^{99m}Tc-MAEC Complexes: New Renal Radiopharmaceuticals Combining Characteristics of ^{99m}Tc-MAG3 and ^{99m}Tc-EC

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^{99m}Tc-Mercaptoacetyltriglycine (^{99m}Tc-MAG3) and ^{99m}Tc-L,L-ethylenedicysteine (^{99m}Tc-LL-EC) are useful renal radiopharmaceuticals; however, both agents have renal clearances less than that of ^{131}I-orthoiodohippurate (^{131}I-OIH), and ^{99m}Tc-LL-EC exists in dianionic and monoanionic forms at physiologic pH. In an effort to develop a superior ^{99m}Tc agent with a rapid clearance comparable with that of ^{131}I-OIH, we have designed a new ligand system, mercaptoacetamide-ethylene-cysteine (MAEC), which combines important structural features of both MAG3 and EC. **Methods:** Biodistribution and clearance studies were performed on Sprague–Dawley rats using *syn*- and *anti*-^{99m}Tc-L- and -D-MAEC coinjected with ^{131}I-OIH. Studies were also performed by coinjecting each isomer (~74 MBq [~2 mCi]) and 7.4–11.1 MBq (200–300 μCi) of ^{131}I-OIH in 3 volunteers with dual-isotope imaging performed using a camera system fitted with a high-energy collimator. Blood samples were obtained from 3 to 90 min after injection and urine samples were obtained at 30, 90, and 180 min. **Results:** In the rats, <10% of the injected dose remained in the blood at 10 min after injection for all isomers, and the urine dose at 60 min ranged from 84% to 99% that of ^{131}I-OIH. The clearances of *syn*- and *anti*-^{99m}Tc-L-MAEC in the rats were higher than the clearances for the D-isomers ($P \leq 0.02$) and were 102% and 105% that of ^{131}I-OIH, respectively. In humans, the plasma protein binding of the ^{99m}Tc-MAEC complexes ranged from 82% to 89%. All 4 complexes provided excellent renal images. The ^{99m}Tc-MAEC complex/^{131}I-OIH plasma clearance ratio in humans ranged from 45% (*anti*-^{99m}Tc-L-MAEC) to 74% (*syn*-^{99m}Tc-D-MAEC) with the 180-min urine excretion equivalent to that of ^{131}I-OIH for all 4 complexes. **Conclusion:** Initial data in humans suggest that *syn*-^{99m}Tc-D-MAEC has a higher clearance than that of ^{99m}Tc-MAG3; however, none of the ^{99m}Tc-MAEC tracers have a clearance equivalent to that of ^{131}I-OIH and further ligand design is needed.

Key Words: ^{99m}Tc-mercaptoacetamide-ethylene-cysteine; ^{99m}Tc-ethylenedicysteine; ^{99m}Tc-mercaptoacetyltriglycine; renal radiopharmaceuticals

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During the past 20 y several ^{99m}Tc complexes have been synthesized and tested as potential alternatives to ^{131}I- or ^{123}I-orthoiodohippurate (OIH) (*1–3*). The most widely used agent is ^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG3), which has been extensively studied and considered by many to be the ^{99m}Tc renal agent of choice (*4–6*). Nevertheless, ^{99m}Tc-MAG3 is still not the 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 (ERPF) (*1*). Furthermore, a small percentage of ^{99m}Tc-MAG3 is transported into the small intestine via the hepatobiliary system in healthy volunteers; this percentage increases in patients with renal failure and can lead to problems in image interpretation (*7–9*). Increased hepatobiliary activity can also occur with suboptimal kit preparation (*10*).

In 1990, Verbruggen et al. (*11*) observed that ^{99m}Tc-L,L-ethylenedicysteine (^{99m}Tc-LL-EC), the polar metabolite of the brain agent ^{99m}Tc-L,L-ethylenedicysteine diethyl ester (^{99m}Tc-LL-ECD), was rapidly and efficiently excreted into the urine in mice. Subsequent studies have shown that the clearance of ^{99m}Tc-LL-EC is higher than that of ^{99m}Tc-MAG3 and ranges from 70% to 76% that of OIH (*12–15*). Nevertheless, the clearance of ^{99m}Tc-LL-EC is significantly less than that of ^{131}I-OIH. Moreover, ^{99m}Tc-LL-EC exists in dianionic (80%) and monoanionic (20%) forms at physiologic pH, and it is highly unlikely that these 2 forms have the same clearance or protein binding affinity (*15*). The pH-dependent distribution between monoanionic and dianionic forms may lead to greater clearance variability if ^{99m}Tc-LL-EC is used to monitor a patient's renal function over time.

The limitations of ^{99m}Tc-MAG3 and ^{99m}Tc-LL-EC coupled with the need for a ^{99m}Tc tracer that can directly measure ERPF have prompted a continuing search for an improved ^{99m}Tc renal imaging agent that has a clearance comparable to that of ^{131}I-OIH. Our previous successful speciation studies with Re-EC complexes led directly to our design of a new series of ligands, mercaptoacetamide-ethylene-cysteine

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(MAEC), which combined important structural features of EC (cysteine moiety) and MAG3 (mercaptoacetamide moiety) (Fig. 1) (*16–22*). This study compares the pharmacokinetics of *syn*- and *anti*-^{99m}Tc-D- and L-MAEC with those of ^{131}I-OIH in rats and healthy volunteers.

