Comparison of Technetium-99m-LL-EC Isomers in Rats and Humans

Andrew Taylor, Lory Hansen, Dennis Eshima, Eugene Malveaux, Russell Folks, Lorie Shattuck, Malgorzata Lipowska and Luigi G. Marzilli

Division of Nuclear Medicine, Department of Radiology, Emory University School of Medicine and Department of Chemistry, Emory University, Atlanta, Georgia

Technetium-99m-L,L-ethylenedicysteine (^{99m}Tc-LL-EC) is a new renal imaging agent with pharmacokinetic properties reported to be slightly superior to those of ^{99m}Tc-mercaptoacetyltriglycine (^{99m}Tc-MAG3); however, to better define the potential of the enantiomer ^{99m}Tc-DD-EC and the diastereomer ^{99m}Tc-DL-EC as renal imaging agents, we compared the three EC stereoisomers with ^{131}I-ortho-iodohippurate (OIH) in a series of rats and humans. **Methods:** Each ^{99m}Tc-EC stereoisomer was coinjected with OIH in six Sprague-Dawley rats for measurements of clearance and extraction fraction. Each stereoisomer was also coinjected with OIH in three human volunteers followed by sequential imaging, plasma clearance measurements and timed urine collections. **Results:** Technetium-99m-DD-EC had the highest clearance and extraction efficiency in rats (p ≤ 0.02). In humans, image quality was good with all three agents. The clearance ratio (EC/OIH) was 82% ± 8% for ^{99m}Tc-DD-EC compared to 70% ± 3% and 40% ± 5% for ^{99m}Tc-LL-EC and ^{99m}Tc-DL-EC, respectively. Technetium-99m-DD and ^{99m}Tc-LL-EC were excreted more rapidly than ^{99m}Tc-DL-EC. **Conclusion:** Technetium-99m-DD-EC has excellent imaging properties and the data suggest that its clearance may approach that of OIH more closely than any other ^{99m}Tc renal agent. A potential limitation is the fact that both ^{99m}Tc-DD and LL-EC exist in dianionic (80%) and monoanionic (20%) forms at physiological pH and it is unlikely that these two forms have the same clearance or protein binding affinity.

Key Words: technetium-99m-DD-EC; technetium-99m-LL-EC; technetium-99m-DL-EC; renal scintigraphy

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During the past 10 yr, several ^{99m}Tc complexes have been synthesized and tested as potential alternatives to ^{131}I or ^{123}I orthoiodohippurate (OIH) (*1*). To date, the most successful agent is ^{99m}Tc-mercaptoacetyltriglycine (MAG3). Technetium-99m-MAG3 has been extensively studied and many consider it to be the ^{99m}Tc renal agent of choice (*2,3*). Nevertheless, ^{99m}Tc-MAG3 is still not the ideal replacement for OIH because its clearance is only 50%–60% that of OIH and it does not provide a direct measurement of effective renal plasma flow. Furthermore, a small percentage of ^{99m}Tc-MAG3 is transported into the small intestine via the hepatobiliary system in normal volunteers. This percentage increases in patients with renal failure and can lead to problems in image interpretation (*4–6*). Increased hepatobiliary activity can also occur with suboptimal kit preparation (*7*). These limitations have prompted a continuing search for improved ^{99m}Tc renal imaging agents.

In 1990, Verbruggen et al. (*8*) observed that the polar metabolite, ^{99m}Tc-L,L-ethylenedicysteine (^{99m}Tc-LL-EC), of the brain agent, ^{99m}Tc-L,L-ethylenedicysteine diethylester, was rapidly and efficiently excreted into the urine in mice. This observation led them to pursue the evaluation of ^{99m}Tc-LL-EC as a renal

imaging agent. Subsequent studies in mice and baboons, showed that the pharmacokinetic properties of ^{99m}Tc-LL-EC more closely approached those of OIH than the properties of MAG3 and also suggested that LL-EC was superior to the enantiomer ^{99m}Tc-DD-EC (*3,9,10*).

Because of the promising animal data, the ^{99m}Tc complexes of LL-EC and DD-EC were compared in a human volunteer. Although renal washout appeared to be faster for the DD complex, DD-EC appeared to show more diffuse tissue localization. Consequently, subsequent studies focused on LL-EC. Studies completed comparing LL-EC and MAG3 in six healthy human volunteers showed that LL-EC had a higher plasma clearance, although the percent dose in the urine at 30 min and 60 min and the renogram curves were almost identical (*11*). A more extensive investigation comparing the clearance of LL-EC with MAG3 in 60 patients showed that the clearance of LL-EC averaged 71% that of OIH, whereas the clearance of MAG3 was only 52% that of OIH (*12*). Other studies have reported similar LL-EC/OIH ratios with values averaging 75%-76% (*13,14*). The imaging properties of LL-EC appear to be similar to those of MAG3. In addition, the time to maximum activity for MAG3, ^{99m}Tc LL-EC and OIH are similar although the time from peak to 50% of peak activity appears to be less for OIH than for the two ^{99m}Tc complexes (*14,15*).

