First Evaluation of a ^{99m}Tc-Tricarbonyl Complex, $^{99m}Tc(CO)_3$(LAN), as a New Renal Radiopharmaceutical in Humans

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^{99m}Tc-Mercaptoacetyltriglycine (^{99m}Tc-MAG3), ^{99m}Tc-DD- and LL-ethylene-dicysteine (^{99m}Tc-EC), and ^{99m}Tc-mercaptoacetamide-ethylene-cysteine (^{99m}Tc-MAEC) contain N_3S or N_2S_2 ligands designed to accommodate the 4 ligating sites of the $(^{99m}TcO)^{3+}$ core; they are all excellent renal imaging agents but have renal clearances lower than that of ^{131}I-orthoiodohippurate (^{131}I-OIH). To explore the potential of the newly accessible but less polar $[^{99m}Tc(CO)_3]^+$ core with 3 ligating sites, we decided to build on the success of ^{99m}Tc-EC, with its N_2S_2 ligand and 2 dangling carboxylate groups; we chose an N_2S ligand that also has 2 dangling carboxylate groups, lanthionine, to form $^{99m}Tc(CO)_3$(LAN), a new renal radiopharmaceutical. **Methods:** Biodistribution studies were performed on Sprague–Dawley rats with $^{99m}Tc(CO)_3$(LAN) isomers, *meso*-LAN and DD,LL-LAN (an enantiomeric mixture), coinjected with ^{131}I-OIH. Human studies also were performed by coinjecting each ^{99m}Tc-labeled product (~74 MBq [~2 mCi]) and ^{131}I-OIH (~7.4 MBq [~0.2 mCi]) into 3 healthy volunteers and then performing dual-isotope imaging by use of 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:** Biodistribution studies in rats revealed rapid blood clearance as well as rapid renal extraction for both preparations, with the dose in urine at 60 min averaging 88% that of ^{131}I-OIH. In humans, both agents provided excellent renal images, with the plasma clearance averaging 228 mL/min for $^{99m}Tc(CO)_3$(*meso*-LAN) and 176 mL/min for $^{99m}Tc(CO)_3$(DD,LL-LAN). At 3 h, both $^{99m}Tc(CO)_3$(*meso*-LAN) and $^{99m}Tc(CO)_3$(DD,LL-LAN) showed good renal excretion, averaging 85% and 77% that of ^{131}I-OIH, respectively. Plasma protein binding was minimal (10% and 2%, respectively), and erythrocyte uptake was similar (24% and 21%, respectively) for $^{99m}Tc(CO)_3$ (*meso*-LAN) and $^{99m}Tc(CO)_3$(DD,LL-LAN). **Conclusion:** Although the plasma clearance and the rate of renal excretion of the $^{99m}Tc(CO)_3$(LAN) complexes were still lower than those of ^{131}I-OIH, the results of this first application of a ^{99m}Tc-tricarbonyl complex as a renal radiopharmaceutical in humans demonstrate that $^{99m}Tc(CO)_3$(LAN) complexes are excellent renal imaging agents and support continued renal radiopharmaceutical development based on the ^{99m}Tc-tricarbonyl core.

Key Words: ^{99m}Tc-tricarbonyl; lanthionine; $^{99m}Tc(CO)_3$(LAN); renal radiopharmaceuticals

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The development of technetium radiopharmaceuticals has relied heavily on the $(TcO)^{3+}$ core, with technetium in its +5 oxidation state, which is readily accessible by pertechnetate reduction in the presence of chelating ligands. Recently, the numerous synthetic advantages of the ^{99m}Tc-labeled water-stable organometallic precursor, $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (with ^{99m}Tc in its low +1 oxidation state), have shifted the focus of ^{99m}Tc-labeled radiopharmaceutical development to agents with a *fac*-$[^{99m}Tc(CO)_3]^+$ core (*1–11*). Both cores are compact, form kinetically inert agents with suitable ligands, and are versatile for labeling many types of bioactive molecules. However, the *fac*-$[^{99m}Tc(CO)_3]^+$ moiety is nonpolar, has an almost spheric shape, and offers only 3 sites on an octahedral face for ligand attachment. N_2S_2 and N_3S ligands designed to accommodate ligand attachment for the 4 coplanar sites of the polar $(TcO)^{3+}$ core are generally unsuitable for the *fac*-$[^{99m}Tc(CO)_3]^+$ core; consequently, new ligands are needed.

To date, renal radiopharmaceuticals designed around the $(TcO)^{3+}$ core have not achieved renal clearance in humans comparable to that of orthoiodohippurate (OIH). Thus, it seemed justified to redirect some of our effort to applying the tricarbonyl core approach to the goal of improving the performance of $(TcO)^{3+}$ renal imaging agents. Our studies are focusing on relatively small ligands containing at least 3 N, O, or S ligating atoms (*12,13*). An important focus of our work was to determine whether the effects of the less polar tricarbonyl core on the biodistribution and pharmacokinetics of a radiopharmaceutical designed around this core would preclude developing tracers with high renal clearance. One of the first tridentately coordinating ligands that we selected to exploit for the *fac*-$[^{99m}Tc(CO)_3]^+$ core in the design of a novel renal radiopharmaceutical was lanthionine (3,3′-thiodialanine; $LANH_2$) (Fig. 1). We selected this design because it mirrors that of one of the best

