Initial Evaluation of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ as a Renal Tracer in Healthy Human Volunteers

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Abstract

Purpose Preclinical studies in rats showed that two of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ isomers (*rac*- and *L*-ASMA) had pharmacokinetic properties equivalent to that of ^{131}I -OIH, the radiopharmaceutical standard for the measurement of effective renal plasma flow. The aim of this study was to evaluate the pharmacokinetics of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ isomers in healthy human subjects.

Methods Three ASMA ligands (*rac*-, *L*- and *D*-ASMA) were labeled with $^{99m}\text{Tc}(\text{CO})_3$ using an IsoLink kit (Covidien), and each formed $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ tracer was co-injected with ^{131}I -OIH into healthy human subjects followed by sequential imaging, plasma clearance measurements and timed urine collection. Plasma protein binding, red cell uptake and percent injected dose in the urine were determined. Urine from each group of volunteers was analyzed for metabolites by HPLC.

Results Image quality was excellent with all three agents. Each $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ preparation was excreted unchanged in the urine. The plasma clearance ratio ($^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})/^{131}\text{I}$ -OIH) was 81 ± 3 % for *D*-ASMA compared to only 20 ± 4 % for *L*-ASMA and 37 ± 7 % for *rac*-ASMA; the 81 % clearance ratio for *D*-ASMA isomer is still ~30 % higher than the ^{99m}Tc -MAG3/ ^{131}I -OIH clearance ratio (~50–60 %). Red cell uptake was similar for all three tracers (6–9 %), and all tracers had a relatively rapid renal excretion; at 3 h, the $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})/^{131}\text{I}$ -OIH urine ratio was 100 ± 3 % for *D*-ASMA, 80 ± 2 % for *L*-ASMA and 88 ± 1 % for *rac*-ASMA.

Conclusions The renal excretion characteristics of $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$ in humans are superior to those of the other two $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ isomers studied, but are

still inferior to ^{131}I -OIH, even though there was no difference in the clearance of two of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ isomers and ^{131}I -OIH in rats. The work described here demonstrates the sensitivity in in vivo biological behavior of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ isomers to their subtle structural differences.

Keywords Technetium · Renal radiopharmaceuticals · Kidney · Imaging · $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ · ^{131}I -o-iodohippurate

Introduction

Renal radionuclide studies can provide a simple, noninvasive method of evaluating kidney function and can assist in the early detection and diagnosis of chronic kidney disease. In view of superior physical characteristics of ^{99m}Tc over ^{131}I with regards to γ -camera imaging and radiation dose to the patients, multiple ^{99m}Tc renal tracers have been evaluated as potential alternatives to ^{131}I -o-iodohippurate (^{131}I -OIH), which has been recognized as the radiopharmaceutical standard for the measurement of effective renal plasma flow (ERPF).

To date, the most widely used alternative to ^{131}I -OIH is ^{99m}Tc -mercaptoacetyltriglycine (^{99m}Tc -MAG3), but its success is largely due to excellent scintigraphic imaging qualities and not an improvement in renal clearance, which is still only ~50–60 % that of ^{131}I -OIH [1–3]. ^{99m}Tc -ethylenedicycysteine (^{99m}Tc -EC) was introduced several years after ^{99m}Tc -MAG3 [4, 5], and subsequent studies have showed that the clearances of ^{99m}Tc -DD-EC and ^{99m}Tc -LL-EC are higher than the clearance of ^{99m}Tc -MAG3, but still less than that of ^{131}I -OIH [6]. In addition, a small percentage of ^{99m}Tc -MAG3 and ^{99m}Tc -LL-EC is eliminated via the hepatobiliary pathway, which can lead to problems in image

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interpretation, particularly in patients with renal failure [7–11]. Moreover, the continuing effort to develop a ^{99m}Tc renal pharmaceutical with both reduced hepatobiliary excretion and superior pharmacokinetic properties has been simulated by a report noting marked variability of ^{99m}Tc -MAG3 clearance compared to para-aminohippuran (PAH), the non-radioactive gold standard for the measurement of ERPF [12], as well as the studies concluding that the ^{99m}Tc -MAG3 clearance is not accurate enough to evaluate a change in kidney function [13–15].