MATERIALS AND METHODS

All chemicals and solvents were of reagent grade and were used without further purification. *N*-(*S*-Benzoylmercaptoacetamide)ethylene-L-cysteine (L-MAEC) was prepared as described previously (*23*). The synthesis of *N*-(*S*-benzoylmercaptoacetamide)ethylene-D-cysteine (D-MAEC) was performed in a similar manner as that of L-MAEC except that D-cysteine was used instead of L-cysteine. ^{99m}Tc-Sodium pertechnetate (Na[$^{99m}TcO_4$]) was eluted from a ^{99}Mo/^{99m}Tc generator (Amersham Health) using 0.9% saline. High-performance liquid chromatography (HPLC) analyses were performed on a Beckman System Gold Nouveau equipped with model 170 Radioisotope Detector, 166 UV Detector, and 32 Karat workstation software.

^{99m}Tc Radiolabeling

Each ligand (1 mg) was dissolved in EtOH (0.1 mL) and glycine buffer (0.1 mL; 50 mg of glycine sodium salt in 10 mL of 0.9% saline). Freshly prepared stannous tartrate solution (4 mmol/L in H_2O, 0.1 mL) was added. The final pH of the solution was ~9. Na[$^{99m}TcO_4$] in generator saline (0.25 mL) was added to the solution and the mixture was heated at 100°C for 10 min. After cooling to room temperature, the pH was adjusted to 6 with 1N HCl. The ^{99m}Tc-MAEC complexes were isolated by reverse-phase HPLC on a Beckman Ultrasphere ODS 5-μm column (4.6 × 250 mm); flow rate, 1 mL/min; mobile phase, 8% EtOH, 0.01 mol/L sodium phosphate buffer, pH 6.5. Stannous reduction of $^{99m}TcO_4^-$ at pH 9 in the presence of either L-MAEC or D-MAEC ligands produced a mixture of two ^{99m}Tc-MAEC products. The two ^{99m}Tc-MAEC products were resolved by HPLC in an approximate ratio of 2:3. The radiochemical purity of the HPLC-separated ^{99m}Tc-MAEC complexes was >99%. The complexes were buffered to pH 7.4 and tested for stability by HPLC up to 6 h; no measurable decomposition was observed.

The first eluting peak was assigned as *anti*-^{99m}Tc-MAEC and the second peak as *syn*-^{99m}Tc-MAEC. We assigned the *syn* configuration to the ^{99m}Tc-MAEC complex because it was favored at high pH, a characteristic found in related systems and confirmed by chemical methods (*21*,*23*,*24*). Although the *anti* isomer formed at pH 12, it converted to the *syn* isomer on heating, a result similar to that obtained for Re-MAEC complex (*23*); a lower pH (~9) must be used to slow the *anti*-to-*syn* conversion rate. At high pH, the *syn* isomer is thermodynamically preferred (Re, 94%; ^{99m}Tc, 94%) and the rate of *anti*-to-*syn* equilibration is fast (reaction completion, <30 min) (*3*,*24*).

Rat Studies

Biodistribution Studies. The animal experiments followed the principles of laboratory animal care and were approved by the Institutional Animal Care and Use Committee of Emory University. *syn*- and *anti*-^{99m}Tc-L- and D-MAEC were each evaluated in 5 Sprague–Dawley rats at 10 and 60 min, respectively; data for 2 rats studied with *anti*-^{99m}Tc-L-MAEC at 10 min and 1 rat studied at 60 min had to be excluded due to problems with injection, standard preparation, or hypotension. A solution of each ^{99m}Tc complex (3.7 MBq/mL [100 μCi/mL]) and ^{131}I-OIH (925 kBq/mL [25 μCi/mL]) was prepared and six 0.2-mL aliquots were drawn with insulin syringes. Five aliquots were used for doses; the sixth aliquot was diluted to 100 mL, and three 1-mL portions of the resulting solution were used as standards. Each rat was anesthetized with ketamine/xylazine (2 mg/kg body weight) injected intramuscularly. The bladder was catheterized using heat-flared PE-50 tubing for urine collection.

The radiopharmaceutical solution was injected intravenously via a tail vein, and 5 animals were killed at 10 min and 5 were killed at 60 min after injection. A blood sample was obtained and the heart, lung, spleen, liver, intestine, stomach, and kidney were removed. The whole liver was weighed and random sections were obtained for counting. Blood, whole organs, and tissue samples were placed in tubes, and each sample was weighed. Radioactivity of the sample and standards was measured in a double-channel well counter with 20% windows centered on the photo peaks of ^{99m}Tc (140 keV) and ^{131}I (360 keV). Counts were corrected for background radiation, physical decay, and spillover of ^{131}I counts into the ^{99m}Tc window. The percentage dose found in each tissue or organ was calculated by dividing the counts in each tissue or organ by the total injected counts. The value given for bowel represents combined stomach and intestine activity. The percentage injected dose in whole blood was estimated assuming a blood volume of 6.5% of total body weight.