Technetium-99m-EC may also exist in DL isomeric forms (Fig. 1). The promising results with the LL isomer prompted us to evaluate the other isomeric forms of ^{99m}Tc-EC in rats and human subjects to determine if one of the other isomeric forms might be superior to ^{99m}Tc-LL-EC. Furthermore, these studies would provide additional data regarding the structural characteristics required for optimal tubular transport and facilitate the design of improved renal tubular agents.

MATERIALS AND METHODS

L,L-ethylenedicysteine, D,D-ethylenedicysteine and a mixture of D,D- L,L- and D,L-ethylenedicysteine were prepared according to literature procedures (*15,16*). L-thiazolidine-4-carboxylic acid and D-thiazolidine-4-carboxylic acid prepared from optically pure L-cysteine and D-cysteine were reductively dimerized to give pure enantiomeric products. When the racemic cysteine was used to obtain the D,L-ethylenedicysteine ligand, the reaction yielded an isomeric mixture consisting of all three isomers.

Technetium-99m Radiolabeling

Each ligand (1 mg) was dissolved in 1 N NaOH (100 µl). Technetium-99m-sodium pertechnetate in generator saline (0.25 ml) was added to the solution along with freshly prepared stannous chloride solution (4 mM, 0.02 M HCl, 100 µl). The mixtures were heated at 100°C for 10 min followed by addition of 1 N HCl (105 µl). The ^{99m}Tc-EC 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 0.05 M NaH₂PO₄, pH 4.3 or 0%–10% EtOH gradient, 0.01 M NaH₂PO₄, pH 3.0 (1-min
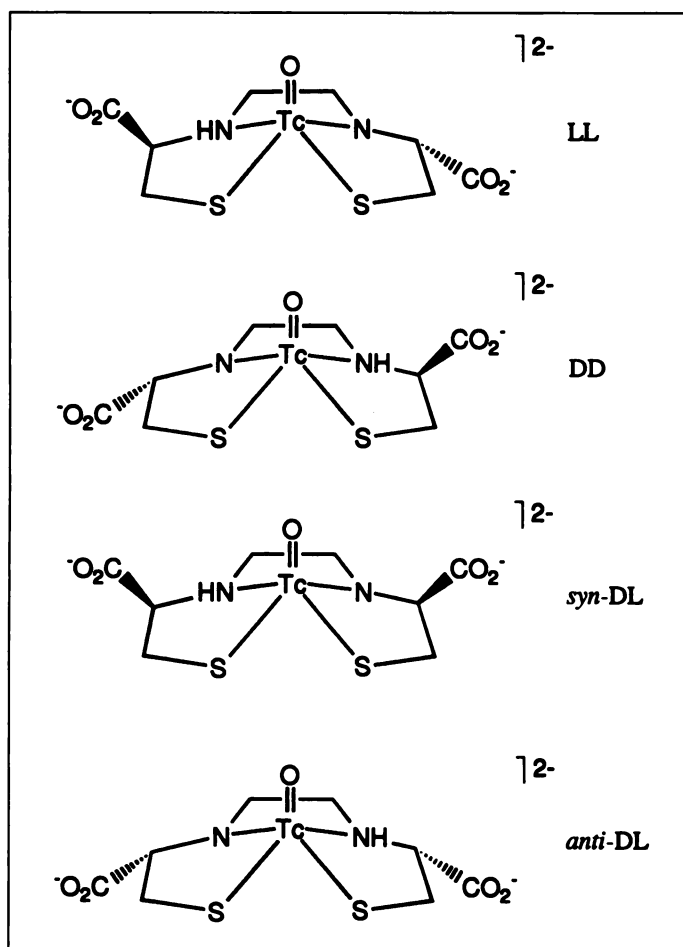
FIGURE 1. Isomers of ^{99m}Tc-EC. The structure shown for ^{99m}Tc-LL-EC (top) represents the predominant form of the complex at physiological pH. The structures for the other isomers (DD, *syn*-DL and *anti*-DL) represent the same protonation state. Each isomer may be axially ligated by solvent. Analogous complexes have been prepared with rhenium.