Further attempts to search for a better renal tubular agent led to the synthesis of $^{99m}\text{Tc}(\text{CO})_3$ -lanthionine [$^{99m}\text{Tc}(\text{CO})_3(\text{LAN})$], which became the first tracer based on a ^{99m}Tc -tricarbonyl core evaluated in humans [16]. Although the image quality was excellent with this agent and $^{99m}\text{Tc}(\text{CO})_3(\text{LAN})$ had a relatively rapid renal excretion, its plasma clearance was substantially lower than the plasma clearance of ^{131}I -OIH [16]. Recently, we showed that a new renal tracer $^{99m}\text{Tc}(\text{CO})_3$ -nitrilotriacetic acid [$^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$] not only gave kidney images of superb quality, but also showed identical pharmacokinetic properties to those of ^{131}I -OIH based on studies in rats, normal humans and patients with chronic kidney disease [17–19].

In a continuation of our efforts to develop renal tubular tracers with a clearance equivalent to that of PAH, which has a clearance 10–15 % higher than that of ^{131}I -OIH, we evaluated analogs of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$, including $^{99m}\text{Tc}(\text{CO})_3$ -aspartic-*N*-monoacetic acid [$^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$] (Fig. 1) and found that $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ and ^{131}I -OIH had comparable pharmacokinetic properties in normal rats; moreover, in rats with simulated renal failure, $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ had even less excretion into the bowel than did ^{131}I -OIH [20]. While in vivo biological characteristics of renal tracers in rats are generally predictive of results in humans, differences may exist, and the potential advantaged properties of

$^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ over ^{131}I -OIH in rats need to be evaluated in humans. To accomplish this goal, we undertook a series of studies to compare the pharmacokinetic properties and imaging characteristics of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ isomers with those of ^{131}I -OIH in healthy human volunteers.

Materials and Methods

General

The ASMA ligand was synthesized as previously described as a racemic mixture of *D*- and *L*-isomers (*rac*-ASMA) [21, 22] and as the enantiomerically pure *L*- and *D*-isomers (*L*-ASMA and *D*-ASMA, respectively) [23]. $[\text{Na}][^{99m}\text{TcO}_4]$ was eluted from a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator (Lantheus Medical Imaging). $[\text{Na}][^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ was prepared using the IsoLink kit (Covidien) by adding $[\text{Na}][^{99m}\text{TcO}_4]$ generator eluent (1 ml, 1.11–3.7 GBq) to the IsoLink vial, heating for 30 min at 100 °C and adding 1 N HCl (120 μl) to neutralize the solution, as described in the manufacturer's insert. ^{131}I -OIH was prepared as previously described by the isotope exchange reaction between non-radioactive hippuran (OIH) and radioactive sodium iodide (Na^{131}I) [17]. Analysis of the radiolabeled compounds was performed on a high-performance liquid chromatography (HPLC) instrument (Beckman System Gold Nouveau) equipped with a Beckman model 170 radiometric detector, a model 166 ultraviolet–visible light detector, and a Beckman C18 RP Ultrasphere octyldecyl silane column (5- μm , 4.6 $\times$ 250 mm). The solvent system was 0.05 M triethylammonium phosphate (TEAP) buffer pH 2.5 (solvent A) and ethanol (solvent B), and the flow rate was 1 ml/min. The gradient method used was the same as reported previously [24]. The same HPLC system and conditions were used for purification and analysis of ^{99m}Tc tracers and for the urine

Fig. 1 Structural comparison of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ and $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ complexes; asterisk denotes asymmetric carbon, and ellipse encloses dangling the carboxylate group

metabolite studies. Urine, plasma and red blood cell radioactivity were measured with an automated 2480 Wizard 2 gamma counter (Perkin-Elmer) with a 3-inch NaI(Tl) detector.