Metabolism Studies. Rats were prepared according to the procedure described for the biodistribution studies. A bolus injection of the radiopharmaceutical (~37 MBq [~1 mCi]) was given, and the urine was collected for 30 min and analyzed by HPLC alone and with purified complex added. Each ^{99m}Tc-MAEC complex was tested in 2 rats.

Renal Clearance, Extraction Fraction (EF), and Plasma Protein Binding (PPB). Each of the ^{99m}Tc-MAEC isomers was compared with ^{131}I-OIH in 6 Sprague–Dawley rats to determine steady-state plasma clearance, EF, and PPB. Each rat was anesthetized with ketamine/xylazine (2 mg/kg body weight) injected intramuscularly and placed on a heated surgical table. After tracheotomy, the left

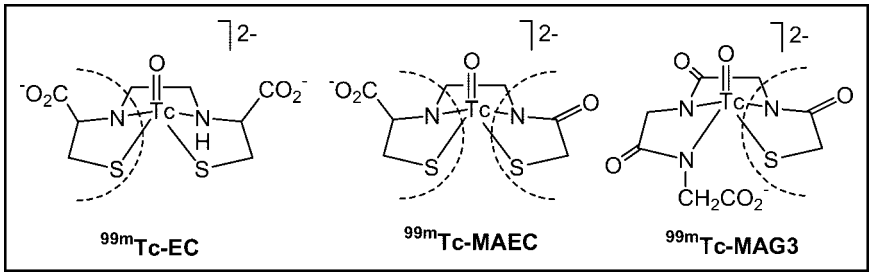

FIGURE 1. ^{99m}Tc-MAEC combines structural characteristics of ^{99m}Tc-MAG3 and ^{99m}Tc-EC.

jugular vein was cannulated with 2 pieces of PE-50 tubing (one for infusion of the radiopharmaceuticals and one for infusion of normal saline [5.2 mL/h] to maintain hydration and additional anesthetic [up to 4 mg/h] as necessary). The right carotid artery was cannulated for blood sampling and the bladder was catheterized using heat-flared PE-50 tubing for urine collection. The core temperature of each animal was continually monitored using a rectal temperature probe. Each ^{99m}Tc complex (370 kBq/mL [10 μCi/mL]) was coinfused with ^{131}I-OIH (185 kBq/mL [5 μCi/mL]) as an internal control at a flow rate of 1.5 mL/h through the left jugular vein for 45–60 min to establish steady-state blood levels. Urine was collected for three 10-min clearance periods, and midpoint blood samples (0.3 mL) were obtained. The following equation was used to calculate renal clearance: Cl (mL/min) = (urine volume/min × urine concentration)/plasma concentration. The average of the three 10-min clearance periods was used as the clearance value.

To measure EF, a left renal venous blood sample (0.5 mL) followed by a carotid artery sample (3 mL) was obtained at the conclusion of the study. The venous sample was centrifuged within 10 min of collection. EF was calculated by the following equation: EF = (arterial concentration – venous concentration)/arterial concentration; there was no correction for leakage of any of the tracers out of the red cells into the plasma. PPB was determined by ultracentrifugation (Centrifree micropartition system; Amicon Inc.) of 1 mL of arterial plasma: PPB = (1.0 – [ultrafiltrate concentration/plasma concentration]) × 100. A Beckman γ-counter system was used to determine the concentration of radioactivity in plasma, in red blood cells, and in urine samples with correction for ^{131}I scatter into the ^{99m}Tc window.

Healthy Volunteer Studies

All studies were performed with the approval of the Human Investigations Committee, and a signed consent form was obtained. *syn*- and *anti*-^{99m}Tc-D- and L-MAEC complexes were each evaluated in 3 healthy volunteers. HPLC-purified complexes and phosphate-buffered saline (pH 7.4) were passed through a Sep-Pak Plus C_{18} cartridge (Waters Co.) (primed with 1 mL of ethanol) and a sterile Millex-GS 22-μm filter (Millipore Co.) into a sterile, pyrogen-free empty vial. The final concentration was 37 MBq/mL (~1 mCi/mL) and the final pH was 7.4. Test samples of each complex were analyzed and determined to be sterile and pyrogen free. Approximately 74 MBq (~2 mCi) of each ^{99m}Tc-MAEC complex were coinjected with 7.4–11.1 MBq (200–300 μCi) ^{131}I-OIH, and imaging was performed using a General Electric camera with a 0.953 cm (0.375 in.) crystal fitted with a high-energy collimator; a 20% window was centered over the 365-keV photopeak of ^{131}I, and a second 20% window was centered over the 140-keV photopeak of ^{99m}Tc. Data were acquired in a 128 × 128 matrix using a 3-phase dynamic acquisition and processed on a General Electric StarCam computer using QuantEM renal software. Blood samples were obtained at 3, 5, 10, 20, 30, 45, 60, and 90 min after injection and plasma clearances for ^{131}I-OIH and each ^{99m}Tc-MAEC complex were determined using the single-injection, 2-compartment model of Sapirstein et al. (*25*). The volunteers voided at 30 and 180 min after injection to determine the percentage dose in the urine at each time period. For metabolism studies, a urine sample from the 30-min urine collection was obtained from each volunteer and analyzed by HPLC alone and with purified complex added.