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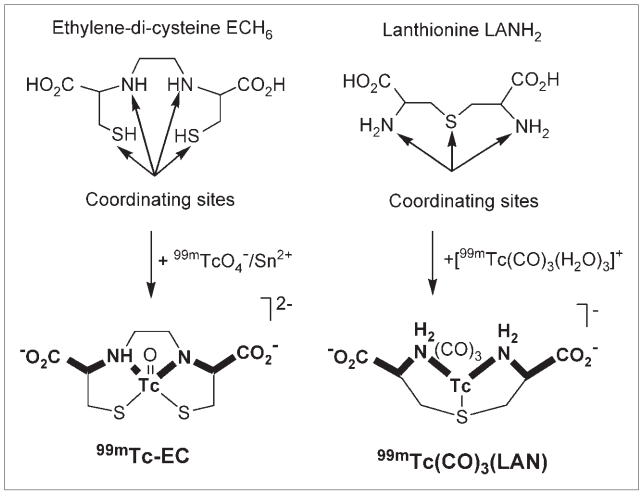

FIGURE 1. Comparison of ^{99m}Tc-EC and ^{99m}Tc(CO)$_3$(LAN), 2 agents with 2-carboxylate structural features.

N_2S_2 renal imaging agents, ^{99m}Tc-ethylene-dicysteine (^{99m}Tc-EC), in that it contains an O_2C-CH_2-NH-Tc-NH-CH_2-CO_2 sequence as well as 2 dangling carboxylate groups (Fig. 1). This similarity to ^{99m}Tc-EC and the promising initial results in rats led us to select this agent among those under study in our laboratories for our first assessment of a ^{99m}Tc-tricarbonyl core agent in humans. This report describing the biodistribution, excretion, and imaging characteristics of the new renal imaging agent, ^{99m}Tc(CO)$_3$(LAN), in fact presents one of the first human studies with any type of radiopharmaceutical containing the ^{99m}Tc-tricarbonyl core.

MATERIALS AND METHODS

All chemicals and solvents were of reagent grade and were used without further purification. $LANH_2$, a mixture of DD-, LL-, and *meso*-(DL)-LAN isomers, was purchased from TCI America. ^{99m}Tc-Pertechnetate ($^{99m}TcO_4^-$) was eluted from a ^{99}Mo/^{99m}Tc generator (Amersham Health) with 0.9% saline. High-performance liquid chromatography (HPLC) analyses were performed by use of a Beckman System Gold Nouveau apparatus (for rat studies) and a Beckman System Gold Bioessential apparatus (for human studies) equipped with a model 170 radiometric detector, a model 166 ultraviolet light–visible light detector, and 32 Karat chromatography software; a Beckman C_{18} RP Ultrasphere octyldecyl silane 5-μm column (4.6 × 250 mm), a flow rate of 1 mL/min, and a mobile phase of ethanol (12%) and tetraethylammonium phosphate buffer (0.05 mol/L; pH 2.5) were used. The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor was prepared directly from $^{99m}TcO_4^-$ in saline solution under 1 atm of CO as described previously (*14*).

^{99m}Tc Radiolabeling

$LANH_2$ (1 mg) was dissolved in 1N HCl (0.1 mL), and the pH of the solution was adjusted to ~9 with 1N NaOH. A sample (0.1 mL) of this solution was added to 1 mL of the freshly prepared $[^{99m}Tc(CO)_3(H_2O)_3]^+$ solution; the mixture was heated at 70°C for 30 min, cooled to room temperature, and analyzed by HPLC to show 3 resolved HPLC peaks with the following retention times: 6, 8, and 10 min (a minor peak). All 3 complexes were isolated by HPLC, and their radiochemical purities were found to be greater than 98%. The first eluting peak was assigned as ^{99m}Tc(CO)$_3$(*meso*-LAN), and the second peak was assigned as ^{99m}Tc(CO)$_3$(DD,LL-LAN) (an enantiomeric mixture of DD- and LL-LAN isomers). Those 2 complexes were buffered to pH 7.4 and tested by HPLC for stability up to 6 h; no measurable decomposition was observed, and they were tested in rats and humans. The third peak represented the less stable isomer containing the *meso*-LAN ligand and was not used in our studies. We assigned those configurations to the ^{99m}Tc(CO)$_3$(LAN) isomers because similar results were obtained for Re(CO)$_3$(LAN) complexes, which we have fully characterized by analytic and spectroscopic methods (*15*).

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. ^{99m}Tc(CO)$_3$(*meso*-LAN) and ^{99m}Tc(CO)$_3$(DD,LL-LAN) complexes were each evaluated in 5 Sprague–Dawley rats at 10 and 60 min. A solution of each ^{99m}Tc-labeled 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 samples were drawn into insulin syringes. Five samples were used for doses; the sixth sample 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 of body weight) injected intramuscularly, with additional supplemental anesthetic as needed. The bladder was catheterized by use of heat-flared PE-50 tubing (Becton, Dickinson and Co.) for urine collection.