gradient with ^{99m}Tc-DD and LL-EC and a 10-min gradient for the isomeric mixture). Stannous reduction of ^{99m}TcO$_4^-$ under basic conditions in the presence of either the LL or DD isomers produced a single radiochemical species, ^{99m}Tc-LL-EC or ^{99m}Tc-DD-EC, respectively, in greater than 90% yield. Labeling of the isomeric mixture gave a mixture of ^{99m}Tc-EC products. Two ^{99m}Tc-EC peaks were resolved by HPLC in an approximate ratio of 1:1. For both buffer systems, the first eluting peak was assigned as ^{99m}Tc-DL-EC since the second peak corresponded to the retention volume of pure ^{99m}Tc-LL-EC and ^{99m}Tc-DD-EC. For the isomeric mixture, only the ^{99m}Tc-DL-EC peak was collected.

The radiochemical purity of the HPLC separated ^{99m}Tc-EC complexes ranged from 96%–100%. Each was diluted with phosphate-buffered saline (pH 7.4) and tested for stability by HPLC analysis using a Beckman Ultrasil AX 10 μm column (4.6 × 250 mm); 0.01 M NaH$_2$PO$_4$, pH 7.4 mobile phase; flow rate of 1 ml/min. No significant decomposition of the ^{99m}Tc complexes was observed up to 6 hr.

Rat Studies

Renal Clearance, Extraction Fraction and Plasma Protein Binding. The LL-, DD- and DL-EC isomers were each evaluated in six Sprague-Dawley rats. The HPLC purified complexes were diluted to 10 μCi/ml with phosphate-buffered saline for steady state plasma clearance, extraction efficiency and plasma protein binding studies. Each rat was anesthetized with ketamine HCl (100 mg/kg intraperitoneal) and placed on a heated surgical table. After tracheostomy, the left jugular vein was cannulated with two pieces

of PE-50 tubing (one for infusion of the radiopharmaceuticals and one to infuse normal saline (5.2 ml/hr) to maintain hydration and additional anesthetic (4 mg/hr) as necessary). The right carotid artery was cannulated for blood sampling and the bladder was catheterized using heat-flared PE-50 tubing. The core temperature of each animal was continually monitored using a rectal temperature probe. The ^{99m}Tc complex was coinfused with ^{131}I OIH (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 then 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 measurements was used as the clearance value.

To measure extraction fraction, 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. Extraction fraction = (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. Red blood cell activity (RBC), as a percentage of whole blood activity, was determined from the arterial sample assuming a hematocrit of 50%; RBC = red blood cell concentration/(red blood cell concentration + plasma concentration). Plasma protein binding was determined by ultracentrifugation (Centrifree® micro-partition system, Amicon Inc., Beverly, MA) of 1 ml of arterial plasma; plasma protein binding = [1.0-(ultrafiltrate concentration/plasma concentration)] × 100. Ultracentrifugation of the ^{99m}Tc complexes in protein-free buffer showed negligible binding to the ultrafiltration membrane. A gamma 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.

Biodistribution Studies. Each rat was anesthetized as described above. Tracheostomy was performed and the left jugular vein was cannulated with one piece of PE-50 tubing for injection of the ^{99m}Tc radiopharmaceutical. The bladder was catheterized using heat flared PE-50 tubing for urine collection. A bolus injection of the radiopharmaceutical (400–500 μCi/0.25 ml) was given and the rat was imaged (1 frame/10 sec) for 22 min (130 frames) using a gamma camera. Each animal was killed at the conclusion of the dynamic study and simultaneous static images of the isolated lungs, heart, liver, spleen, stomach, kidneys, bowel and bladder with urine were acquired as well as a static image of the rat carcass without the organs. Total counts for the injected dose were determined from the sum of the isolated organ counts and the carcass counts decay corrected to the time of organ imaging. ROIs were drawn around each organ to determine the percent injected dose in each organ.

Metabolism Studies. Rats were prepared according to the procedure described for the biodistribution studies. A bolus injection of the radiopharmaceutical (1–2 mCi) was given and the urine was collected for 30 min. The urine was centrifuged to settle any particulates and analyzed by HPLC alone and with purified complex added. Each ^{99m}Tc-EC complex was tested in two rats.