Statistical Analysis

The statistical analysis was based on a 1-way ANOVA and paired *t* test. $P \leq 0.05$ was considered to be significant. Datasets with $n \leq 3$ were not analyzed.

RESULTS

Rat Studies

Biodistribution Studies. All 4 ^{99m}Tc-MAEC isomers (Fig. 2) showed a rapid blood clearance in rats with <10% of the injected dose remaining in the blood at 10 min after injection (Table 1). There was rapid urine excretion at 10 min as well as high specificity for renal excretion, with the dose in the urine at 60 min ranging from 84% to 99% that of ^{131}I-OIH (Table 1). *anti*-^{99m}Tc-D-MAEC and *anti*-^{99m}Tc-L-MAEC showed more hepatic uptake than ^{131}I-OIH at 10 min after injection and significantly more bowel activity at 60 min than ^{131}I-OIH ($P < 0.05$), indicating hepatobiliary transport. Uptake in the spleen, heart, and lungs was ≤ 0.4% of the injected dose for all isomers at both time periods.

Renal Clearance, EF, and PPB. The renal clearances, EFs, and PPB studies of the 4 complexes in rats are summarized in Table 2; historical ^{99m}Tc-MAG3 data are shown for comparison (*26*). To minimize the effect of the experimental conditions on the results, the clearance and EFs for each complex were normalized to the corresponding ^{131}I-OIH value in each rat. The ^{99m}Tc-MAEC/^{131}I-OIH clearance ratio for *syn*- and *anti*-^{99m}Tc-L-MAEC was significantly higher than that of *syn*- and *anti*-^{99m}Tc-D-MAEC ($P \leq 0.02$); similarly, the EF ratio of *syn*- and *anti*-^{99m}Tc-L-MAEC/^{131}I-OIH was also higher than that of the D-complexes ($P \leq 0.02$). PPB was moderately high (68%–96%) for all ^{99m}Tc-MAEC complexes. On the basis of the animal data, *syn*-^{99m}Tc-L-MAEC was the most promising complex, with a 78% protein binding, minimal bowel activity, and a clearance and extraction ratio comparable with those of ^{131}I-OIH.

Metabolism Studies in Rats. The urine was analyzed by HPLC to determine if the complexes were excreted intact. The ^{99m}Tc-L-MAEC complexes demonstrated *syn*-to-*anti* interconversion that was catalyzed in vivo. Approximately

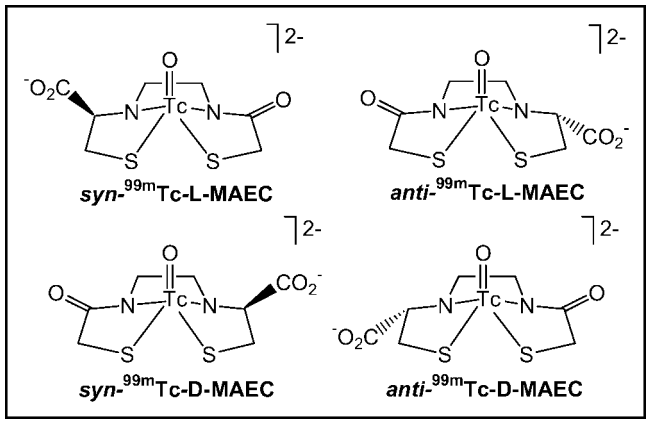

FIGURE 2. Isomers of ^{99m}Tc-MAEC.

TABLE 1
Percentage Injected Dose in Rats of *syn*- and *anti*-^{99m}Tc-D- and L-MAEC Isomers in Blood, Urine, and Selected Organs at 10 and 60 Minutes Compared with ^{131}I-OIH