The radiopharmaceutical solution was injected intravenously via a tail vein; 5 animals were sacrificed at 10 min after injection, and 5 animals were sacrificed at 60 min after injection. A blood sample was obtained, and the heart, lungs, spleen, liver, intestines, stomach, and kidneys 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. The radioactivity of the sample and standards was measured by use of a dual-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 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 value given for the bowel represents combined stomach and intestine activities. The percentage injected dose in whole blood was estimated by assuming a blood volume of 6.5% of total body weight.

Metabolism Studies. Rats were prepared according to the procedure described above for the biodistribution studies. A bolus injection of each ^{99m}Tc(CO)$_3$(LAN) complex (~7.4 MBq [0.2 mCi]) was intravenously administered to 2 rats; urine was collected for 30 min and analyzed by HPLC alone and with a purified complex added to determine whether the complex was metabolized or excreted unchanged in the urine.

Healthy Volunteer Studies

All studies were performed with the approval of the Radioactive Drug Research Committee and the Emory University Institutional Review Board; signed consent was obtained from each

volunteer. Six healthy volunteers (4 men and 2 women; mean ± SD age, 30.3 ± 5.5 y; range, 25–38 y) participated in this study. Inclusion criteria were the absence of any history of kidney and bladder diseases and a normal review of systems. Pregnancy was excluded in women by means of a urine pregnancy test. Measurements of blood pressure, heart rate, and temperature were taken before and after injection for each volunteer; in addition, a complete blood count, standard chemistry panel, and urinalysis were obtained before and 24 h after injection. Volunteers were requested to drink approximately 500 mL of water before the study. $^{99m}Tc(CO)_3$(*meso*-LAN) and $^{99m}Tc(CO)_3$(DD,LL-LAN) 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 4 mL of ethanol) and a sterile Millex-GS 22-μm filter (Millipore Corp.) (primed with 4 mL of saline) 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 $^{99m}Tc(CO)_3$(LAN) complex were coinjected with 7.4–11.1 MBq (200–300 μCi) of ^{131}I-OIH, and imaging was performed by use of a General Electric Infinia camera with an ~1-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 with a 3-phase dynamic acquisition and processed on a General Electric Xeleris computer with 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 $^{99m}Tc(CO)_3$(LAN) complex were determined by use of the single-injection, 2-compartment model of Sapirstein et al. (*16*). The volunteers voided at 30, 90, and 180 min after injection to determine the percentage of the dose in urine at each time period. Plasma protein binding (PPB) was determined by ultrafiltration (Centrifree micropartition system; Amicon Inc.) of 1 mL of plasma: PPB = (1.0 − [ultrafiltrate concentration/plasma concentration]) × 100. A Beckman γ-counter system was used to determine the concentrations of radioactivity in plasma, in erythrocytes, and in urine samples, with correction for ^{131}I scatter into the ^{99m}Tc window. To determine whether the complex was metabolized or excreted unchanged in the urine, a 1-mL urine sample from the 30-min urine collection was obtained from each volunteer and analyzed by HPLC alone and with a purified complex added.

RESULTS

^{99m}Tc Radiolabeling

Lanthionine was effectively radiolabeled with ^{99m}Tc under mild conditions (30 min at 70°C, pH ~9) to form well-defined complexes with the ^{99m}Tc-tricarbonyl core at a high yield. In all complexes, the $LANH_2$ ligand coordinated tridentately and facially to yield a $^{99m}Tc(CO)_3(N_2S)$ coordination sphere, leaving both carboxyl groups uncoordinated. $^{99m}Tc(CO)_3$(*meso*-LAN) is a stable product of the *meso*-LAN ligand (there is also a less stable isomer containing the *meso*-LAN ligand that converts to a more stable product), and $^{99m}Tc(CO)_3$(DD,LL-LAN) is an enantiomeric mixture of DD- and LL-LAN isomers.

TABLE 1
Percentage Injected Dose in Rats of $^{99m}Tc(CO)_3$(*meso*-LAN) and $^{99m}Tc(CO)_3$(DD,LL-LAN) Compared with ^{131}I-OIH in Blood, Urine, and Selected Organs at 10 and 60 Minutes (n = 5)

Isomer	Blood		Kidneys		Urine			Liver		Bowel	
	^{99m}Tc	^{131}I-OIH	^{99m}Tc	^{131}I-OIH	^{99m}Tc	^{131}I-OIH	^{99m}Tc/^{131}I-OIH ratio	^{99m}Tc	^{131}I-OIH	^{99m}Tc	^{131}I-OIH
meso-LAN											
10 min	4.1 ± 0.4	3.3 ± 0.5	6.5 ± 2.8	4.9 ± 3.5	41.0 ± 7.3	59.9 ± 11.5	69 ± 6	4.2 ± 0.6	2.0 ± 0.2	1.2 ± 0.3	1.0 ± 0.2
60 min	0.6 ± 0.1	0.5 ± 0.1	1.2 ± 0.3	0.7 ± 0.3	77.9 ± 3.2	87.5 ± 8.2	89 ± 6	1.2 ± 0.5	0.8 ± 0.5	1.7 ± 0.1	0.9 ± 0.2
DD,LL-LAN											
10 min	5.7 ± 0.7	4.0 ± 0.6	9.5 ± 0.9	4.6 ± 1.0	33.4 ± 3.9	59.2 ± 3.6	56 ± 4	3.9 ± 0.5	2.3 ± 0.2	1.7 ± 0.4	1.1 ± 0.2
60 min	0.7 ± 0.2	0.4 ± 0.1	1.2 ± 0.3	0.3 ± 0.1	76.4 ± 7.2	88.0 ± 7.8	87 ± 4	0.8 ± 0.3	0.5 ± 0.3	4.6 ± 0.7	0.9 ± 0.1

Data are mean ± SD.

