling

Both *rac*-ASMA and L-ASMA were labeled in a similar manner to form their ^{99m}Tc complexes. The ^{99m}Tc-tricarbonyl precursor, $[^{99m}Tc(CO)_3(H_2O)_3]^+$, was prepared fresh daily by adding 1.0 mL of a $Na^{99m}TcO_4$ saline solution to an IsoLink kit and heating at 100°C for 20 min. After neutralization with 0.12 mL of 1 M HCl, 0.5 mL of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor was added to a sealed vial containing approximately 0.2 mg of ASMA in 0.2 mL of water. The pH of the solution was adjusted to about 7 with 1 M NaOH, and the mixture was heated at 70°C for 30 min and then was cooled to room temperature. ^{99m}Tc(CO)$_3$(ASMA) was then purified by HPLC. The 2 diastereomers of ^{99m}Tc(CO)$_3$(ASMA) formed during the labeling process could not be separated by HPLC, and the tracer was collected in a single fraction and was further evaluated as a mixture of products. The aqueous solution of ^{99m}Tc(CO)$_3$(ASMA) collected was diluted in a physiologic phosphate buffer at pH 7.4 to obtain a concentration of 3.7 MBq/mL for in vivo experiments. The sample of the isolated ^{99m}Tc complex was mixed with the aqueous solution of its rhenium analog, Re(CO)$_3$(ASMA), and the mixture was analyzed by HPLC to confirm the identity of the ^{99m}Tc tracer.

In Vitro Stability

A solution of ^{99m}Tc(CO)$_3$(ASMA) was buffered in a physiologic phosphate buffer at pH 7.4 and evaluated by HPLC for up to 24 h to assess complex integrity. In addition, the isolated ^{99m}Tc(CO)$_3$(ASMA) complex (0.1 mL in the buffered solution) was incubated with 0.1 M solutions of histidine and cysteine (0.9 mL), respectively, at 37°C, and aliquots of the incubation mixture were analyzed by HPLC at 2 and 4 h to determine the degree of decomposition.

Biodistribution Studies

^{99m}Tc(CO)$_3$(ASMA) was evaluated in 2 experimental groups of rats (Sprague–Dawley, 203–350 g each; Charles River). Rats in both groups were anesthetized with intramuscularly injected ketamine–xylazine (2 mg/kg of body weight), with additional supplemental anesthetic used as needed. In the first group of 8 normal rats (group A), the bladder was catheterized by use of heat-flared polyethylene-50 tubing (Becton, Dickinson and Co.) for urine collection. The second group of 4 rats (group B) was prepared to produce a model of renal failure. In that group, the abdomen was opened by a midline incision and both renal pedicles were identified and ligated just before radiotracer administration; thus, no urine was collected.

Each rat was injected via a tail vein with 0.2 mL of a solution containing $^{99m}Tc(CO)_3$(ASMA) (3.7 MBq/mL [100 μCi/mL]) and ^{131}I-OIH (925 kBq/mL [25 μCi/mL]) in phosphate-buffered saline, pH 7.4. One additional aliquot of the ^{99m}Tc and ^{131}I tracer solution (0.2 mL) for each time point was diluted to 100 mL, and three 1-mL portions of the resulting solution were used as standards.

In group A, 4 animals were sacrificed at 10 min and 4 animals at 60 min after injection. A blood sample was obtained, and the kidneys, heart, lungs, spleen, stomach, and intestines were removed and placed in counting vials. The whole liver was weighed, and random sections were obtained for counting. Samples of blood and urine were also placed in counting vials and weighed. Each sample and the standards were counted for radioactivity using an automated γ-counter; counts were corrected for background radiation, physical decay, and spillover of ^{131}I counts into the ^{99m}Tc window. The percentage of the dose in each tissue or organ was calculated by dividing the counts in each tissue or organ by the total injected counts. The percentage injected dose in whole blood was estimated by assuming a blood volume of 6.5% of total body weight. Three rats probably became hypotensive during the study since 2 rats in the 10-min group A study of $^{99m}Tc(CO)_3$(L-ASMA) and 1 rat in the 60-min group A study of $^{99m}Tc(CO)_3$(*rac*-ASMA) produced very little urine; although these 3 rats could be considered as a model of reduced function, they were eliminated from the combined data analysis even though the ratio of ^{99m}Tc/^{131}I in the urine ranged from 99% to 103%. The group B rats were sacrificed 60 min after injection. Selected organs and blood were collected and counted.