	Blood		Kidney		Urine		Urine	Liver		Bowel	
Isomer	^{99m}Tc	^{131}I-OIH	^{99m}Tc	^{131}I-OIH	^{99m}Tc	^{131}I-OIH	% ^{99m}Tc/^{131}I-OIH	^{99m}Tc	^{131}I-OIH	^{99m}Tc	^{131}I-OIH
D-MAEC											
anti, 10 min	8.1 ± 1.1	4.3 ± 1.0	5.8 ± 0.3	4.3 ± 0.4	42.6 ± 5.0	59.2 ± 7.4	72	6.8 ± 1.1	2.3 ± 0.8	3.3 ± 0.7	0.9 ± 0.5
anti, 60 min	3.2 ± 0.2	0.6 ± 0.1	3.3 ± 0.2	0.4 ± 0.2	75.0 ± 4.5	89.4 ± 7.5	84	1.3 ± 0.0	0.4 ± 0.2	5.0 ± 1.8	0.8 ± 0.2
syn, 10 min	8.5 ± 2.2	5.3 ± 1.2	5.2 ± 3.7	5.4 ± 4.0	37.7 ± 6.7	48.1 ± 5.6	78	7.9 ± 4.3	2.9 ± 1.1	3.2 ± 0.2	1.6 ± 0.5
syn, 60 min	0.6 ± 0.2	0.6 ± 0.1	0.8 ± 0.3	0.3 ± 0.2	85.1 ± 9.7	91.9 ± 7.2	93	0.8 ± 0.3	0.3 ± 0.2	3.1 ± 0.8	0.7 ± 0.1
L-MAEC											
anti, 10 min	4.6 ± 0.6	4.4 ± 0.6	14.9 ± 2.5	5.7 ± 2.1	45.4 ± 6.0	58.8 ± 10.5	77	4.8 ± 0.9	2.8 ± 0.6	2.7 ± 0.5	1.0 ± 0.3
anti, 60 min	0.5 ± 0.1	0.6 ± 0.1	4.3 ± 0.7	0.4 ± 0.2	83.0 ± 4.3	91.3 ± 4.6	91	1.2 ± 0.2	0.4 ± 0.2	5.8 ± 3.6	0.8 ± 0.3
syn, 10 min	4.3 ± 0.9	4.3 ± 0.4	10.8 ± 2.4	6.8 ± 2.2	49.4 ± 7.8	56.7 ± 7.0	87	2.6 ± 0.6	2.2 ± 0.4	1.6 ± 0.2	1.1 ± 0.2
syn, 60 min	0.5 ± 0.7	0.7 ± 0.4	2.2 ± 0.4	0.5 ± 0.5	78.6 ± 23.4	78.9 ± 24.4	99	0.3 ± 0.2	0.5 ± 0.2	1.7 ± 0.6	0.8 ± 0.2

n = 5 for each data point with exception of *anti*-^{99m}Tc-D-MAEC at 10 min, where n = 3, and *anti*-^{99m}Tc-D-MAEC at 60 min, where n = 4.
Data are presented as mean ± SD.

20% of each injected agent converted to the corresponding isomer (*syn* to *anti* and *anti* to *syn*), giving the ratio of isomers in urine of 80% injected agent/20% corresponding isomer. The ^{99m}Tc-D-MAEC complexes did not demonstrate *syn*-to-*anti* conversion and each isomer was excreted unchanged in the urine.

Healthy Volunteer Studies

The PPB of the 4 ^{99m}Tc-MAEC isomers ranged from 82% to 89%; red cell binding ranged from 19% to 23% (Table 3). With the exception of *anti*-^{99m}Tc-L-MAEC, almost all the injected dose was recovered in the urine at 3 h. The clearance of *syn*-^{99m}Tc-D-MAEC averaged 74% that of ^{131}I-OIH compared with 49% for *syn*-^{99m}Tc-L-MAEC and 45% and 59% for *anti*-^{99m}Tc-L- and D-MAEC, respectively (Table 3), and is higher than the 50%–60% ratio reported for ^{99m}Tc-MAG3 (*1*). Image quality was excellent with all agents. Relative function measured with the 4 ^{99m}Tc-MAEC complexes was equivalent to relative function measured with ^{131}I-OIH; renogram parameters (time to peak [TTP] and the 20 min/max ratio for whole kidney regions of interest [ROIs]) were slightly more prolonged with the ^{99m}Tc-MAEC complexes than with ^{131}I-OIH (Table 4). Representative *syn*-^{99m}Tc-L-MAEC images, renogram curves, and simultaneous ^{131}I-OIH images and curves are shown in Fig. 3.

Metabolism Studies in Humans. A urine sample from the 30-min urine collection for each complex was obtained for HPLC analysis. The results were similar to those of the metabolism studies performed in rats. As observed in the rat, the *syn*-to-*anti* conversion in vivo occurred only for ^{99m}Tc-L-MAEC complexes and was present in a ratio of 80% injected isomer to 20% of the corresponding isomer. ^{99m}Tc-D-MAEC complexes showed no *syn*-to-*anti* conversion and each injected isomer was excreted unchanged in the urine.

DISCUSSION

^{131}I-OIH is typically used as the benchmark for new ^{99m}Tc agents because it provides a measure of ERPF and it is easy to measure in plasma and urine; however, the clearance of ^{131}I-OIH is about 10% less than the clearance of *p*-aminohippuric acid (PAH) and it is no longer commercially available in the United States. A new ^{99m}Tc tubular agent with a clearance comparable with that of ^{131}I-OIH would optimize renal imaging and allow a direct measurement of ERPF.