Rat Biodistribution Studies

Both $^{99m}Tc(CO)_3$(*meso*-LAN) and $^{99m}Tc(CO)_3$(DD,LL-LAN) showed rapid blood clearance in rats, with less than 6% of the injected dose remaining in the blood at 10 min after injection (Table 1). Both complexes also demonstrated rapid renal extraction and high specificity for renal excretion; the mean ± SD doses in urine at 60 min (as a percentage of ^{131}I-OIH) were 89% ± 6% for $^{99m}Tc(CO)_3$ (*meso*-LAN) and 87% ± 4% for $^{99m}Tc(CO)_3$(DD,LL-LAN). Less than 1% of the total activity was present in the spleen, heart, and lungs; moreover, there was minimal gastrointestinal activity: 4.6% for $^{99m}Tc(CO)_3$(DD,LL-LAN) and 1.7% for $^{99m}Tc(CO)_3$(*meso*-LAN).

Healthy Volunteer Studies

There was no evidence of any toxicity, as determined by measurements of blood pressure, heart rate, or temperature, complete blood count, standard chemistry panel, or urinalysis, for any of the volunteers. The clearance of $^{99m}Tc(CO)_3$(*meso*-LAN) averaged 228 mL/min, and that of $^{99m}Tc(CO)_3$(DD,LL-LAN) was 176 mL/min (Table 2); both clearances were substantially lower than the clearance of 538 mL/min for ^{131}I-OIH. PPB was minimal for both $^{99m}Tc(CO)_3$(LAN) isomers and averaged 10% for *meso*-LAN and 2% for DD,LL-LAN. Erythrocyte uptake levels were similar for the 2 isomers: 24% for *meso*-LAN and 21% for DD,LL-LAN. Both complexes had relatively rapid renal excretion, with the difference being that the DD,LL-LAN isomer was excreted more slowly than the *meso*-LAN isomer; the activities in urine [as a percentage of ^{131}I-OIH, i.e., $^{99m}Tc(CO)_3$(LAN)/^{131}I-OIH] at 30 and 180 min were 57% ± 6% and 85% ± 6%, respectively, for *meso*-LAN and 45% ± 3% and 77% ± 6%, respectively, for DD,LL-LAN (Table 2). Image quality was excellent with both agents (Fig. 2). The time to peak appeared to be slightly more prolonged with $^{99m}Tc(CO)_3$)(LAN) complexes than with ^{131}I-OIH, and ratios of counts in kidneys at 20 min after injection to maximum counts for whole-kidney and cortical regions of interest appeared to be higher (Table 3). Representative $^{99m}Tc(CO)_3$) (LAN) images and renogram curves, as well as simultaneous ^{131}I-OIH images and curves, are shown in Figure 2.

Metabolism Studies

Urine was analyzed by HPLC to determine whether the complexes were excreted intact. Greater than 95% of the activity recovered in urine from both rats and humans coeluted with the respective HPLC-purified $^{99m}Tc(CO)_3$(*meso*-LAN) and $^{99m}Tc(CO)_3$(DD,LL-LAN) tracers, proving that each complex was excreted unchanged (Fig. 3).

DISCUSSION

A major focus of our research has been to develop radiopharmaceuticals possessing high renal clearance (*13,17–22*). To obtain an agent with high renal clearance, ^{99m}Tc-labeled peptides and ligands are designed to target the organic anion tubular transporter of the proximal tubule (*17,22,23*). Small peptides are easy to synthesize and modify, are less likely than typical ligands to be immunogenic, and are more likely to exhibit rapid blood clearance. In most cases, the primary sites of interactions of the peptides are specific receptors on the outer surface of the cell membrane (extracellular). Thus, ^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG3), ^{99m}Tc-EC, and ^{99m}Tc-mercaptoacetamide-ethylene-cysteine (^{99m}Tc-MAEC) (*22*) are excreted primarily by tubular secretion, whereas the nonpeptide ^{99m}Tc-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) is excreted by glomerular filtration and has a relatively low clearance compared with the other ^{99m}Tc-labeled renal agents.

All of these factors make small peptides excellent candidates for the development of target-specific radiopharmaceuticals. However, as mentioned earlier, agents based on the newer peptide ligands, although having clearances higher than that of ^{99m}Tc-MAG3, still have clearances lower than those of ^{131}I-OIH and *p*-aminohippurate.

In an effort to define new cores for exploring ligands that could produce a superior ^{99m}Tc-labeled tubular agent, we decided to investigate the potential of the $[^{99m}Tc(CO)_3]^+$ core. This core recently attracted growing interest, particularly after Alberto et al. reported an aqueous preparation of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ precursor (*14,24*) and the introduction of the IsoLink boranocarbonate kit (Mallinckrodt).