Normal Volunteer Studies

Technetium-99m-LL-EC, DD-EC and DL-EC were each evaluated in three normal male volunteers. The HPLC purified complexes and phosphate-buffered saline (pH 7.0) were passed through a Sep-Pak Plus C18 cartridge (primed with 1 ml ethanol) and a sterile Millex-GS 0.22 μm filter unit into a sterile, pyrogen-free empty vial. The final concentration was ~ 2.0 mCi/2.5 ml and the final pH ranged from 5.7 to 7.4. Test samples of each complex were sterile and pyrogen free. Approximately 2 mCi of each ^{99m}Tc

TABLE 1
Biodistribution Results: Percent of Total Activity* in Rats at 22 Minutes

Complex	Liver	Intestines	Kidneys	Bladder + Urine	Carcass
^{99m}Tc-LL-EC	4.5 ± 0.2	1.8 ± 0.5	24.4 ± 4.8	45.6 ± 5.1	22.6 ± 1.1
^{99m}Tc-DD-EC	2.9 ± 0.7	1.7 ± 0.5	5.4 ± 1.1	63.2 ± 5.0	25.4 ± 3.3
^{99m}Tc-DL-EC	2.6 ± 0.4	1.1 ± 0.3	19.3 ± 6.5	54.4 ± 5.1	21.6 ± 2.7

*<1% in spleen, stomach, heart and lung, n = 3.

complex were coinjected with 200 μCi of [^{131}I]OIH and plasma samples were obtained at 5, 10, 15, 20, 30, 45, 60 and 90 min postinjection. The plasma clearances of [^{131}I]OIH and each EC complex were determined using the single injection, two-compartment model of Sapirstein et al. (17). The volunteers voided at 30, 90 and 180 min postinjection to determine the percent dose in the urine at each time period. A urine sample from the 30 min urine collection was obtained for HPLC analysis from one of the normal volunteers and analyzed for each complex. One volunteer was imaged with each complex using a simultaneous acquisition with a 20% window centered over the 363-keV photopeak of ^{131}I and a second 20% window over the 140-keV photopeak of ^{99m}Tc. Data were acquired in a 128 × 128 matrix using a three-phase dynamic acquisition: Phase 1 consisted of twenty-four 2-sec frames, Phase 2 consisted of sixteen 15-sec frames and Phase 3 consisted of forty 30-sec frames. Data were acquired using a computer fitted with a high-energy collimator. All studies were performed with the approval of the Human Investigations Committee and a signed consent form was obtained.

Statistical Analysis

The statistical analysis was based on an analysis of variance (ANOVA) and the independent t-test. A $p \leq 0.02$ was considered to be significant. Data with an $n \leq 3$ were not analyzed.

RESULTS

Rat Studies

Biodistribution Studies. Technetium-99m-DD-EC appeared to be excreted more rapidly in the urine than the other two complexes (Table 1). Technetium-99m-LL-EC had a slightly higher uptake in liver and intestine (6.3%) than DD-EC and DL-EC, which had combined uptakes in liver and intestine of 4.6 and 3.7%, respectively. Of note, 24.4% of the total LL-EC activity and 19.3% for DL-EC activity were retained in the

kidney at 22 min compared to only 5.4% for DD-EC. This finding is also illustrated by the representative left kidney renograms using each complex (Fig. 2). For all three complexes, less than 1% of total activity was found in spleen, stomach, heart or lung.

Renal Clearance, Extraction Fraction and Plasma Protein Binding. The renal clearances, extraction fractions and plasma protein binding studies of the three complexes in rats are summarized in Table 2. The clearances of ^{99m}Tc-LL-EC, DD-EC and DL-EC were 60%, 108% and 67% of OIH, respectively; extraction fractions were 109%, 125% and 93% of OIH, respectively. To minimize the effect of the experimental conditions on the results, the clearance and extraction fractions in each rat were normalized to the corresponding OIH value before comparison. The ^{99m}Tc-EC/OIH clearance ratio for DD-EC was significantly higher than that of LL- and DL-EC ($p < 0.01$); similarly, the extraction fraction ratio of DD-EC/ OIH was also higher than that of the other two complexes ($p < 0.02$). The differences between the LL-EC and DL-EC clearance and extraction fraction ratios were not significant.

Only 4%–7% of blood activity was found in red blood cells for the ^{99m}Tc-EC complexes compared to 30% for OIH. We did not attempt to correct for the leakage of the tracer out of the red cell in the venous sample and this probably accounts for the high EC extraction efficiencies relative to OIH. Plasma protein binding was moderately high (64%–74%) for all three complexes. Although not significantly different than for the LL and DL complexes, ^{99m}Tc DD-EC had the lowest red blood cell uptake (4% of whole blood activity) and the lowest plasma protein binding (64%).

