

Evaluation of Technetium-99m-L,L-EC in Renal Transplant Recipients: A Comparative Study with Technetium-99m-MAG3 and Iodine-125-OIH

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The clinical usefulness of kit-formulated ^{99m}Tc -L,L-EC, a new renal tubular tracer agent based on a diaminodithiol ligand was evaluated in a large population of renal transplant recipients. **Methods:** Fifty patients with transplants were studied. Five patients with renal insufficiency and five normal volunteers were also included to extend the range of renal function values. The labeling efficiency of ^{99m}Tc -L,L-EC in routine conditions, i.e., without HPLC purification, and the safety of the tracer were evaluated. **Results:** The mean radiochemical purity of ^{99m}Tc -L,L-EC determined by thin-layer chromatography was 97.4%. No side effects or significant biochemical changes were observed. The clearance of ^{99m}Tc -L,L-EC and ^{125}I -OIH ranged from 10.7 to 417.5 and from 27.6 to 602.7 ml/min/1.73 m², respectively. The clearance of ^{99m}Tc -L,L-EC and ^{99m}Tc -MAG3 averaged respectively 71% and 52% of that of ^{125}I -OIH. **Conclusion:** The labeling procedure of kit-formulated ^{99m}Tc -L,L-EC is easy and efficient. This tracer is safe and suitable for both imaging and quantitative measurement of the renal tubular function. Technetium-99m-L,L-EC represents an excellent alternative to ^{99m}Tc -MAG3.

Key Words: LLEC; MAG3; ERPF; tubular tracer; kidney transplant

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Renal function can be evaluated noninvasively and accurately by simple methods using radiopharmaceuticals. Iodine-131-labeled orthoiodohippurate (hippuran, ^{131}I -OIH) was introduced in 1960 as a radiolabeled analog of paraaminohippuric acid (PAH) for the measurement of effective renal plasma flow (ERPF) (1). However, ^{131}I has several drawbacks, such as its long half-life and the emission of beta particles and high-energy photons, which result

in a high radiation dose and a suboptimal detection by gamma cameras. Iodine-123 was introduced to substitute for ^{131}I in the labeling of OIH, resulting in a lower radiation dose and better imaging characteristics (2). However, ^{123}I -OIH has not found widespread use because of the high cost, short half-life and noncontinuous availability. Finally, ^{125}I -OIH is not suitable for scintigraphic imaging. Therefore, ^{99m}Tc -labeled agents with biologic properties similar to those of OIH were developed as alternatives to radiolabeled OIH.

Technetium-99m-mercaptoacetyltriglycine (^{99m}Tc -MAG3), the most attractive of these agents up to now, has found a large-scale clinical use. Despite the excellent imaging properties caused by the physical properties of ^{99m}Tc , ^{99m}Tc -MAG3 has pharmacokinetic characteristics rather different from those of OIH. The plasma clearance of ^{99m}Tc -MAG3 amounts to only 49%—67% of that of OIH (3–8). Moreover, problems with labeling efficiency, kit formulation and stability have been encountered with this molecule (9).

Technetium-99m-L,L-ethylenedicycysteine (^{99m}Tc -L,L-EC) is a renal tubular tracer based on a diaminodithiol (N_2S_2) ligand. This tracer is the diacid derivative of the brain agent ^{99m}Tc -L,L-ethylenedicycysteine diethylester (Neurolite, du Pont-Merck, N. Billerica, MA) (10). The labeling of L,L-EC with ^{99m}Tc can be achieved easily and rapidly at room temperature, starting from a lyophilized kit (10). Animal (11) and preliminary human (11–13) studies conducted with high-performance liquid chromatography (HPLC)-purified ^{99m}Tc -L,L-EC showed that its renal clearance is significantly closer to that of OIH than is the case with ^{99m}Tc -MAG3.

This study evaluated the labeling efficiency of kit-formulated L,L-EC in routine conditions, i.e., without HPLC purification, the safety of this tracer and its pharmacokinetics by comparison with ^{125}I -OIH and ^{99m}Tc -MAG3 in a large population of renal transplant recipients. The imaging properties of ^{99m}Tc -L,L-EC were also compared with those of ^{99m}Tc -MAG3.