As a relatively soft receptor, the $[^{99m}Tc(CO)_3]^+$ core prefers ligands with soft sp^2 aromatic nitrogen and thioether donors (*25–28*). A bifunctional approach that incorporates ligating groups, such as pyridyl or imidazole groups, into amino acids or peptides has proved successful in labeling of the $[^{99m}Tc(CO)_3]^+$ core (*2,29*). However, we avoided incorporating pyridine rings into ligands to

TABLE 2
Clearance, Protein Binding, Erythrocyte Binding, and Urine Excretion of $^{99m}Tc(CO)_3$(LAN) Complexes Compared with ^{131}I-OIH in Humans (n = 3)

Isomer	$^{99m}Tc(CO)_3$(LAN) clearance (mL/min)	^{131}I-OIH clearance (mL/min)	$^{99m}Tc(CO)_3$(LAN)/^{131}I-OIH clearance ratio (%)	Protein binding (%)	Erythrocyte binding (%)	$^{99m}Tc(CO)_3$(LAN)/^{131}I-OIH 30-min urine excretion ratio (%)	$^{99m}Tc(CO)_3$(LAN)/^{131}I-OIH 180-min urine excretion ratio (%)
meso-LAN	228 ± 33	548 ± 37	42 ± 5	10 ± 0.6	24 ± 3.6	57 ± 6	85 ± 6
DD,LL-LAN	176 ± 8	528 ± 13	33 ± 2	2 ± 0.0	21 ± 8.6	45 ± 3	77 ± 6

Data are mean ± SD.