Normal Volunteer Studies

The clearance of ^{99m}Tc-DD-EC averaged 480 ml/min compared to 320 ml/min for LL-EC and 233 ml/min for DL-EC
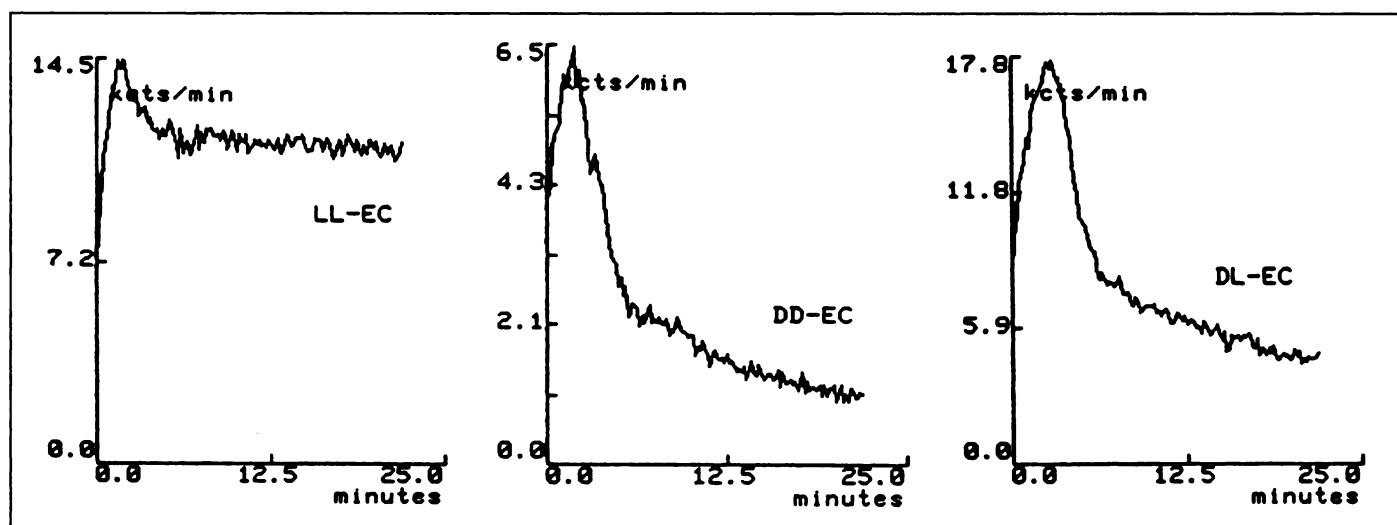
FIGURE 2. Representative renogram curves of the ^{99m}Tc-LL-EC (right), ^{99m}Tc-DD-EC (middle) and ^{99m}Tc-DL-EC complexes in a rat kidney (left). The ^{99m}Tc-DD-EC isomer shows the earliest peak and most rapid washout. There is retention of the ^{99m}Tc-LL-EC isomer in the renal parenchyma.

TABLE 2
Renal Plasma Clearance, Extraction Efficiency and Plasma Protein Binding of Technetium-99m
Renal Agents in Rats (n = 6): Comparison with OIH

Complex	Clearance (ml/min/100 g)	Clearance % Tc/OIH	EE%	EE % Tc/OIH	PPB %
99mTc-LL-EC	1.69 ± 0.44	60 ± 9	76 ± 3	109 ± 10	66 ± 10
99mTc-DD-EC	3.31 ± 0.64	108 ± 6*	91 ± 1	128 ± 11†	64 ± 4‡
99mTc-DL-EC	2.13 ± 0.48	67 ± 13	73 ± 12	93 ± 15	74 ± 5

*p < 0.01 (99mTc-DD-EC compared to LL- and DL-EC).
†p < 0.02 (99mTc-DD-EC compared to LL- and DL-EC).
‡p < 0.01 (99mTc-DD-EC compared to DL-EC).
EE = extraction efficiency; PPB = plasma protein binding.

(Table 3). Representative 99mTc-EC images and renogram curves are shown in Figure 3. When these values were normalized for the corresponding [131I]OIH clearance, the ratio of the clearance of DD-EC to OIH averaged 82% compared to 70% for LL-EC and 40% for DL-EC. The plasma protein binding of DD-EC was 28% compared to 47% for LL-EC and 72% for DL-EC. Red cell uptake was minimal, averaging 7% for DD- and DL-EC and 12% for LL-EC. Both DD- and LL-EC were excreted at essentially the same rate as OIH; DL-EC was excreted more slowly than OIH (Table 4).

Metabolism Studies

In the rat, greater than 95% of the activity recovered in the urine coeluted with the HPLC purified 99mTc-LL-EC and DD-EC complexes. Approximately 81% of the urine activity coeluted with the purified 99mTc-DL-EC complex, 4% of the activity eluted at the solvent front and the remaining 15% represented a less polar species. In the normal volunteers one 30-min urine sample was analyzed for each complex and similar results were obtained. Technetium-99m-LL-EC and DD-EC were excreted unchanged; a less polar metabolite accounted for 8% of the activity in the 99mTc-DL-EC urine.