Radiosynthesis of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$

All three ASMA ligands (*rac*-, *L*- and *D*-ASMA) were labeled according to the previously described procedure [20] to yield their respective dianionic $\text{fac-}[^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})]^{2-}$ complexes, which we depict further as $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$. Briefly, $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ (0.5 ml, ~ 2 GBq/ml) and an aqueous solution of the corresponding ASMA ligand (0.2 ml, 1 mg/ml) were combined in a sealed vial, and the pH was adjusted to ~ 7 with 1 M NaOH. After heating for 30 min at 70°C , the vial was cooled to room temperature. $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ was purified by HPLC as described above, and the radiochemical purity was $>98\%$. The two diastereomers of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ formed during the labeling process for each *L*- and *D*-ASMA ligand could not be separated by HPLC, and the tracer was collected in a single fraction and further evaluated as a mixture of products. Ethanol was partially removed by N_2 gas. The collected $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ fraction was subsequently diluted in phosphate-buffered saline (PBS) by a factor of 20 to ensure that any remaining organic solvent comprised less than 5 % of the solution (v/v). The HPLC-purified product was sterilized by filtration (sterile filter Millex-GS, $22\ \mu\text{m}$, Millipore), and the final concentration was 37 MBq/ml. After formulation, the identity and the chemical and radiochemical purities of the $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ tracer were checked using HPLC and $\text{Re}(\text{CO})_3(\text{ASMA})$ as a nonradioactive standard [25]. The bubble point test of the sterilization filter was performed before the injection of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$. Each final preparation (pH 7.4) was tested and determined to be sterile and pyrogen free.

Healthy Volunteer Studies

All studies were performed with the approval of the Emory University Institutional Review Board and conducted under the auspices of IND 112,322 from the US Food and Drug Administration. Written informed consent was obtained from each volunteer. Nine healthy volunteers (4 male, 5 females; mean age $\pm$ SD, 28 ± 7 years; range 18–40 years) participated in this study and were divided randomly into three groups of three subjects each. Enrolled participants had no history of kidney or bladder diseases, and pregnancy was excluded in female volunteers using a urine pregnancy test. Each volunteer was asked to drink a glass of water before the study. In addition, safety assessments for each volunteer included the measurement of vital signs at pre-injection baseline and after imaging. Also, clinical laboratory tests were performed at pre-

and post-injection and included complete blood count, standard chemistry panel and urinalysis.

Imaging was performed using an Infinia camera (GE Healthcare) with 3/8-inch crystal and a high-energy collimator after each volunteer had been coinjected with $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ (~ 74 MBq) and ^{131}I -OIH (~ 9.25 MBq). Each subject was imaged supine with the kidneys and bladder within the field of view. At the conclusion of the 24-min acquisition, a postvoid image of the kidneys was obtained as well as an anterior image over the gallbladder and abdomen. Data were acquired and processed on a Xeleris computer (GE Healthcare) with an extensive in-house upgrade of the original QuantEM renal software (GE Healthcare). Renogram curves were generated using cortical (parenchymal) and whole-kidney regions of interest (ROIs), and the time-to-peak height (TTP) and 20-min-to-maximum count ratios (20 min/max) were calculated. Plasma clearances for $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ and ^{131}I -OIH were determined using the single injection, two-compartment model of Sapirstein et al. [26] after ten venous blood samples had been obtained at 3, 5, 10, 20, 30, 45, 60, 90, 120 and 180 min post-injection. The value of plasma clearance (Cl) was normalized for body surface area (BSA), which was calculated by means of the Haycock formula [27], and expressed in $\text{ml/min}/1.73\ \text{m}^2$ (Cl/BSA).

The volunteers voided at 30, 90 and 180 min post-injection to determine the percent dose in the urine. Plasma protein binding (PPB) and the percent uptake in the erythrocytes (RBC) were determined as described previously [18] in duplicate, and the mean values were reported. No correction was made for plasma trapped in the red blood cell sample.

In vivo stability and metabolism of the complex were determined by comparing the HPLC analysis of a 30-min urine sample for each $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ preparation with that of the purified complex.

Statistical Analysis

Statistical analyses were performed using a two-tailed Student's *t* test. A *P*-value of <0.05 was considered statistically significant. All results are expressed as the mean $\pm$ SD.