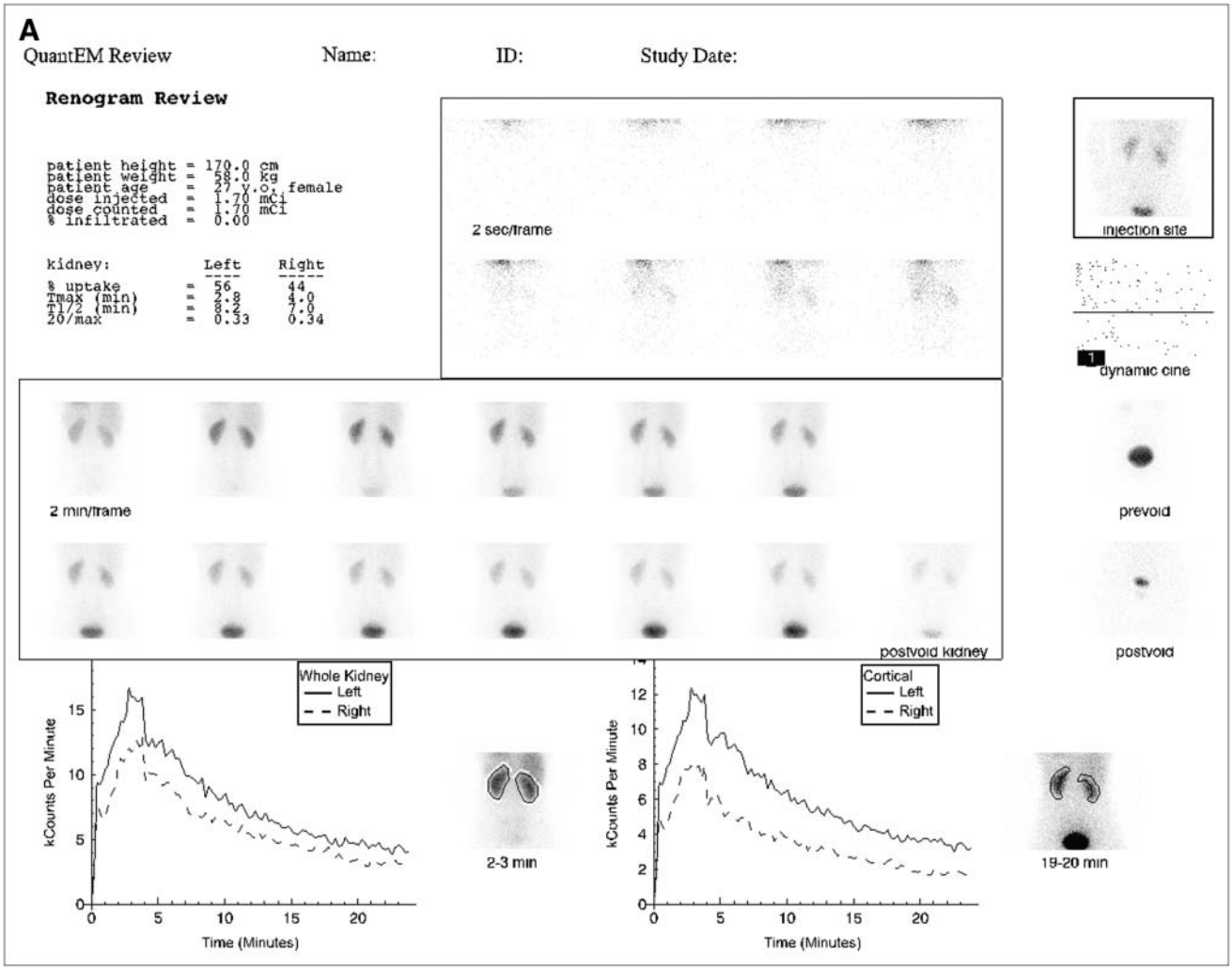

FIGURE 2. (A) $^{99m}Tc(CO)_3$(LAN) images and curves obtained from 27-y-old female volunteer who received an intravenous injection containing 62.9 MBq (1.7 mCi) of $^{99m}Tc(CO)_3$(*meso*-LAN) and 7.03 MBq (0.19 mCi) of ^{131}I-OIH before 24 min of data acquisition. Demographics and renogram data—relative uptake (% uptake), time to maximum counts [Tmax (min)], time to half-maximum counts [T1/2 (min)], and ratio of counts at 20 min to maximum counts (20/max) for whole-kidney region of interest—are displayed in upper left panel. Upper middle panel shows flow images at 2 s per frame. Image containing injection site in volunteer's arm (upper right panel) showed no infiltration. Center panel shows good uptake by kidneys bilaterally and prompt excretion into bladder. Whole-kidney renogram curves are shown in lower left panel, and cortical renogram curves are shown in lower right panel. (B) ^{131}I-OIH images and renogram curves obtained from volunteer described in A by use of identical regions of interest over whole kidney and cortex. ^{131}I-OIH renogram curves are much noisier than $^{99m}Tc(CO)_3$(LAN) renogram curves because of lower dose of ^{131}I-OIH and because camera was not optimized to image high-energy photons of ^{131}I.

enhance labeling because pyridine rings tend to raise the overall lipophilicity of a complex; the latter situation usually leads to labeled agents with high levels of hepatobiliary uptake, an undesirable property in a renal radiopharmaceutical (*30*).

Lanthionine (Fig. 1) is a small peptide (dipeptide) containing 2 free carboxyl groups that would be recognized by the anionic renal tubular transport system. Moreover, it is a simple N_2S ligand that efficiently produces uniform products when labeled with the ^{99m}Tc-tricarbonyl core. In humans, only 10% of $^{99m}Tc(CO)_3$(*meso*-LAN) and 2% of $^{99m}Tc(CO)_3$(DD,LL-LAN) are protein bound. These protein-binding levels are much lower than those for ^{99m}Tc-MAG3 (PPB, ~80%), ^{99m}Tc-DD-EC (PPB, ~28%), or *syn*-^{99m}Tc-D-MAEC (PPB, ~87%). Reduced protein binding is a desirable property in a renal radiopharmaceutical because it facilitates clearance by glomerular filtration as well as tubular extraction (*31*). The clearance of both ^{99m}Tc-tricarbonyl agents exceeds the glomerular filtration rate; this fact indicates that these complexes must be transported by the renal tubules and, as anionic tracers, they likely share the same tubular transport process as ^{131}I-OIH, ^{99m}Tc-MAG3, ^{99m}Tc-EC, and ^{99m}Tc-MAEC.

All 3 renal tubular agents with the $(^{99m}TcO)^{3+}$ core and high renal clearance in humans, ^{99m}Tc-MAG3, ^{99m}Tc-DD-EC, and *syn*-^{99m}Tc-D-MAEC, contain an oxo-technetium-glycyl sequence with a CO_2^- group *syn* to the oxo ligand (*syn*-CO_2^-); structure–distribution relationships suggest that