Renal Clearance

Four male rats were anesthetized and placed on a heated surgical table. After tracheostomy, the left jugular vein was cannulated with 2 pieces of polyethylene-50 tubing (one for infusion of radiopharmaceuticals and the other to infuse normal saline [5.8 mL/h] to maintain hydration and additional anesthetic [5 mg/h] as necessary). The right carotid artery was cannulated for blood sampling, and the bladder was catheterized by use of polyethylene-50 tubing. The core temperature of each animal was continually monitored throughout the study using a rectal temperature probe. $^{99m}Tc(CO)_3$(*rac*-ASMA) (3.7 MBq/mL [100 μCi/mL]) and ^{131}I-OIH (1.85 MBq/mL [50 μCi/mL]) were coinfused at a flow rate of 1.7 mL/h for 60 min to establish steady-state blood levels. Urine was then collected for three 10-min clearance periods, and midpoint blood samples (0.5 mL) were obtained. The blood samples were centrifuged for 15 min, and plasma samples were obtained. Renal clearance (Cl) was determined using the equation Cl (mL/min) = UV/P, where U is the urine radioactivity concentration, V is the urine volume excreted per minute, and P is the plasma radioactivity concentration. The average of the three 10-min clearance measurements was used as the clearance value.

Metabolism Studies

Each $^{99m}Tc(CO)_3$(ASMA) preparation was administrated via the tail vein as a bolus injection (18.5 MBq [0.5 mCi]) in 2 additional rats. Urine was collected for 15 min and analyzed by HPLC to determine whether the tracer was metabolized or excreted unchanged in the urine.

Statistical Analysis

All results are expressed as the mean ± SD. To determine the statistical significance between the measured variables of the 2 groups, comparisons were made using the 2-tailed Student *t* test for paired data. A *P* value of less than 0.05 was considered to be statistically significant.

RESULTS

^{99m}Tc Radiolabeling

The radiolabeling of both L-ASMA and *rac*-ASMA ligands with the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor was performed under mild, aqueous pH 7 conditions and provided the appropriate $^{99m}Tc(CO)_3$(ASMA) tracer with high radiochemical purity (>99%) in both preparations, after HPLC isolation. During the purification process, the ^{99m}Tc tracer was separated from the excess of unlabeled ligand. Since all in vivo studies presented here were done at physiologic pH, we have omitted the radiotracer's charge in our discussion and will simply refer to it as $^{99m}Tc(CO)_3$(ASMA). However, our evidence indicates that at physiologic pH the tracer is dianionic, $[^{99m}Tc(CO)_3(ASMA)]^{2-}$, with trianionic ASMA bound via two 5-membered chelate rings.

The coordination of the L-ASMA ligand possessing an asymmetric carbon to the symmetric ^{99m}Tc-tricarbonyl core generates an asymmetric ^{99m}Tc center and as a consequence the formation of 2 diastereomers of $^{99m}Tc(CO)_3$(ASMA) (Fig. 2). The dangling carboxyl group projects away from the carbonyl ligands (exo) in one diastereomer and toward the carbonyl ligands (endo) in the other diastereomer (Fig. 2). The presence of diastereomers was evident on the radiochromatograms of the HPLC-purified $^{99m}Tc(CO)_3$(L-ASMA). Representative chromatograms of the reaction mixture (Fig. 3A) and of the purified radiolabeled compound (Fig. 3B) are shown in Figure 3. In chromatograms of the reaction mixture (Fig. 3A), the radioactive peak eluting at about 9 min coincides with the peak of residual ^{99m}Tc-pertechnetate, typically present at about 1%–2%. Almost identical retention times of diastereomers prevented their separation; thus, the $^{99m}Tc(CO)_3$(L-ASMA) tracer was collected in a single fraction and was further evaluated as a mixture of 2 diastereomers. The $\{^{99m}Tc(CO)_3\}^+$ labeling of *rac*-ASMA (a mixture of L and D isomers) produced 4 stereoisomeric forms: 2 enantiomeric pairs of diastereomers. A less likely formulation would interchange the roles of the aspartic acid carboxyl groups. However, this would produce a less favorable 6-membered chelate ring.