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MATERIALS AND METHODS

Patients and Design of Study

Patients referred for routine evaluation of the renal graft function were divided into two groups. The first group (Group A) included 25 patients (12 male and 13 female, age: 41 ± 10 yr) with unstable renal function (recently transplanted [less than 15 days] or patients with post-transplantation complications), and a dual-tracer study with $^{99m}\text{Tc-L,L-EC}$ and $^{125}\text{I-OIH}$ was performed. In a second group (Group B), which included 25 patients (17 male and 8 female, age: 37 ± 9 yr) with stable renal function (patients at long-term follow-up after transplantation), a first dual-tracer study to compare $^{99m}\text{Tc-L,L-EC}$ and $^{125}\text{I-OIH}$ was performed, followed 48 hr later by a second study to compare $^{99m}\text{Tc-MAG3}$ and $^{125}\text{I-OIH}$. To cover a wide range of renal function values, four patients on chronic hemodialysis (three anuric), one potential kidney living related donor and five healthy volunteers were also evaluated using the protocol described for Group A. The study was approved by the Ethics Committee of the University of Louvain Medical School and informed consent was obtained from all subjects.

Preparation of Radiopharmaceuticals

Technetium- $^{99m}\text{Tc-L,L-EC}$ was prepared starting from a labeling kit (Laboratory of Radiopharmaceutical Chemistry F.F.W., K.U. Leuven, Belgium) containing, in a 10-ml vial, the lyophilized residue of a solution of 500 μg of L,L-EC and 100 μg of $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 1 ml of 0.05 M phosphate buffer, pH 12. Labeling with ^{99m}Tc was performed by adding 1850 MBq [^{99m}Tc]-sodium pertechnetate eluted from a commercial $^{99}\text{Mo}/^{99m}\text{Tc}$ generator (Ultratechnekow FM, Mallinckrodt Medical, Petten, Holland) in 5 ml of isotonic saline. After incubation for at least 1 min at room temperature, the pH was adjusted from 7 to 8 by addition of 0.2 ml of a 0.5 M phosphate buffer, pH 5. The final pH was controlled using pH indicator strips (Neutralit pH 5–10, Merck, Darmstadt, Germany). The radiochemical purity of $^{99m}\text{Tc-L,L-EC}$ was checked using thin-layer chromatography (TLC) immediately after each labeling procedure ($n = 28$) and HPLC on 16 occasions. TLC was performed on Whatman 4Chr chromatographic paper (Maidstone, UK) with acetone as the solvent (System A) and on ITLC-SG (Gelman Sciences, Ann Arbor, MI) with 0.5 M acetic acid as the solvent (System B). The R_f values of $^{99m}\text{Tc-L,L-EC}$; colloidal ^{99m}Tc ; and $^{99m}\text{TcO}_4^-$ are respectively 0, 0 and 1 in System A and 1, 0 and 1 in System B. HPLC was performed as previously described (10).

Technetium- $^{99m}\text{Tc-MAG3}$ was freshly prepared using commercial labeling kits (TechneScan MAG3, Mallinckrodt Medical, Petten, Holland) following the manufacturer's instructions. The radiochemical purity of $^{99m}\text{Tc-MAG3}$ was measured randomly on five occasions by TLC using the method described by Hung et al. (14).

Iodine-125-labeled OIH ($^{125}\text{I-OIH}$) with an activity of 37 MBq/ml at the calibration date (Amersham plc, UK) was diluted with phosphate-buffered saline, pH 7, to a radioactive concentration of 555 kBq/ml. Quality control was performed on each vial supplied using the chromatographic technique described by Zimmer and Pavel (15).

Iodine-125-OIH was added to the solution of $^{99m}\text{Tc-L,L-EC}$ or $^{99m}\text{Tc-MAG3}$ to a final concentration of 125 kBq/ml of ^{125}I and 12.5 MBq/ml of ^{99m}Tc . An aliquot of the mixture was withdrawn to prepare a standard solution.