Results

The ASMA ligand was effectively radiolabeled with the $[\text{}^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ precursor under mild conditions (30 min at 70°C , pH ~ 7) to give well-defined complexes with the ^{99m}Tc -tricarbonyl core and with high radiochemical purity of $>98\%$ [20]. In all complexes, the ASMA ligand coordinated tridentately and facially to form a $^{99m}\text{Tc}(\text{CO})_3(\text{ONO})$ coordination sphere, leaving one carboxyl group uncoordinated (Fig. 1). All three preparations, $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$,

Table 1 Plasma clearance (Cl, ml/min/1.73 m²), plasma protein binding (PPB, %), red cell binding (RCB, %) and urine excretion (% ID) of ^{99m}Tc(CO)₃(ASMA) in healthy volunteers (n=3) compared to simultaneously injected ¹³¹I-OIH. Data are presented as mean±SD

	Cl	^{99m} Tc/ ¹³¹ I % Cl	PPB	RBC	30 min Urine	^{99m} Tc/ ¹³¹ I % Urine	180 min Urine	^{99m} Tc/ ¹³¹ I % Urine
^{99m} Tc- <i>D</i> -ASMA	327 ± 56	81 ± 3	39±4	9 ± 5	67 ± 8	91 ± 8	92 ± 4	100 ± 3
¹³¹ I -OIH	403 ± 64		74±2	18 ± 2	73 ± 7		92 ± 2	
^{99m} Tc- <i>L</i> -ASMA	90±6	20±4	77±2	5±2	40±2	53±3	74±2	80±2
¹³¹ I -OIH	448±53		73±4	18±4	75±5		92±2	
^{99m} Tc- <i>rac</i> -ASMA	142±5	37±7	54±15	6±4	52±2	72±3	82±1	88±1
¹³¹ I -OIH	390±71		72±4	18±3	73±2		93±2	

^{99m}Tc(CO)₃(*L*-ASMA) and ^{99m}Tc(CO)₃(*rac*-ASMA), are obtained as a mixture of diastereomers because of the chirality of the ASMA ligand [25]. The ^{99m}Tc tracers are very hydrophilic and dianionic at physiological pH since the dangling carboxylate group is deprotonated at that condition.

In our human studies, each ^{99m}Tc(CO)₃(ASMA) preparation was co-injected with ¹³¹I-OIH. There was no evidence of toxicity based on measurements of vital signs, complete blood count, standard chemistry panel and urinalysis for any of the volunteers. No adverse events were reported.

The plasma clearance of ^{99m}Tc(CO)₃(*D*-ASMA) averaged 327±56 ml/min/1.73 m², compared to 90±6 ml/min/1.73 m² for ^{99m}Tc(CO)₃(*L*-ASMA) and 142±5 ml/min/1.73 m² for ^{99m}Tc(CO)₃(*rac*-ASMA) (Table 1). The red cell uptake (RBC) was similar for all three tracers (6–9 %), but the plasma

protein binding (PPB) of ^{99m}Tc(CO)₃(*D*-ASMA) was the lowest among three ^{99m}Tc tracers, 39±4 % for *D*-ASMA compared to 77±2 % for *L*-ASMA and 54±15 % for *rac*-ASMA, respectively, and it was significantly less than the PPB of ¹³¹I-OIH (74±2 % *P*<0.002).

All ^{99m}Tc tracers had a relatively rapid renal excretion. The mean value of the injected dose of ^{99m}Tc(CO)₃(*D*-ASMA) excreted in the urine at 30 min was 67±8 % compared to 40±2 % for ^{99m}Tc(CO)₃(*L*-ASMA) and 52±2 % for ^{99m}Tc(CO)₃(*rac*-ASMA). At 3 h, the urine excretion was similar for all tracers with a ^{99m}Tc(CO)₃(ASMA)/¹³¹I-OIH urine ratio of 100±3 % for *D*-ASMA, 80±2 % for *L*-ASMA and 88±1 % for *rac*-ASMA (Table 1).

An aliquot from the 30 min urine sample from each group of volunteers was analyzed by HPLC to determine whether

Fig. 2 Urine samples from human volunteers injected with ^{99m}Tc(CO)₃(*D*-ASMA) (a), ^{99m}Tc(CO)₃(*L*-ASMA) (b) and ^{99m}Tc(CO)₃(*rac*-ASMA) (c) were subjected to reverse-phase HPLC analysis. Corresponding reference HPLC traces show that all complexes were excreted unchanged in the urine

Fig. 3 (a) Two-minute kidney images and renogram curves after simultaneous injection of 87.3 MBq (2.36 mCi) of $^{99m}\text{Tc}(\text{CO})_3(D\text{-ASMA})$ and 10.4 MBq (0.28 mCi) of $^{131}\text{I}\text{-OIH}$ in a healthy 40-year-old female volunteer. (b) Two-minute kidney images and renogram curves after simultaneous injection of 69.2 MBq (1.87 mCi) of $^{99m}\text{Tc}(\text{CO})_3(L\text{-ASMA})$ and 12.2 MBq (0.33 mCi) of $^{131}\text{I}\text{-OIH}$ in healthy 18-year-old female volunteer.