DISCUSSION

Technetium-99m-LL-EC is primarily secreted by the tubules (18) and can be competitively inhibited by probenecid (3,10). Therefore, 99mTc-LL-EC is thought to share a common transport protein with OIH, 99mTc-MAG3 and syn-99mTc-bis(mercaptoacetamido)propanoate (syn-99mTc-CO2DADS), which are all cleared rapidly by the renal tubules and can also be inhibited by probenecid (19,20). All three 99mTc complexes and 99mTc-DD-EC contain an oxo-technetium-glycyl sequence (O = Tc-N-C-CO2−) with a CO2− group syn to the oxo ligand (syn-CO2−) (21–23). The anti-99mTc-CO2DADS isomer with an anti-CO2− group is not rapidly excreted in the urine (19). These structure-distribution relationships strongly suggest that the oxo group and a syn-CO2− are responsible for receptor recognition.

However, unlike 99mTc MAG3 and syn-99mTc-CO2DADS, 99mTc-LL-EC and 99mTc-DD-EC exist as a mixture of forms

under physiological conditions. The two forms differ in charge, coordination number and ligand denticity. A mixture of species is usually disadvantageous since the two forms are likely to be cleared at different rates. The predominant form (Fig. 1) is a five-coordinate dianionic species. Both carboxyl groups are deprotonated and dangling; one CO2− is syn and the second is anti to the oxo ligand; the anti-N is deprotonated. Based on the pKa value of 6.8 reported for 99Tc-LL-EC (24), approximately 20% of 99mTc-LL-EC and 99mTc-DD-EC exist as six-coordinate monoanionic species with the anti-CO2− group coordinated trans to the oxo ligand. Coordination of the anti-CO2− results from protonation of the anti-N (22). Two 99mTc-DL-EC isomers were predicted in which both CO2− groups were either syn or anti to the oxo ligand; however, we found that when rhenium DL-EC is prepared under high pH conditions, only one DL isomer is formed (25).

Verbruggen et al. (3) reported that labeling of LL-EC with 99mTc at neutral pH did not yield the same product as was obtained from high pH reaction solutions. Both the syn- and anti-rhenium DL-EC isomers were obtained when the pH of the reaction solution was <7 (26). However, anti-rhenium DL-EC easily converted to syn-rhenium DL-EC at high pH and did not reform when the pH was lowered. This result explains why only the syn isomer of 99mTc-DL-EC or rhenium DL-EC was isolated from high pH reaction solutions. Stannous reduction of 99mTcO4− in the presence of the isomeric DL-EC mixture at pH <7 produced a solution that gave erratic results when analyzed by reverse phase HPLC. In most instances, even with HPLC buffer systems that gave good results for the DD, LL and syn-DL complexes prepared at high pH, almost all of the radioactivity was retained on the column; consequently, an anti-99mTc-DL-EC species was not isolated.

In rats, the plasma protein binding of 99mTc-DL-EC (66%) and the extraction fraction and renal clearance ratios (93% and 67% of OIH, respectively) were similar to the values obtained for LL-EC. Since the presence of a syn-CO2− group is considered a fundamental requirement for efficient tubular transport of [99mTc(V)O]3+ complexes (3), the data correlate well with the

TABLE 3
Clearances, Clearance Ratio (EC/OIH), Plasma Protein Binding and Red Cell Uptake of Technetium-99m-LL-EC, Technetium-99m-DD-EC, Technetium-99m-DL-EC and OIH in Normal Volunteers (n = 3)