The virtually identical coordination parameters and physical properties of ^{99m}Tc and rhenium (Re) complexes facilitate understanding of the chemistry and structure of the radioactive ^{99m}Tc agents. To confirm the diastereomeric formulation of the $^{99m}Tc(CO)_3$(ASMA) tracers (Fig. 2), rhenium analogs have been prepared, and their synthesis and full characterization will be published elsewhere. As for the ^{99m}Tc radiotracer, $Re(CO)_3$(ASMA) is also formed exclusively as a mixture of 2 diastereomers with structures shown in Figure 2 for the $^{99m}Tc(CO)_3$(ASMA) isomers. Both ^{99m}Tc and Re complexes showed nearly identical retention time under the HPLC conditions of this study when they were coinjected (Figs. 3B and 3D, respectively), confirming the chemical identity of the $^{99m}Tc(CO)_3$(ASMA) tracers.

To assess the tracer's stability, $^{99m}Tc(CO)_3$(ASMA) was incubated at physiologic pH for 24 h, and no measurable

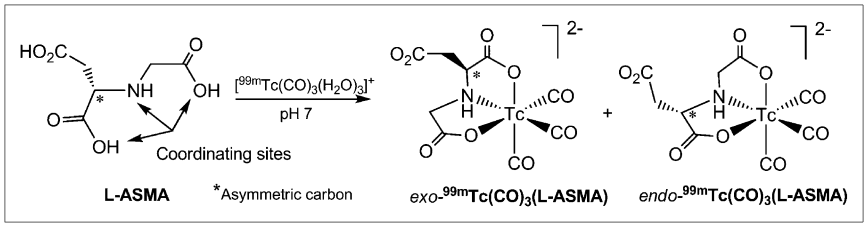

FIGURE 2. Preparation of 2 diastereomers of ^{99m}Tc(CO)$_3$(L-ASMA).

decomposition was observed when an aliquot of the incubated sample was analyzed by HPLC. In addition, the ^{99m}Tc(CO)$_3$(ASMA) tracer was analyzed by HPLC for stability against an excess of cysteine or histidine. In neither case were additional peaks observed in HPLC studies, proving that no transchelation by cysteine and histidine occurred and that ^{99m}Tc(CO)$_3$(ASMA) was completely robust over 4 h under these conditions.

In Vivo Biodistribution and Metabolism of ^{99m}Tc(CO)$_3$(ASMA)

In vivo biodistribution results of ^{99m}Tc(CO)$_3$(L-ASMA) and ^{99m}Tc(CO)$_3$(*rac*-ASMA) expressed as a percentage of injected dose (%ID) for selected tissue and organs are summarized in Table 1 and Figure 4. We compared the biologic behavior of ^{99m}Tc(CO)$_3$(ASMA) to that of ^{131}I-OIH, the clinical standard for the measurement of effective renal plasma flow, in normal rats and in a model of complete renal failure (rats with renal pedicle ligation).