(c) Two-minute kidney images and renogram curves after simultaneous injection of 79.9 MBq (2.16 mCi) of $^{99m}\text{Tc}(\text{CO})_3(rac\text{-ASMA})$ and 11.5 MBq (0.31 mCi) of $^{131}\text{I}\text{-OIH}$ in a healthy 33-year-old female volunteer. [In each panel, the $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ tracer is displayed in the top of the panel and co-injected $^{131}\text{I}\text{-OIH}$ is displayed below]

the complex was excreted intact. As shown in Fig. 2, the retention time of $^{99m}\text{Tc}(\text{CO})_3(D\text{-ASMA})$, $^{99m}\text{Tc}(\text{CO})_3(L\text{-ASMA})$ and $^{99m}\text{Tc}(\text{CO})_3(rac\text{-ASMA})$ before injection and in

the urine were almost identical, confirming each tracers' in vivo stability since only the intact radiolabeled parent compound was observed.

Image quality was excellent with all three ^{99m}Tc agents. A representative study for each tracer is presented in Fig. 3. There was no significant difference in relative uptake in the left and right kidneys for all three $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ preparations compared to the kidney uptake of ^{131}I -OIH ($P=0.51$ for *D*-ASMA, $P=0.12$ for *L*-ASMA and $P=0.07$ for *rac*-ASMA) (Table 2). Average time to peak (TTP) for $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$ for whole kidney was 2.63 ± 0.35 min, which was less than those observed for $^{99m}\text{Tc}(\text{CO})_3(\text{L-ASMA})$ and $^{99m}\text{Tc}(\text{CO})_3(\text{rac-ASMA})$ (5.00 ± 1.61 min and 3.60 ± 0.36 min, respectively). Similarly, the 20-min-to-maximum (20 min/max) count ratio of 0.19 ± 0.05 for $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$ was less than the ratios for two other ^{99m}Tc tracers (0.39 ± 0.13 for *L*-ASMA and 0.30 ± 0.18 for *rac*-ASMA). For comparison, the normal value for whole kidney 20 min/max ratio for ^{99m}Tc -MAG3 was 0.24 ± 0.14 based on an earlier study of 106 subjects referred for potential kidney donation [3]. Since the ^{131}I -OIH renogram curves were quite noisy because of the relatively low counting rate resulting from the lower administered dose, poor capture of 364-keV photon of ^{131}I by 9.5 mm crystal and the fact that the high-energy collimator is not optimized for 364-keV photons, it was not possible to calculate reliable TTP and 20 min/max for ^{131}I -OIH.

Finally, none of the subjects had activity identified in the gall bladder or intestines on the anterior images obtained at 30 min over the gall bladder and abdomen.

Discussion

The chemical structure of the radiolabeled chelate must be known to determine the lipophilicity, geometric shape, net charge and charge distribution of the agents since these properties have a major influence on protein binding, renal specificity, rate of excretion and *in vivo* distribution. The ASMA ligand used in our studies for preparation of ^{99m}Tc -tricarbonyl radiotracers is a geometrical isomer of the nitrilotriacetic acid (NTA) ligand. Based on the structural similarity of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ and $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ (Fig. 1), we anticipated a comparable renal handling of those two isomers. These tracers have very similar properties at physiological pH.