TABLE 2
Renal Clearance (mL/min/100 g), EF, and PPB of ^{99m}Tc-MAEC Isomers in Rats Compared with Simultaneously Injected ^{131}I-OIH (n = 6)

^{99m}Tc-MAEC	Clearance	Clearance ratio (^{99m}Tc/^{131}I-OIH)	EF (%)	EF ratio (^{99m}Tc/^{131}I-OIH)	% PPB
anti-L-MAEC	2.24 ± 0.24	85 ± 3	79 ± 6	105 ± 10	68 ± 4
syn-L-MAEC	2.86 ± 0.69	93 ± 11	76 ± 7	102 ± 10	78 ± 8
anti-D-MAEC	1.27 ± 0.52	41 ± 17	44 ± 18	57 ± 22	81 ± 6
syn-D-MAEC	1.77 ± 0.19	65 ± 10	62 ± 8	89 ± 10	96 ± 1
MAG3*	2.62 ± 0.46	92	82 ± 6	111	78 ± 4

*^{99m}Tc-MAG3 data are based on literature values (*2*).
Data are presented as mean ± SD.

TABLE 3
Clearance, Protein Binding, Red Cell Binding, and Urine Excretion of ^{99m}Tc-MAEC Complexes in Humans Compared with Simultaneously Injected ^{131}I-OIH (n = 3)

^{99m}Tc-MAEC	^{99m}Tc-MAEC clearance (mL/min)	^{131}I-OIH clearance (mL/min)	^{99m}Tc-MAEC/^{131}I-OIH clearance ratio (%)	Protein binding (%)	Red cell binding (%)	^{99m}Tc-MAEC/^{131}I-OIH 30-min urine ratio (%)	^{99m}Tc-MAEC/^{131}I-OIH 180-min urine ratio (%)
syn-L	305 ± 35	621 ± 20	49 ± 4	89.3 ± 0.6	20.3 ± 5.5	86 ± 2	98 ± 6
anti-L	254 ± 71	556 ± 133	45 ± 4	87.7 ± 1.5	19.0 ± 6.1	73 ± 12	97 ± 6
syn-D	431 ± 84	591 ± 150	74 ± 6	86.5 ± 2.1	20.3 ± 2.1	96 ± 2	104 ± 1
anti-D	277 ± 92	472 ± 87	59 ± 20	82.0 ± 5.2	22.7 ± 5.0	79 ± 16	100 ± 2

Data are presented as mean ± SD.

Wide clinical applicability would be enhanced by a radiopharmaceutical that contains only one isomer and exists as a single species under physiologic conditions. This goal is complicated by the observation that optimal tubular transport appears to require a pendant carboxyl group; this carboxyl group is often attached via a chiral carbon, and the coordination of a ligand bearing such pendant carboxyl group to the metal produces 2 isomers. (The carboxyl group can wrap close to the oxo group, *syn*, or away from the oxo group, *anti*.) (Fig. 2).

Quadridentate N_2S_2 and N_3S ligands used for chelation of the metals Tc and Re are linear and form stable M(V)=O complexes with the N and S donor atoms normally coordinating in the equatorial plane with the oxo ligand in an axial position. The S donors are terminal; the 2 N donors are interior and each anchors 2 chelate rings on complexation to the metal (in the N_3S system the third N is terminal). The N donors can be amide groups, amines, or a combination of the 2 donor types. Complexes with 2 secondary amine donors such as ^{99m}Tc-LL-EC and ^{99m}Tc-DD-EC have higher clearances than those of ^{99m}Tc(N_2S_2) and ^{99m}Tc(N_3S) renal agents that contain exclusively amide N donors (*1–3, 15,27*). However, solution studies of Re-LL-EC and its tetramethyl analog, Re-DD-TMEC, have shown that the complexes exist as a mixture of monoanionic CO_2^- ligated and dianionic CO_2^- deligated forms at physiologic pH (*16,17*). These ligated and deligated forms differ in charge, denticity, and structure; because of these differences, the 2 forms are highly unlikely to have comparable protein binding affinities, nor are they likely to be cleared at the same rate. The apparent protein binding and clearance of ^{99m}Tc-LL-EC reflects an average of the 2 species. Because the partition between the 2 species is pH dependent in the physiologic range, changes in renal clearance could reflect changes in pH rather than changes in renal function.

Each (LL, DD, or DL) EC ligand forms Re- and Tc-complexes with 2 geometric forms (*3*). In contrast, Re- and Tc-MAG3 have only one geometric form but, because they lack C_2 symmetry, they have 2 chiral forms. These forms have been separated and evaluated independently, but there are only small differences in plasma clearance and renal transit (*28*). The search for new agents led us to investigate analogs of these species with the goal of identifying the underlying chemistry that would permit us to develop agents that exist in only one form at physiologic pH.