Complex	99mTc-EC clearance (ml/min)	OIH clearance (ml/min)	Clearance EC/OIH (%)	Plasma protein binding (%)	Red cell uptake (%)
99mTc-LL-EC	320 ± 21	458 ± 40	70 ± 3	47 ± 6	12 ± 7
99mTc-DD-EC	480 ± 94	581 ± 63	82 ± 8	28 ± 13	7 ± 1
99mTc-DL-EC	233 ± 31	585 ± 53	40 ± 5	72 ± 2	7 ± 2
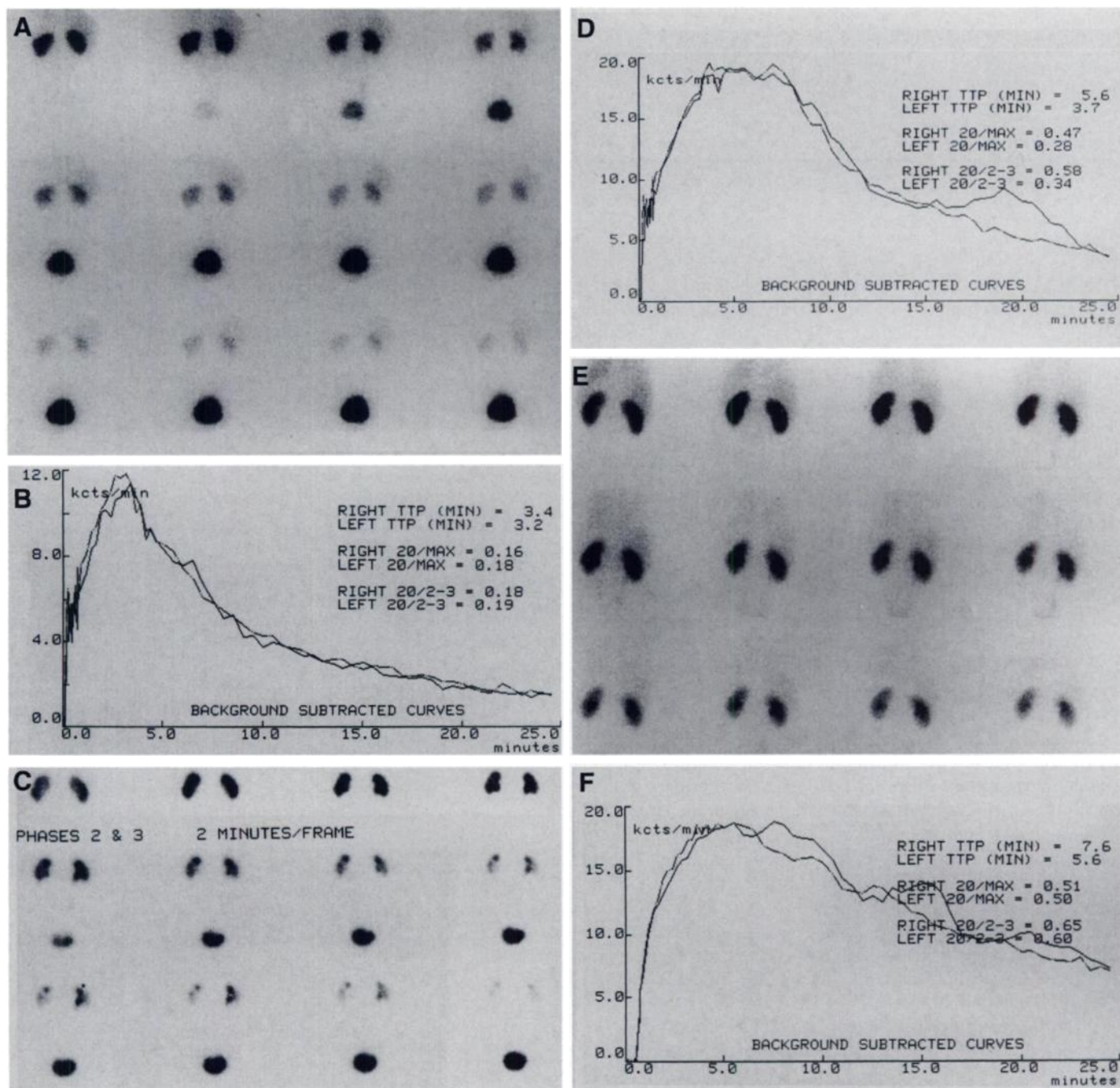
FIGURE 3. (A) Two-minute [99mTc]-DD-EC images and (B) renogram curves. Data were acquired after the simultaneous injection of 1.80 mCi [99mTc]-DD-EC and 185 μCi of OIH. Although the [^{131}I]OIH images are not shown, the time-to-peak activity (TTP), the 19–20 min to maximal activity ratio (20/max) and the 19–20 min to the 2–3 min activity ratio (20/2-3) were similar for both agents. (C) Two-minute [99mTc]-LL-EC images and (D) renogram curves. Data were acquired after the simultaneous injection of 1.80 mCi [99mTc]-DD-EC and 185 μCi OIH. Although the [^{131}I]OIH images are not shown the TTP, the 19–20 min to maximal activity ratio (20/max) and the 19–20 min to the 2–3 min activity ratio (20/2-3) were similar for both agents. (E) Two-minute [99mTc]-DL-EC images and (F) renogram curves. Data were acquired after the simultaneous injection of 1.80 mCi [99mTc]-DD-EC and 185 μCi OIH. Although the [^{131}I]OIH images are not shown, the TTP of the [99mTc]-DL-EC images was more prolonged and the 19–20 min to maximal activity ratio (20/max) and the 19–20 min to the 2–3 min activity ratio (20/2-3) were higher for [99mTc]-DL-EC compared to OIH. The TTP, 20/max and 20/2-3 values for OIH were 4.2 min, 0.15 and 0.23 and 4.2 min, 0.22 and 0.42 for the right and left kidneys, respectively.