Clearance Measurements Method

Studies were performed with the patient in supine position on a Perspex table. A butterfly infusion set with a three-way connector was placed in a peripheral vein, and 4 ml of the mixture containing 50 MBq of $^{99m}\text{Tc-agent}$ and 500 kBq of $^{125}\text{I-OIH}$ was injected as a bolus. The syringe was weighed before and after injection to determine the exact amount of the injected solution. Then 3-ml blood samples were drawn into heparin-coated tubes before injection (blank sample) and at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min postinjection. Before each sample, 3 ml of blood was discarded to avoid dilution or contamination by the previous sample. Then 1-ml plasma samples were obtained after centrifugation for 10 min at 1500 g. The radioactivity of the plasma samples and standard was determined in a well-type scintillation detector, immediately after the test for ^{99m}Tc and after complete decay of ^{99m}Tc (at least 5 days later) for ^{125}I . The raw data were corrected for background radiation, radioactivity in the blank samples and ^{99m}Tc decay during counting.

The pharmacokinetic properties of the three tracer agents were calculated using the open two-compartment model described by Sapirstein et al. (16). The following parameters were obtained: the plasma clearance, the biologic half-lives $T_{50\alpha}$ and $T_{50\beta}$, respectively, and the distribution volumes V_1 , V_2 and V_{TOT} (for more details, see Appendix). The values were normalized to 1.73 m² of body surface calculated using the nomogram of Du Bois and Du Bois (17).

Gamma Camera Imaging

Simultaneously with the clearance study, a dynamic acquisition centered on the kidney(s) (anterior projection for the grafts and posterior projection for native kidneys) was initiated at the moment of injection. Data were recorded for 30 min (15×4 , 24×10 and 25×60 sec) by means of a computer-linked gamma camera (Apex 410, Elscint, Haifa, Israel) equipped with a low-energy high-resolution collimator. Renal first-pass perfusion was evaluated by means of a visual score (0 = no activity, + = activity lower than that in the iliac artery [IA] during the first minute, ++ = activity equal to IA, +++ = activity higher than IA). Renograms were obtained from regions of interest (ROIs) drawn on a 30-min composite image. The following parameters were determined: the time to peak (T_{max}), the $T_{1/2}$ of the excretion phase ($T_{M/2}$, calculated using an exponential fit of the activity data from the peak to 30 min; indeed, in some patients, the residual activity was greater than 50%, which means that the $T_{M/2}$ was not reached yet) and the residual activity at 30 min (expressed as the percentage of peak activity). After the 30-min dynamic acquisition, a 2-min static image of the upper abdomen was obtained to evaluate the liver activity, followed by a 1-min image over the injection site to exclude infiltration of the dose. To compare the liver activity with both $^{99m}\text{Tc-tracers}$, rectangular ROIs of 16 pixels were drawn over the liver and an adjacent area of background. The ROIs were created on the $^{99m}\text{Tc-MAG3}$ data and were repositioned at the same place on the $^{99m}\text{Tc-L,L-EC}$ data. From the counts in these ROIs, a liver activity index was calculated as ($\text{counts}_{\text{liver}} - \text{counts}_{\text{background}}$), corrected for the injected dose. Data observed for $^{99m}\text{Tc-MAG3}$ and $^{99m}\text{Tc-L,L-EC}$ were compared by paired Student's t-test.

In the five healthy volunteers, a total-body scan was obtained 40 to 150 min after injection of $^{99m}\text{Tc-L,L-EC}$ (Diacam, Siemens Medical Systems Inc, Hoffman Estates, IL).

Safety Studies

In 54 patients, systolic and diastolic blood pressure, heart and respiratory rates and body temperature were monitored before, 5 min and 30 min after injection of $^{99m}\text{Tc-L,L-EC}$. In 23 patients of Group B, blood was drawn before and 24 hr after the first study for measurement of hemogram, urea, creatinine, electrolytes, bilirubin and liver enzymes.