In normal rats, the %ID remaining in the blood at 10 min for both ^{99m}Tc(CO)$_3$(ASMA) preparations was similar, 5.2% for *rac*-ASMA and 4.7% for L-ASMA, and those results were comparable to results obtained with ^{131}I-OIH ($P = 0.07$). Both ^{99m}Tc tracers demonstrated rapid renal extraction and high specificity for renal excretion. The activity of ^{99m}Tc tracer in the urine, as a percentage of ^{131}I-OIH, was 106% ± 6% for ^{99m}Tc(CO)$_3$(*rac*-ASMA) and 100% ± 3% for ^{99m}Tc(CO)$_3$(L-ASMA) at 10 min, and 106% ± 0% for ^{99m}Tc(CO)$_3$(*rac*-ASMA) and 107% ± 2% for ^{99m}Tc(CO)$_3$(L-ASMA) at 60 min. There was no significant difference in the %ID in the urine for both ^{99m}Tc(CO)$_3$(ASMA) preparations and ^{131}I-OIH tracers at 10 min ($P > 0.1$), although both ^{99m}Tc(CO)$_3$(ASMA) tracers were eliminated at a faster rate than ^{131}I-OIH at 60 min ($P < 0.001$). The %ID in the blood at 60 min ranged from 0.2% to 0.3% for the two ^{99}Tc tracers, with both values significantly less than the 0.7%–0.8% obtained with ^{131}I-OIH ($P < 0.003$) (Table 1). In addition, there was minimal hepatic and gastrointestinal activity, with lower uptake of both ^{99m}Tc tracers in the liver ($P < 0.01$) and intestines ($P < 0.05$) at 60 min than was seen with ^{131}I-OIH (Table 1). This low activity is consistent with the highly hydrophilic nature of negatively charged ^{99m}Tc complexes at physiologic pH as well as with their small size. All other organs (heart, spleen, lungs, and stomach) showed negligible uptake of the ^{99m}Tc tracers.

Because both ^{99m}Tc tracers showed similar tissue and organ biodistribution in normal rats, we evaluated only ^{99m}Tc(CO)$_3$(*rac*-ASMA) in a rat model of renal failure. Biodistribution studies comparing ^{99m}Tc(CO)$_3$(*rac*-ASMA) with ^{131}I-OIH in rats with renal pedicle ligation demonstrated an increase in liver and intestinal activity of both ^{99m}Tc and ^{131}I agents at 60 min after injection. However, those increases were significantly higher ($P = 0.032$ [liver] and $P = 0.019$ [bowel], respectively) for the ^{131}I tracer, indicating that renal failure results in more hepatobiliary excretion or intestinal secretion of ^{131}I-OIH than of ^{99m}Tc(CO)$_3$(*rac*-ASMA) (Table 1; Fig. 4). In addition, ^{99m}Tc(CO)$_3$(*rac*-ASMA) was significantly better retained in the blood than was ^{131}I-OIH (17.9% vs. 16.7%; $P = 0.017$).

The renal clearance of ^{99m}Tc(CO)$_3$(*rac*-ASMA) was comparable to that of ^{131}I-OIH (2.85 mL/min/100 g vs. 2.97 mL/min/100 g, $P = 0.481$). The clearance value reported in this paper for ^{131}I-OIH was almost identical to those reported by Müller-Suur and Müller-Suur, 2.76 mL/min/100 g (*29*); Taylor and Eshima, 2.84 mL/min/100 g (*30*); and Lipowska et al., 2.96 mL/min/100 g (*24*), when it was measured in similar experiments.