We designed and investigated Re-MAEC because it has a combination of amide and amine donors and only one noncoordinating carboxyl group (*23*). Because the carboxyl group is electron withdrawing and separated by only 2 bonds from the NH group, the acidity of the NH group is

TABLE 4
Renogram Parameters of ^{99m}Tc-MAEC Complexes in Humans Compared with Simultaneously Injected ^{131}I-OIH Using Whole Kidney ROIs (n = 3)

	Left kidney				Right kidney		Left kidney		Right kidney	
^{99m}Tc-MAEC	% ^{99m}Tc-MAEC	% ^{131}I-OIH	TTP* (min) ^{99m}Tc-MAEC	TTP* (min) ^{131}I-OIH	TTP* (min) ^{99m}Tc-MAEC	TTP* (min) ^{131}I-OIH	20 min/max† ^{99m}Tc-MAEC	20 min/max† ^{131}I-OIH	20 min/max† ^{99m}Tc-MAEC	20 min/max† ^{131}I-OIH
syn-L	50 ± 2	52 ± 3	4.2 ± 1.7	3.2 ± 1.3	5.7 ± 2.7	3.2 ± 1.3	0.34 ± 0.12	0.16 ± 0.13	0.3 ± 0.06	0.14 ± 0.08
anti-L	58 ± 1	59 ± 4	6.1 ± 1.7	3.7 ± 0.6	6.5 ± 1.6	5.4 ± 1.7	0.63 ± 0.10	0.23 ± 0.14	0.64 ± 0.14	0.26 ± 0.15
syn-D	55 ± 1	55 ± 3	4.3 ± 0.9	3.8 ± 0.5	4.5 ± 0.5	3.6 ± 0.9	0.18 ± 0.06	0.12 ± 0.05	0.18 ± 0.05	0.12 ± 0.05
anti-D	54 ± 4	52 ± 5	4.7 ± 2.0	4.2 ± 2.0	6.0 ± 1.4	4.8 ± 2.3	0.41 ± 0.24	0.26 ± 0.19	0.43 ± 0.23	0.31 ± 0.18

*TTP = time-to-peak height of renogram curve.
†20 min/max ratio = counts in kidney at 20 min after injection divided by maximum counts.
Data are presented as mean ± SD.