Statistical Analysis

Unless otherwise stated, data are given as the mean \pm s.d. Perfusion indices were compared by Wilcoxon's nonparametric test. Paired Student's t-test and linear-regression analysis were applied to compare the other data. The correlations between $^{99m}\text{Tc-L,L-EC}$ and OIH and between $^{99m}\text{Tc-MAG3}$ and OIH were compared by paired t-test applied on the individual errors of estimate, provided by the least-squares linear regression. A probability value of less than 0.05 was considered significant.

RESULTS

Radiochemical Purity

Twenty-eight labeling procedures of L,L-EC with ^{99m}Tc were performed. The pH of the final solution ranged in all cases between 7.0 and 8.0. TLC demonstrated a mean radiochemical purity of $97.4 \pm 0.8\%$. Free $^{99m}\text{TcO}_4^-$ and colloidal ^{99m}Tc amounted to $0.9\% \pm 0.6\%$ and $1.7\% \pm 0.4\%$, respectively. Radiochemical purity determined by HPLC ($n = 16$) was $99.5\% \pm 0.5\%$.

The mean radiochemical purity of $^{99m}\text{Tc-MAG3}$ was $97.9\% \pm 1.4\%$ ($n = 5$). Free radioiodide in the $^{125}\text{I-OIH}$ solution never exceeded 1.0% ($n = 5$).

Comparison Between Technetium-99m-L,L-EC and Iodine-125-OIH: Pharmacokinetics

A total of 60 patients were available for this comparison. The results of the two-compartment pharmacokinetic analysis are shown in Table 1. A wide range of $^{125}\text{I-OIH}$ clearances ($27.6\text{--}602.7$ ml/min/ 1.73 m 2) was observed. A close correlation was found between the clearance values of $^{99m}\text{Tc-L,L-EC}$ and $^{125}\text{I-OIH}$ ($r = 0.99$, $p < 0.001$, s.e.e. = 15.9 ml/min/ 1.73 m 2) (Fig. 1). The mean clearance of $^{99m}\text{Tc-L,L-EC}$ amounted to $70.9 \pm 10.3\%$ of the $^{125}\text{I-OIH}$ clearance with a range from 34.7% to 103.8% . In the three anuric patients who were undergoing chronic hemodialysis, the ratio of $^{99m}\text{Tc-L,L-EC}$ to $^{125}\text{I-OIH}$ clearance was $43.1\% \pm 7.4\%$. In these patients, the $^{125}\text{I-OIH}$ clearance ranged from 25 to 38 ml/min/ 1.73 m 2 , whereas the $^{99m}\text{Tc-L,L-EC}$ clearance ranged from 10 to 15 ml/min/ 1.73 m 2 . If these three patients were excluded from the analysis, the ratio $^{99m}\text{Tc-L,L-EC}$ to $^{125}\text{I-OIH}$ clearance was $72.4\% \pm 8.2\%$, with a range from 53.7% to 103.8% . The ratio of 103.8% was found in a patient with a severe acute rejection episode associated with anuria and edema. In the five normal volunteers, the plasma clearance of $^{99m}\text{Tc-L,L-EC}$ was 354.4 ± 34.2 ml/min/ 1.73 m 2 (range $324.2\text{--}417.5$ ml/min/ 1.73 m 2) and that of $^{125}\text{I-OIH}$ was 507.4 ± 54.6 ml/min/ 1.73 m 2 (range $446.4\text{--}602.7$ ml/min/ 1.73 m 2).

The total volume of distribution (V_{TOT}) of both tracers was not significantly different and was about 20% of the body weight. The T_{50} of the fast exponential ($T_{50\alpha}$ see

TABLE 1
Pharmacokinetic Data of Iodine-125-OIH and Technetium-99m-L,L-EC in 60 Subjects*

		$^{125}\text{I-OIH}$	$^{99m}\text{Tc-L,L-EC}$	Paired t-Test
Cl	(ml/min) [†]	263.6 ± 138.9 (27.6–602.7)	187.3 ± 95.0 (10.7–417.5)	$p < 0.0001$
$T_{50\alpha}$	(min)	5.3 ± 1.2	5.7 ± 2.4	ns
$T_{50\beta}$	(min)	64.8 ± 62.1	97.2 ± 125.1	$p < 0.005$
V_1	(l) [†]	6.7 ± 1.1	7.0 ± 1.1	$p < 0.0005$
V_2	(l) [†]	6.5 ± 1.6	6.5 ± 1.7	ns
V_{TOT}	(l) [†]	13.1 ± 2.4	13.5 ± 2.4	ns
	(% BW)	19.9 ± 3.7	20.5 ± 4.1	

*Mean \pm s.d. Values in brackets are ranges.
[†]Related to 1.73 m 2 body surface.
 BW = body weight; ns = nonsignificant.
 For explanation of symbols, see Appendix.