They are both very hydrophilic due to an overall dianionic charge resulting from an identical ONO coordination mode of the ligands to the $[\text{}^{99m}\text{Tc}(\text{CO})_3]^+$ core and the presence of a deprotonated dangling carboxylate group. However, in contrast to a symmetrical NTA ligand that forms a single complex upon coordination to the ^{99m}Tc tricarbonyl core with a well-established structure based on the characterization of its Re analog [17], the ASMA ligand with the asymmetric carbon produces a mixture of diastereomers, which differ only in the position and orientation of the uncoordinated carboxylate group on the aspartate chelate ring (*endo* when the dangling group projects toward the face defined by the carbonyl ligands or *exo* when projecting away from the tricarbonyl face; please see the cited references for a full depiction of diastereomers) [25, 20]. Briefly, since *endo*- and *exo*- $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$ diastereomers are also enantiomers of *endo*- and *exo*- $^{99m}\text{Tc}(\text{CO})_3(\text{L-ASMA})$ complexes, respectively, these radiotracers have identical HPLC chromatograms. Taking into account that the *rac*-ASMA ligand is a racemic mixture of *D*- and *L*-ASMA, the HPLC chromatogram for the $^{99m}\text{Tc}(\text{CO})_3(\text{rac-ASMA})$ tracer is also identical to the chromatograms of *D*-ASMA and *L*-ASMA radiotracers (Fig. 2). Nevertheless, even with differences in their structural features, and the fact that $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ is a mixture of diastereomers, our previous studies in normal rats demonstrated that $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ and $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ tracers had pharmacokinetic properties comparable to those of ^{131}I -OIH including high specificity for renal excretion. Also, they both had less hepatic and intestinal activity than ^{131}I -OIH in an animal model of renal failure [17, 20]. Since there are interspecies differences and the pharmacokinetics of renal tracers in humans does not perfectly mirror those obtained in rats, evaluation of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ isomers in humans was warranted.

Our present studies in healthy volunteers showed differences not only in the pharmacokinetics of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ in comparison to $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$, but also among the $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ isomers. Although there was no direct comparison in this study of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ to $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ or to ^{99m}Tc -MAG3, several conclusions can be made on the basis of available data. First, image quality was excellent for all three $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ preparations (Fig. 3). Second, the best

Table 2 Relative kidney uptake of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ and ^{131}I -OIH in healthy volunteers ($n=3$ for each ^{99m}Tc tracer). Data are presented as mean $\pm$ SD; ^a*P* represents comparison of kidney uptake between $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ and ^{131}I -OIH

Complex	Left kidney uptake (%)		Right kidney uptake (%)		<i>P</i> ^a
	^{99m}Tc -ASMA	^{131}I -OIH	^{99m}Tc -ASMA	^{131}I -OIH	
^{99m}Tc - <i>D</i> -ASMA	48 $\pm$ 6	50 $\pm$ 2	52 $\pm$ 6	50 $\pm$ 2	0.5101
^{99m}Tc - <i>L</i> -ASMA	50 $\pm$ 2	52 $\pm$ 3	50 $\pm$ 2	48 $\pm$ 3	0.1181
^{99m}Tc - <i>rac</i> -ASMA	47 $\pm$ 3	45 $\pm$ 3	53 $\pm$ 3	55 $\pm$ 3	0.0742

Fig. 4 Comparison of clearance ratios ($^{99m}\text{Tc}/^{131}\text{I}$, %) of $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$, $^{99m}\text{Tc}(\text{CO})_3(\text{L-ASMA})$, $^{99m}\text{Tc}(\text{CO})_3(\text{rac-ASMA})$ and $^{99m}\text{Tc-MAG3}$ [2, 28, 29] vs. simultaneously injected $^{131}\text{I-OIH}$ in normal volunteers

tracer appears to be $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$ with a plasma clearance of 81 % that of $^{131}\text{I-OIH}$ (Table 1); that ratio is substantially better than the 50–60 % $^{99m}\text{Tc-MAG3}/^{131}\text{I-OIH}$ clearance ratio (Fig. 4) [2, 28, 29], although it is still inferior to the 100 % $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})/^{131}\text{I-OIH}$ clearance ratio [18]. The mean plasma clearance of $^{131}\text{I-OIH}$ in all our nine healthy subjects (413 ± 61 ml/min/ 1.73 m²) was lower than the mean $^{131}\text{I-OIH}$ clearance we obtained in an earlier study of normal volunteers (530 ml/min) [2]. The possibility of clearance variations points out the need for an internal standard such as $^{131}\text{I-OIH}$ when evaluating the clearance of new ^{99m}Tc renal tracers.