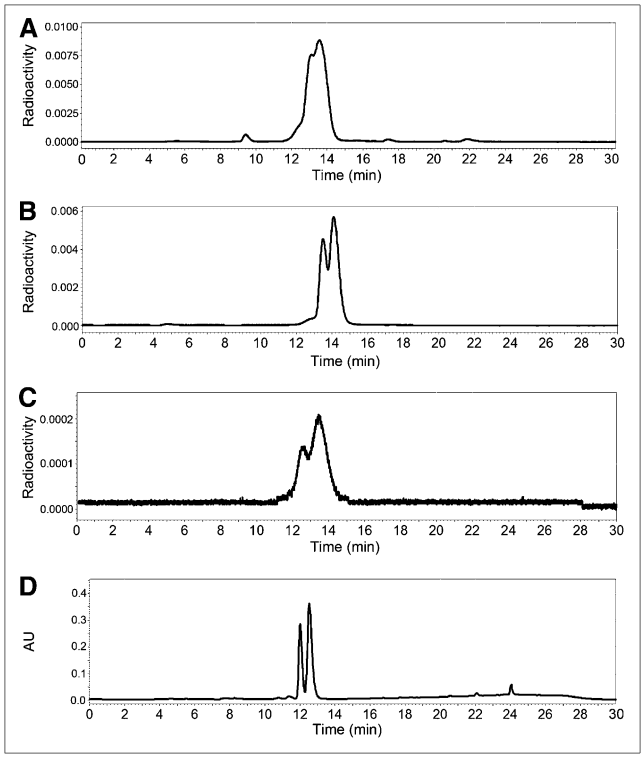

FIGURE 3. HPLC of ^{99m}Tc(CO)$_3$(L-ASMA): labeling mixture (A), before injection (B), in urine at 15 min after injection (C), and ultraviolet trace (254 nm) of Re(CO)$_3$(L-ASMA) (D). AU = absorbance units.

TABLE 1
Biodistribution (%ID) of $^{99m}Tc(CO)_3$(ASMA) and ^{131}I-OIH in Normal (Group A) and Renal Pedicle–Ligated (Group B) Rats

Parameter	Blood	Liver	Stomach	Intestine	Kidney	Urine	% urinary $^{99m}Tc/^{131}I$
10 min, group A							
^{99m}Tc-*rac*-ASMA*	5.2 ± 1.7	2.5 ± 0.3	0.2 ± 0.0	0.5 ± 0.1	6.7 ± 1.0	51.3 ± 6.2	106 ± 6
^{131}I-OIH	6.7 ± 1.9	3.7 ± 0.6	0.3 ± 0.1	1.7 ± 0.3	6.5 ± 0.7	48.3 ± 4.9	
P	0.0711	0.0178		0.0041		0.1104	
^{99m}Tc-L-ASMA†	4.7 ± 0.9	3.7 ± 1.2	0.2 ± 0.0	0.8 ± 0.2	4.3 ± 2.2	51.1 ± 7.3	100 ± 3
^{131}I-OIH	5.1 ± 1.0	2.9 ± 0.3	0.3 ± 0.1	1.5 ± 0.3	4.0 ± 1.8	50.9 ± 6.0	
P	0.0704	0.4576		0.0792		0.9003	
60 min, group A							
^{99m}Tc-*rac*-ASMA‡	0.2 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.4 ± 0.1	0.2 ± 0.1	94.4 ± 1.0	106 ± 0
^{131}I-OIH	0.7 ± 0.1	0.7 ± 0.1	0.2 ± 0.0	1.3 ± 0.3	0.3 ± 0.1	89.1 ± 0.9	
P	0.0002	0.0050		0.0413		0.0008	
^{99m}Tc-L-ASMA*	0.3 ± 0.1	0.3 ± 0.0	0.0 ± 0.0	0.5 ± 0.2	0.7 ± 0.3	95.1 ± 4.1	107 ± 2
^{131}I-OIH	0.8 ± 0.1	0.6 ± 0.2	0.1 ± 0.1	1.3 ± 0.3	0.7 ± 0.3	88.8 ± 2.7	
P	0.0034	0.0061		0.0001		0.0007	
60 min, group B							
^{99m}Tc-*rac*-ASMA*	17.9 ± 1.1	6.2 ± 0.6	0.7 ± 0.2	4.4 ± 0.6	0.6 ± 0.2	—	—
^{131}I-OIH	16.7 ± 1.3	7.9 ± 0.5	0.5 ± 0.2	8.9 ± 2.2	0.5 ± 0.2	—	
P	0.0175	0.0323		0.0189			

*Four rats.
†Two rats.
‡Three rats.
Data are presented as mean ± SD.