Appendix) corresponding to the mixing throughout the distribution volume was not significantly different for $^{99m}\text{Tc-L,L-EC}$ and $^{125}\text{I-OIH}$ while T_{50} of the slow exponential ($T_{50\beta}$), which reflects excretion once mixing has been completed, was significantly longer for $^{99m}\text{Tc-L,L-EC}$ than for $^{125}\text{I-OIH}$ (97.2 versus 64.8 min, $p < 0.005$). However, the correlation between these values was significant ($r = 0.90$, $p < 0.001$).

Comparison Between Technetium-99m-L,L-EC and Technetium-99m-MAG3: Pharmacokinetics

In the 25 patients in Group B, the clearance and biodistribution characteristics obtained from the $^{125}\text{I-OIH}$ studies performed 2 days apart were not significantly different (Table 2), and a direct comparison of $^{99m}\text{Tc-L,L-EC}$ and $^{99m}\text{Tc-MAG3}$ was thus possible.

The plasma clearance of $^{99m}\text{Tc-MAG3}$ was systematically lower than that of $^{99m}\text{Tc-L,L-EC}$ and $^{125}\text{I-OIH}$

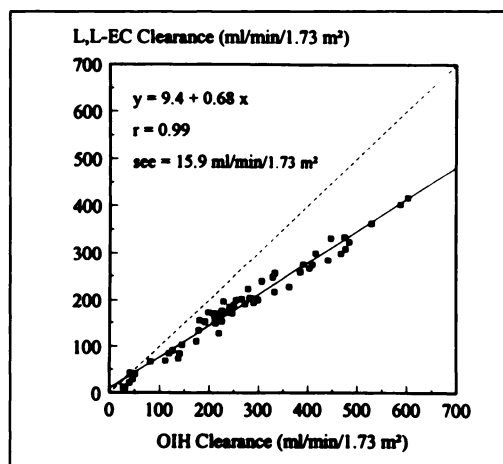


FIGURE 1. Correlation between the clearance of $^{125}\text{I-OIH}$ and $^{99m}\text{Tc-L,L-EC}$ clearance in 60 subjects. The dashed line indicates the line of identity.

TABLE 2
Comparison Between the Pharmacokinetic Data of Iodine-125-OIH at Day 0 and Day 2 in 25 Patients (Group B)*

		Day 0	Day 2	Paired t-Test
Cl	(ml/min) [†]	286.1 ± 108.4 (119.3–588.2)	287.5 ± 101.2 (122.5–546.3)	ns
T _{50α}	(min)	5.3 ± 1.0	5.6 ± 2.0	ns
T _{50β}	(min)	40.5 ± 12.0	41.1 ± 14.7	ns
V ₁	(l) [†]	6.4 ± 0.7	6.5 ± 1.0	ns
V ₂	(l) [†]	5.8 ± 1.3	5.7 ± 1.2	ns
V _{TOT}	(l) [†]	12.2 ± 1.6	12.2 ± 2.0	ns
	(% BW)	19.0 ± 3.1	19.0 ± 3.2	

*Mean ± s.d. Values between brackets are ranges.

[†]Related to a 1.73-m² body surface.

[‡]Cl_{Day 2} = 0.875 Cl_{Day 0} + 37.1 (ml/min/1.73 m²); r = 0.94; s.e.e. = 35.9 ml/min/1.73 m².

BW = body weight; ns = nonsignificant.

For explanation of symbols, see Appendix.