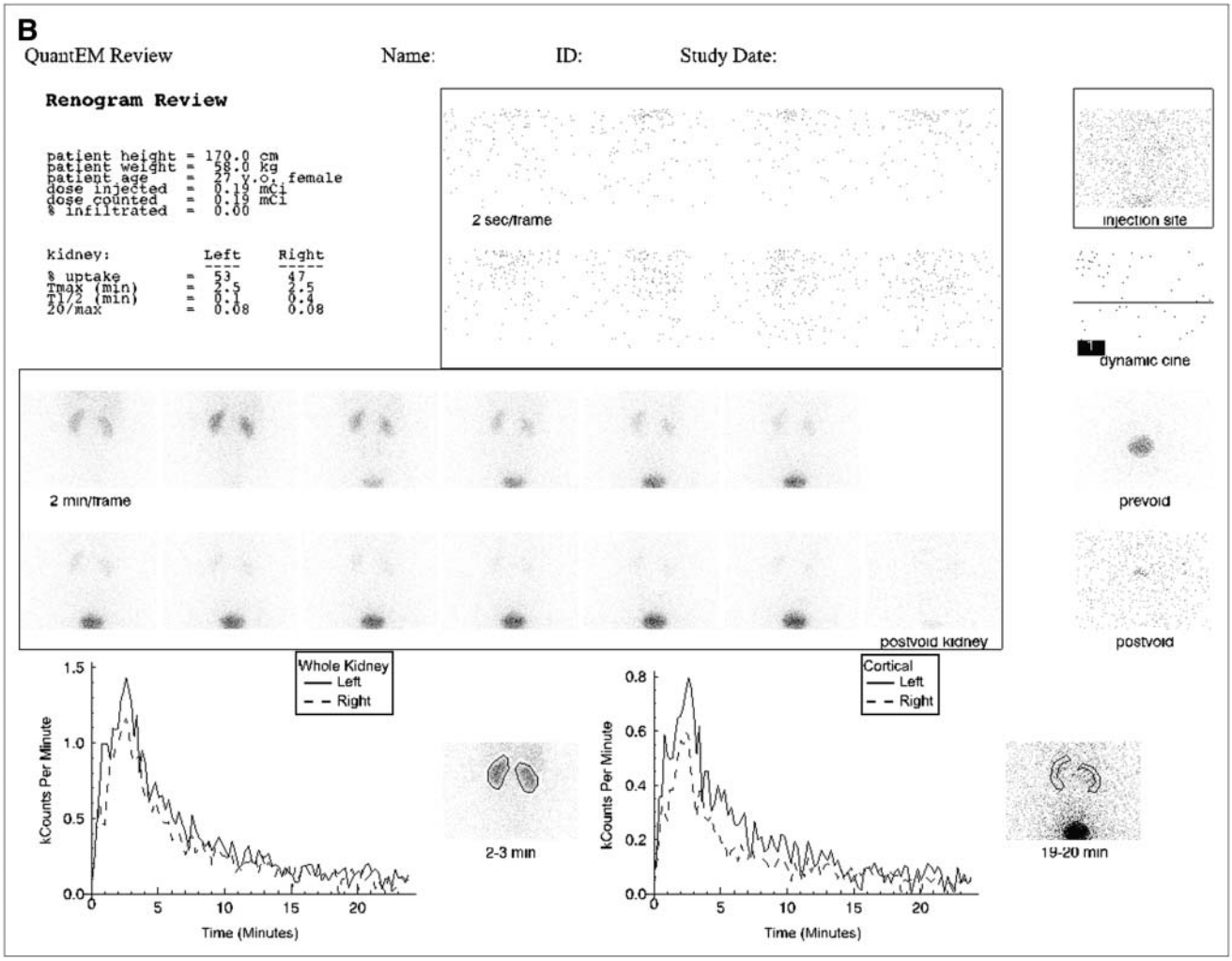

FIGURE 2. (Continued)

the combination of the oxo and *syn*-CO_2^- groups is responsible for receptor recognition (*32*). Results in rodents generally show a similar dependence, with *syn* isomers showing higher clearance than *anti* isomers for agents with 1 carboxyl group. In rodents, however, the results for ^{99m}Tc-EC isomers do not show this dependence.

Labeling of a mixture of DD-, LL-, and DL-EC ligands resulted in a mixture of products that were resolved by HPLC into 3 peaks, one for the complexes with the chiral ligands (^{99m}Tc-DD-EC and ^{99m}Tc-LL-EC) and one for each of the 2 *meso* forms (*syn*- and *anti*-^{99m}Tc-DL-EC). In mice, biodistribution studies showed no significant differences in renal excretion, hepatobiliary excretion, or blood clearance for any of the 3 peaks (*33–35*). In rats, clearance, extraction efficiency, and biodistribution results were almost identical for all 4 separated ^{99m}Tc-EC isomers (*17,36*). In humans, however, our results showed that ^{99m}Tc-DD-EC and ^{99m}Tc-LL-EC had similar clearances (^{99m}Tc-EC/^{131}I-OIH: 82% and 70%, respectively), which were significantly higher than the 40% clearance for *syn*-^{99m}Tc-DL-EC (*17*). Similarly, the percentage injected doses (^{99m}Tc-EC/^{131}I-OIH) in urine at 0–30 min were 90% and 92% for ^{99m}Tc-DD-EC and ^{99m}Tc-LL-EC, respectively; that for *syn*-^{99m}Tc-DL-EC was 57%.

Our new ^{99m}Tc-tricarbonyl agents are based on a completely different core with different physical properties and do not contain the oxo-technetium-glycyl sequence, but they still exhibit a high specificity for renal excretion. In rats, there was no difference in the excretion of the ^{99m}Tc(CO)$_3$(LAN) isomers at 60 min despite the absolute configurations of the asymmetric carbons; however, in humans, the *meso*-LAN isomer appeared to be superior to the DD,LL-LAN isomer (Table 2). It should be noted that ^{99m}Tc(CO)$_3$(DD,LL-LAN) should have been a superior tracer relative to ^{99m}Tc(CO)$_3$(*meso*-LAN) on the basis of a superficial analogy to ^{99m}Tc-EC biodistribution, because both agents contain 2 dangling carboxylate groups. This similar lack of dependence on stereochemistry in rodent biodistribution, combined with a different dependence on chiral versus *meso* stereochemistry, led us to analyze more thoroughly all of these structures to understand better the relationship between a particular structure and its renal clearance.

TABLE 3

Renogram Parameters for Whole-Kidney Regions of Interest with $^{99m}Tc(CO)_3$(*meso*-LAN) and $^{99m}Tc(CO)_3$(DD,LL-LAN) Compared with ^{131}I-OIH in Humans (n = 3)

	Left kidney				Right kidney		Left kidney		Right kidney	
Isomer	% ^{99m}Tc	% ^{131}I-OIH	TTP (min) for ^{99m}Tc	TTP (min) for ^{131}I-OIH	TTP (min) for ^{99m}Tc	TTP (min) for ^{131}I-OIH	20 min/max for ^{99m}Tc	20 min/max for ^{131}I-OIH	20 min/max for ^{99m}Tc	20 min/max for ^{131}I-OIH
meso-LAN	47 ± 8.1	46 ± 7.0	4.66 ± 2.49	2.65 ± 0.53	3.58 ± 0.32	3.08 ± 0.59	0.35 ± 0.10	0.12 ± 0.09	0.29 ± 0.03	0.07 ± 0.02
DD,LL-LAN	60 ± 2.1	62 ± 4.2	3.17 ± 0.62	3.15 ± 0.75	3.01 ± 0.57	3.55 ± 0.19	0.39 ± 0.07	0.12 ± 0.06	0.33 ± 0.08	0.08 ± 0.04

TTP = time to peak height of renogram curve; 20 min/max = ratio of counts in kidney at 20 min after injection to maximum counts.
Data are mean ± SD.