Because these ^{99m}Tc tracers are excreted in the urine, we examined the metabolic stability of $^{99m}Tc(CO)_3$(ASMA) by analyzing the urine collected from rats during the first 15 min after a bolus injection of 18.5 MBq (0.5 mCi). Figure 3 shows typical HPLC chromatograms for $^{99m}Tc(CO)_3$(L-ASMA). As in the parent radiotracer, two $^{99m}Tc(CO)_3$(L-ASMA) diastereomers are clearly evident in the urine radiochromatogram (Fig. 3C). The elution times of $^{99m}Tc(CO)_3$(L-ASMA) before injection (Fig. 3B) and in the urine (Fig. 3C) are almost identical, confirming in vivo stability of both diastereomers of the ^{99m}Tc tracer. Similar results were obtained for $^{99m}Tc(CO)_3$(*rac*-ASMA).

DISCUSSION

Amino acid analogs, including aminopolycarboxylic acids, have been proposed for use as effective chelating ligands for the $\{M(CO)_3\}^+$ core (M = ^{99m}Tc, Re) (*31–36*). Such amino acid analogs can provide 3 potential coordinating groups positioned to coordinate tridentately and facially to the $\{M(CO)_3\}^+$ center to form stable ^{99m}Tc/Re complexes. Our analysis of the charge and charge distribution of the tricarbonyl complexes suggests that a negative inner coordination sphere and an overall dianionic charge at physiologic pH favor rapid renal tubular secretion (*24,28,37*). The best example of a ^{99m}Tc-tricarbonyl renal tracer meeting these charge requirements and having an aminopolycarboxylate ligand is $^{99m}Tc(CO)_3$(NTA). The renal clearance and timed urine excretion of $^{99m}Tc(CO)_3$(NTA) were comparable to those of ^{131}I-OIH in normal rats (*24*) and subjects with normal renal function (*21*), and hepatobiliary excretion was less than that of ^{131}I-OIH in rats with simulated renal failure (*24*).

ASMA's chelating ability for $^{99m}Tc/Re(CO)_3$ cores has not been investigated previously. The ligand used here is a geometric isomer of the NTA ligand; one of the carboxymethyl groups is shifted from the central amine atom in NTA to the neighboring α-carbon in ASMA (Fig. 1). Both ligands have 3 carboxyl groups and an amine function and can form two 5-membered chelate rings with the core. ASMA has the potential to form a 5- and a 6-membered chelate ring. For either ASMA binding mode, the very hydrophilic ligand should form very stable, dianionic $^{99m}Tc/Re(CO)_3$ complexes having a dangling carboxylate group needed for rapid renal excretion (*38*).

Ideally, a renal tubular radiopharmaceutical should be designed to exist as a single species at physiologic pH since introduction of a carboxyl group into the ligand core can result in the formation of chelate ring stereoisomers on ^{99m}Tc labeling, each with different pharmacokinetic properties. One of the best examples of this phenomenon is ^{99m}Tc-*N,N′*-bis (mercaptoacetyl)-2,3-diaminopropionate (^{99m}Tc-CO_2-DADS); in healthy volunteers 81% of one isomer was excreted into the urine in 30 min, compared with only 18.5% of the second isomer (*12*). Similarly, we showed that ^{99m}Tc-DD-ethylene-dicysteine (^{99m}Tc-DD-EC) had a higher clearance than ^{99m}Tc-LL-EC in humans (*18*). These examples clearly show that minor configurational changes can alter the tubular extraction of the radiotracers.