In humans, only 39 % of $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$ is protein bound and represents the lowest protein binding of the three $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ preparations (Table 1); it is similar to that of $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ (PPB, 43 %), but it is much lower than that of $^{99m}\text{Tc-MAG3}$ (PPB, ~80 %). As a result of lower protein binding, a renal tracer can be eliminated by glomerular filtration in addition to tubular extraction [30]. Because the clearance of $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$ exceeds the maximum possible glomerular filtration rate, this complex must be excreted through the proximal tubular cells and, as an anionic tracer, is likely transported by the organic anion transporter in the same manner as $^{131}\text{I-OIH}$, $^{99m}\text{Tc}(\text{CO})_3(\text{NTA})$ and $^{99m}\text{Tc-MAG3}$. We expect that the other two $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ preparations share a similar route of elimination due to their structural similarity and due to the fact that the urine activity ratios of $^{99m}\text{Tc}(\text{CO})_3(\text{L-ASMA})/^{131}\text{I-OIH}$ and $^{99m}\text{Tc}(\text{CO})_3(\text{rac-ASMA})/^{131}\text{I-OIH}$ at 10 min [20] exceeded the expected 40 % filtration fraction in rats [31].

Since we have demonstrated that $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$ has a higher clearance and rate of urine excretion than $^{99m}\text{Tc}(\text{CO})_3(\text{L-ASMA})$ and $^{99m}\text{Tc}(\text{CO})_3(\text{rac-ASMA})$ in humans, it can be assumed that it fits more efficiently to the renal transport proteins and that the chelate ring stereochemistry plays an important role in renal handling. It has been previously shown that the pharmacokinetic properties of renal

tracers are susceptible to the presence of functional groups, chelate ring stereochemistry of the ligand and positional isomerism of the functional group. For example, one of the isomers of an early ^{99m}Tc renal tracer, *syn*- $^{99m}\text{Tc-N,N'}$ -bis-(mercaptoacetyl)-2,3-diaminopropionic acid (*syn*- $^{99m}\text{Tc-CO}_2\text{-DADS}$), is excreted faster than the *anti*-isomer in normal subjects [32, 15, 33]. Similar to our findings, pharmacokinetic properties in rats were almost identical for all separated $^{99m}\text{Tc-EC}$ isomers, but in humans, the clearance of $^{99m}\text{Tc-DD-EC}$ was significantly higher than the clearance of $^{99m}\text{Tc-DL-EC}$ [6]. Other studies reported that *syn*- $^{99m}\text{Tc-D-mercaptoacetamide-ethylene-cysteine}$ (*syn*- $^{99m}\text{Tc-D-MAEC}$) had a higher clearance in humans than its *L-MAEC* isomer [34]. It was also shown that the efficiency of renal handling of the derivatives of $^{99m}\text{Tc-MAG3}$ was influenced to a great extent by the configuration of isomers and the site of substitution [35–37].

Conclusions

Image quality was uniformly excellent for all three $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ preparations. Preliminary data in humans suggest that the pharmacokinetic properties of $^{99m}\text{Tc}(\text{CO})_3(\text{D-ASMA})$ are superior to those of the other ASMA isomers and also superior to those of $^{99m}\text{Tc-MAG3}$, but they are still not equivalent to those of $^{131}\text{I-OIH}$. Even though studies in rats demonstrated no difference in the clearance of $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ isomers and $^{131}\text{I-OIH}$, the plasma clearance of the $^{99m}\text{Tc}(\text{CO})_3(\text{ASMA})$ isomers in humans was less than that of $^{131}\text{I-OIH}$.

The development of radiopharmaceuticals for clinical application in humans is critically dependent on evaluation and testing in animal models. However, the results of this study are a testament to the fact that subtle yet significant differences may exist between tracer pharmacokinetics in animal models and in humans and emphasize that the animal model can only serve as a guide and not a replacement for human testing.

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Conflict of Interest Andrew T. Taylor and Russell D. Folks receive royalties from the sale of QuantEM software. This arrangement has been reviewed and approved by Emory University in accordance with its conflict-of-interest policy. Malgorzata Lipowska and Jeffrey Klenc declare that they have no conflict of interest.

Ethics Statement All studies were performed with the approval of the Emory University Institutional Review Board and conducted under the auspices of IND 112,322 from the United States Food and Drug Administration. Written informed consent was obtained from each volunteer.

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