FIGURE 3. Urine samples from human volunteers injected with $^{99m}Tc(CO)_3$(*meso*-LAN) (A) and $^{99m}Tc(CO)_3$(DD,LL-LAN) (B) were subjected to γ-radioactive reversed-phase HPLC analysis. Corresponding reference HPLC traces show that both complexes were excreted unchanged in urine.

The 2 CO_2 groups project in opposite directions in the isomer with the higher clearance and higher rate of excretion in urine, $^{99m}Tc(CO)_3$(*meso*-LAN), and in the same direction in the isomer with the lower clearance and lower rate of excretion in urine, $^{99m}Tc(CO)_3$(DD,LL-LAN) (Fig. 4). In this regard, our new results parallel those obtained with ^{99m}Tc-EC agents. For both DD- and LL-EC isomers, the 2 CO_2 groups are on opposite sides of the structures. The

FIGURE 4. Schematic drawing of spatial relationships of carboxylate groups (CO_2) to each other and to plane defined by donor atoms in ^{99m}Tc-EC and $^{99m}Tc(CO)_3$(LAN) complexes.

lower extraction efficiency for the DL-EC isomer and ^{99m}Tc(CO)$_3$(DD,LL-LAN) may be attributable to the steric properties of 2 bulky carboxylate groups (CO_2) on the same side of the molecule or to electrostatic effects because the 2 CO_2 groups are ionized and in close proximity to each other (Fig. 4). This feature appears to affect biodistribution in humans but not in rodents for agents with 2 carboxyl groups. This difference in the way in which the carboxyl groups project between the more classical ^{99m}Tc-EC agents and our new ^{99m}Tc(CO)$_3$(LAN) agents is a direct consequence of the stereochemistry imposed by the cores; the Tc-tricarbonyl core imposes a triangular facial ligand coordination in an agent with a pseudooctahedral geometry, and the Tc-oxo core imposes a planar square-like ligand coordination in an agent with a pseudosquare-pyramidal geometry. It is interesting that 1 carboxyl group in the *meso* compound is situated very close to a carbonyl group, yet this agent has very high clearance. These findings offer hope that the effects of the relatively nonpolar carbonyl groups may not have an adverse effect on the recognition of the tracer by the proximal tubular receptor.

Another approach toward understanding the effects of changes at the Tc center on the biologic properties of ^{99m}Tc-labeled radiopharmaceuticals involves a comparison of complexes containing the same chelating ligand but different Tc cores. In recent biodistribution experiments with mice, Rattat et al. (*37*) studied the characteristics of 3 different DTPA complexes: ^{99m}Tc-DTPA (with a Tc-oxo core), ^{99}Tc(CO)$_3$(DTPA) (with a Tc-tricarbonyl core), and ^{99m}Tc(CO)$_2$(NO)(DTPA) (with a Tc-dicarbonyl-nitrosyl core). ^{99m}Tc-DTPA, a renal imaging radiopharmaceutical with the "classic" core, was excreted rapidly by the kidneys and had a low overall uptake in all other organs. Labeling of DTPA with the ^{99m}Tc-tricarbonyl core led to an agent with a decreased excretion rate, a slightly higher liver uptake, and a longer retention in blood. Introduction of the ^{99m}Tc-dicarbonyl-nitrosyl core resulted in a significant increase in liver uptake, whereas excretion by the kidneys dropped to a negligible level, compared with the results for ^{99m}Tc-DTPA. These 3 different DTPA agents showed different physical and biological characteristics, and these differences can be attributed to the consequences of the modifications at the Tc center. However, because the exact chemical speciation of ^{99m}Tc(CO)$_3$(DTPA) and ^{99m}Tc(CO)$_2$(NO)(DTPA) has not been defined (*38*), the extent to which the spatial relationships of the carboxyl groups to each other and to the different cores influence biodistribution is unclear, and further studies are needed.

CONCLUSION

Results in rats showed that both ^{99m}Tc(CO)$_3$(LAN) isomers are rapidly excreted in the urine and have a high specificity for renal excretion. Moreover, we described the first application of a ^{99m}Tc-tricarbonyl renal radiopharmaceutical in humans, and our results offer promise that a complex based on the $[Tc(CO)_3]^+$ core could be an excellent renal imaging agent with a high plasma clearance. Although the plasma clearance and the rate of renal excretion were still lower than those for ^{131}I-OIH, these data provide support for the continued development of renal and other radiopharmaceuticals based on the ^{99m}Tc-tricarbonyl core. Additional ligand design and testing will be required to develop a ^{99m}Tc-labeled renal tracer that will provide a direct measurement of effective renal plasma flow.

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