We also demonstrated that both ^{99m}Tc-DD- and ^{99m}Tc-LL-EC exist in carboxyl ligated monoanionic (20%) and deligated dianionic (80%) forms in rapid equilibrium at physiologic

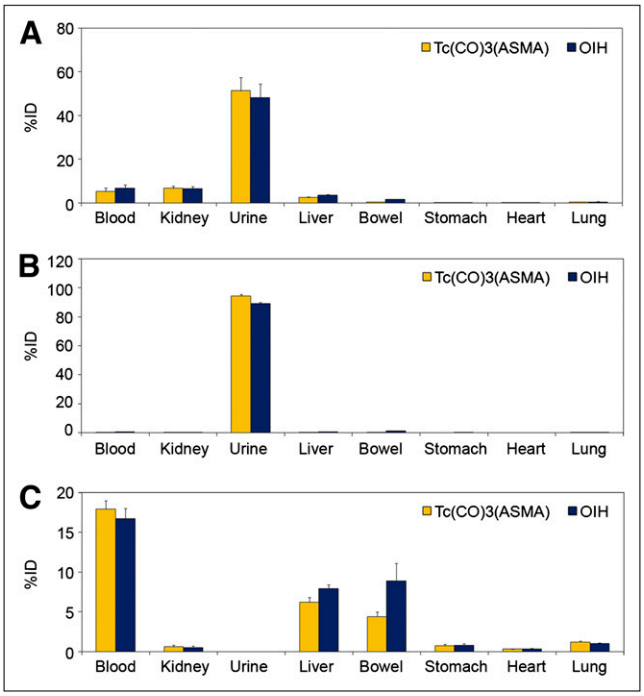

FIGURE 4. Biodistribution of $^{99m}Tc(CO)_3$(*rac*-ASMA) and ^{131}I-OIH in normal rats at 10 min (A) and 60 min (B) after injection and in rats with renal pedicle ligation at 60 min after injection (C), expressed as %ID per organ, blood, and urine.

pH; these 2 forms differ distinctly in structure and charge and almost certainly have different protein binding and tubular receptor affinities. Changes in pH could shift the proportion between the ligated and deligated forms, resulting in a change in clearance that might be interpreted as a change in renal function; also, clearance of these 2 forms may vary in different disease states.

We demonstrate that our new dianionic $^{99m}Tc(CO)_3$(ASMA) tracer not only has a clearance comparable to that of ^{131}I-OIH but is also rapidly extracted by the kidney and eliminated in the urine as rapidly as ^{131}I-OIH. The fact that all $^{99m}Tc(CO)_3$(ASMA) stereoisomers exhibit rapid renal extraction is expected because the dangling carboxylate group for each stereoisomer is far from the coordination sphere (unlike ^{99m}Tc-CO_2-DADS) and there are no possibilities for carboxyl ligated and deligated forms (unlike ^{99m}Tc-DD- and ^{99m}Tc-LL-EC).

Finally, results obtained from the ligated renal pedicle model show that $^{99m}Tc(CO)_3$(ASMA) is highly specific for renal excretion and actually has less liver and intestinal activity than ^{131}I-OIH. Hepatobiliary elimination of ^{99m}Tc-mercaptoacetyltriglycine has been demonstrated in humans (*39*) and rats (*40*) and is likely the mechanism for the increased intestinal activity in our study, although this mechanism cannot be definitely established from our data. Nevertheless, the high specificity of $^{99m}Tc(CO)_3$(ASMA) for renal excretion is an important feature; if impaired renal function accelerates hepatobiliary elimination, the plasma clearance will overestimate renal function, and gallbladder activity may compromise scan interpretation (*39*).

CONCLUSION

The ASMA ligand, which binds via two 5-membered chelate rings, can be efficiently radiolabeled in high yields with the $\{^{99m}Tc(CO)_3\}^+$ core to produce a robust, dianionic $^{99m}Tc(CO)_3$(ASMA) complex. This complex showed pharmacokinetic properties comparable to those of ^{131}I-OIH in normal rats and had less hepatic and intestinal activity than ^{131}I-OIH in an animal model of renal failure. These favorable pharmacokinetic properties warrant studies to determine whether $^{99m}Tc(CO)_3$(ASMA) will have pharmacokinetic properties in humans comparable to or superior to those of ^{99m}Tc-mercaptoacetyltriglycine and ^{131}I-OIH.

DISCLOSURE STATEMENT

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