assignment of a *syn* structure to [99mTc]-DL-EC. Renal clearance of the *anti* isomer would be expected to be much lower. Technetium-99m-DD-EC had the highest extraction efficiency in rats (125% of OIH) and highest renal clearance (108% of OIH) of all three isomers. The major difference between [99mTc]-LL-EC and DD-EC was the retention of LL-EC in the rat kidneys. At 22 min, the activity of [99mTc]-LL-EC in the kidney was 4.5 times greater than the activity of [99mTc] DD-EC. The renal retention would not affect the plasma clearance but would certainly affect the rate of excretion and the renogram curve, as

illustrated in Figure 2. Similar observations have been found by Van Nerom et al. (*10*) and Muller-Suur (personal communication, 1995).

In general, the rat has been a good model for predicting the behavior of [99mTc] renal radiopharmaceuticals. However, interspecies differences do occur and there is no substitute for human testing. The fact that the clearance of the [99mTc]-EC complexes exceeds the glomerular filtration rate indicates that they must be transported by the renal tubules. Furthermore, they appear to share the same tubular transport process as OIH and

TABLE 4
Percent Dose of Technetium-99m-LL-EC, Technetium-99m-DD-EC and Technetium-99m-DL-EC in the Urine at 0–30 min, 30–90 min
and 90–180 min Postinjection Compared to OIH in Normal Volunteers (n = 3)

Complex	^{99m}Tc-EC 0–30 min	^{99m}Tc-EC 30–90 min	^{99m}Tc-EC 90–180 min	^{99m}Tc-EC 0–180 min	OIH 0–180 min	EC/OIH 0–30 min	EC/OIH 0–180 min
^{99m}Tc-LL-EC	68 ± 5	17 ± 5	4 ± 3	89 ± 3	92 ± 14	92 ± 10	97 ± 9
^{99m}Tc-DD-EC	61 ± 8	18 ± 2	6 ± 2	85 ± 9	86 ± 4	90 ± 9	99 ± 8
^{99m}Tc-DL-EC	45 ± 6	24 ± 5	10 ± 2	80 ± 7	96 ± 7	57 ± 8	83 ± 6

^{99m}Tc-MAG3 since, like these tracers, the excretion of ^{99m}Tc-LL-EC can be competitively inhibited by probenecid (10,20). Since the ^{99m}Tc-EC complexes are not as highly protein bound as ^{99m}Tc-MAG3, the ^{99m}Tc-EC complexes should be filtered by the glomerulus to a greater extent than ^{99m}Tc-MAG3. This has, in fact, been documented by micropuncture studies in the rat. Furthermore, tubular reabsorption of ^{99m}Tc-LL-EC from the ultrafiltrate does not occur (18) and this is almost certainly true of ^{99m}Tc-DD-EC.

In the human studies, the clearance of ^{99m}Tc-DD-EC averaged 480 ml/min compared to 320 ml/min for LL-EC. Both clearances were higher than that of DL-EC. When normalized to the OIH clearance, the clearance ratio of DD-EC still appeared to be higher than that of LL-EC, 82 ± 8% compared with 70 ± 3%. Both of these ratios compare favorably to the MAG3/OIH clearance ratios which have ranged from 49% to 70% with an average around 56% (1). Although ^{99m}Tc-DD-EC appeared to have a higher clearance than ^{99m}Tc-LL-EC, the rates of excretion in the urine were essentially the same. In our limited series of subjects, there was no difference in image quality (Fig. 3). We did not obtain delayed anterior views to evaluate hepatobiliary excretion. However, preliminary results with ^{99m}Tc-LL-EC suggest that there is some hepatobiliary excretion similar to that observed with MAG3 (4,13). Future studies of the ^{99m}Tc-EC complexes should include delayed anterior views; these images will be most relevant in azotemic patients.

CONCLUSION

These data provide a better understanding of the structure/function relationships of the ^{99m}Tc-EC complexes and will be helpful in the design of future renal radiopharmaceuticals. The pharmacokinetics of ^{99m}Tc-DD-EC appear to be closer to OIH than those of ^{99m}Tc-LL-EC but both ^{99m}Tc-DD- and LL-EC exist in monoanionic and dianionic forms at physiological pH. We have characterized the solution structure of these two forms (22) and it is highly unlikely that they have similar clearances. The protein binding affinities are also likely to be different. An optimal renal tubular agent should exist as a single species at physiological pH.

ACKNOWLEDGMENT

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