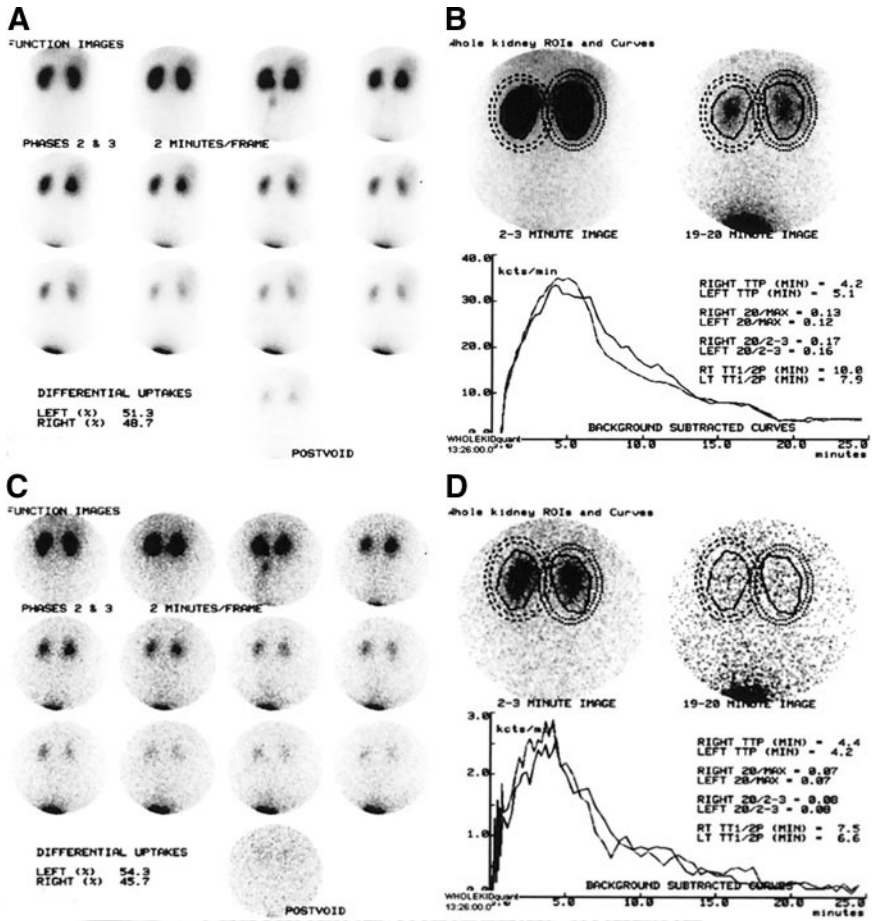

FIGURE 3. Data were acquired in 1 volunteer after simultaneous injection of 69.93 MBq (1.89 mCi) *syn*-^{99m}Tc-D-MAEC and 11.47 MBq (0.31 mCi) ^{131}I-OIH; postvoid images were acquired ~30 min after injection. The 2-min *syn*-^{99m}Tc-D-MAEC images (A) and renogram curves (B) can be compared with simultaneously acquired ^{131}I-OIH images (C) and renogram curves (D). (A) Sequential 2-min images after injection of 69.93 MBq (1.89 mCi) *syn*-^{99m}Tc-D-MAEC; differential (relative) uptake is 51.3% in left kidney and 48.7% in right kidney. Final image is postvoid image obtained ~30 min after injection. (B) Renogram curves and curve parameters based on whole kidney ROIs assigned over kidneys in A. Whole kidney and background ROIs are superimposed over the 2- to 3- and 19- to 20-min images at top. TTP refers to time-to-peak height of renogram curve and values are expressed in minutes; 20/max expression refers to ratio of counts at 20 min divided by maximum counts; 20/2–3 expression refers to ratio of counts at 19–20 min divided by counts at 2–3 min; TT1/2P refers to time (minutes) for counts in renogram curve to fall to half of peak value. RT = right; LT = left. (C) Sequential 2-min images after injection of 11.47 MBq (0.31 mCi) ^{131}I-OIH. ^{131}I-OIH images were acquired simultaneously as *syn*-^{99m}Tc-D-MAEC images in A. Differential (relative) uptake is 54.3% in left kidney and 45.7% in right kidney. Final image is postvoid image obtained ~30 min after injection. (D) Renogram curves and curve parameters based on whole kidney ROIs assigned over kidneys in A and applied to ^{131}I-OIH image data (C). Whole kidney and background ROIs are superimposed over 2- to 3- and 19- to 20-min images at top. TTP, 20/max expression, 20/2–3 expression, and TT1/2P are as described in B.

enhanced. Moreover, the amide donor bears a negative charge and is deprotonated; consequently, the metal–MAEC complex has only one dissociable NH group, and it was reasonable to expect that dissociation of the NH would occur outside the physiologic pH range. This is, in fact, what occurs. We found that the pK_a of the single amine in the Re-MAEC complexes was near pH 6 and the dianionic form predominates (96%) at physiologic pH (*23*,*24*).

In general, the rat has been a good model for predicting the behavior of ^{99m}Tc renal radiopharmaceuticals; however, interspecies differences do occur and there is no substitute for human testing. For example, *syn*-^{99m}Tc-L-MAEC is the best ^{99m}Tc-MAEC complex in rats, whereas *syn*-^{99m}Tc-D-MAEC is clearly the best isomer in humans. The fact that the clearance of the ^{99m}Tc-MAEC complexes exceeds the glomerular filtration rate indicates that these complexes must be transported by the renal tubules and, as anionic tracers, they likely share the same tubular transport process as for ^{131}I-OIH, ^{99m}Tc-MAG3, and ^{99m}Tc-EC. ^{99m}Tc-MAG3, ^{99m}Tc-LL- and -DD-EC, and *syn*-^{99m}Tc-CO_2DADS (*syn*-bis-mercaptoacetamidopropanoate) are all cleared rapidly by the renal tubules and, like the best ^{99m}Tc-MAEC complex in humans (*syn*-^{99m}Tc-D-MAEC), all contain an oxo-technetium-glycyl sequence (O=Tc-N-C-CO_2^-) (*1–3*,*27*) with, except for ^{99m}Tc-MAG3, a CO_2^- group *syn* to the oxo ligand. However, for ^{99m}Tc-MAG3, the terminal glycyl res-

idue is conformationally flexible and conformers are possible with a *syn* relationship of the carboxyl to the oxo ligand. The *anti*-^{99m}Tc-CO_2DADS isomer is not rapidly excreted in the urine (*27*) and the *anti*-^{99m}Tc-MAEC complexes have prolonged half-time values compared with the *syn*-complexes. These structure–distribution relationships strongly suggest that the combination of the oxo group and the carboxyl moiety is responsible for receptor recognition, with the *syn* relationship being the preferred structure in humans.

The fact that *syn*- and *anti*-^{99m}Tc-L-MAEC complexes convert to the corresponding isomers (*syn* to *anti* and *anti* to *syn*) in vivo is an interesting observation. Since this conversion does not occur in vitro at physiologic pH, the conversion must be catalyzed by enzymatic activity. Furthermore, the enzyme only recognizes the L configuration since *syn*-to-*anti* interconversion does not occur for the ^{99m}Tc-D-MAEC isomers. The enzyme is probably located in the proximal tubular cell, although further testing must be performed to confirm this hypothesis. If the enzyme is identified and confirmed to reside in the cells of the renal tubules, *syn*-^{99m}Tc-L-MAEC could potentially be used as a probe to monitor enzyme activity in disease states.

CONCLUSION

In summary, these data provide a better understanding of the structure–function relationships of the ^{99m}Tc-MAEC complexes and will be helpful in the design of future renal radiopharmaceuticals. *syn*-^{99m}Tc-D-MAEC can be prepared as a single species (94% *syn*) when labeled at high pH. Initial data in humans suggest that its clearance is greater than that of ^{99m}Tc-MAG3 and is comparable to the clearance reported for ^{99m}Tc-LL-EC. Nevertheless, the clearance of ^{99m}Tc-MAEC is still less than those of ^{131}I-OIH and PAH, and further ligand design and testing are required to develop a ^{99m}Tc renal tracer that will provide a direct measurement of ERPF.

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