(Table 3). Accordingly, the ^{99m}Tc-MAG3 to ¹²⁵I-OIH clearance ratio was lower than the ^{99m}Tc-L,L-EC to ¹²⁵I-OIH clearance ratio (51.7% ± 8.0% versus 70.6% ± 6.2%, p < 0.0001, Fig. 2). The ^{99m}Tc-L,L-EC to ^{99m}Tc-MAG3 clearance ratio was 138.9% ± 19.3% (range 112.6%–187.7%). Although both ^{99m}Tc-L,L-EC and ^{99m}Tc-MAG3 clearances correlated well with the clearance of ¹²⁵I-OIH (r = 0.98, p < 0.0001 and r = 0.94, p < 0.0001, respectively), the s.e.e. was significantly smaller (p < 0.0001) for the correlation ^{99m}Tc-L,L-EC/¹²⁵I-OIH clearance (s.e.e. = 15.2 ml/min/1.73 m²) than for the correlation ^{99m}Tc-MAG3/¹²⁵I-OIH clearance (s.e.e. = 22.3 ml/min/1.73 m², Fig. 3).

The total volume of distribution of ^{99m}Tc-MAG3 was smaller than that of ^{99m}Tc-L,L-EC (8.0 ± 1.4 versus 12.3 ± 1.5 l/1.73 m²).

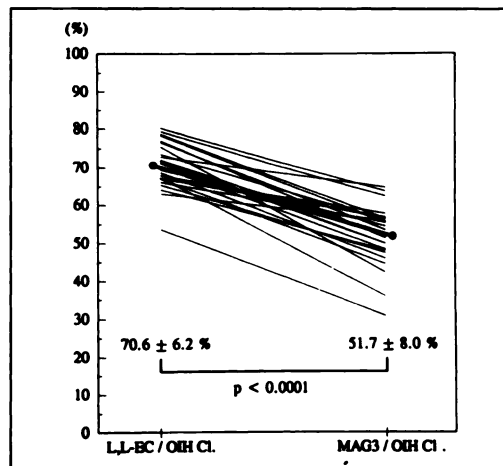


FIGURE 2. Relationship between the ^{99m}Tc-L,L-EC to ¹²⁵I-OIH clearance ratio and the ^{99m}Tc-MAG3 to ¹²⁵I-OIH clearance ratio in the 25 patients of Group B. The mean values are bold.

Comparison Between Technetium-99m-L,L-EC and Technetium-99m-MAG3: Scintigraphic Data

The quality of the scintigraphic images obtained with ^{99m}Tc-L,L-EC and ^{99m}Tc-MAG3 was similar and provided high contrast in the urinary tract structures in most patients, except in those with severe renal failure (Figs. 4 and 5). No cases of significant difference were observed between the two tracers with regard to the image quality, the perfusion index and the semiquantitative parameters derived from the renograms (Table 4). However, the liver activity corrected for the background and the injected dose was significantly smaller with ^{99m}Tc-L,L-EC than with ^{99m}Tc-MAG3 (2.8 ± 2.2 versus 7.7 ± 4.0 cpm/pixel, p < 0.0001). Even in anuric patients, no significant activity was seen in the bowel and gallbladder in the ^{99m}Tc-L,L-EC studies, whereas the liver activity remained minimal. Total body scans obtained in healthy volunteers after injection of ^{99m}Tc-L,L-EC demonstrated intense activity in the kid-

TABLE 3
Pharmacokinetic Data of Iodine-125-OIH, Technetium-99m-L,L-EC and Technetium-99m-MAG3 in 25 Patients (Group B)*

		¹²⁵ I-OIH	^{99m} Tc-L,L-EC	^{99m} Tc-MAG3
Cl	(ml/min) [†]	286.1 ± 108.4 (119.3–588.2)	200.5 ± 71.6 [‡] (73.9–403.5) [‡]	148.6 ± 53.2 [§] (44.7–251.0) [§]
T _{50α}	(min)	5.3 ± 1.0	6.0 ± 1.6	5.7 ± 1.8
T _{50β}	(min)	40.5 ± 12.0	54.6 ± 19.6 [¶]	49.7 ± 21.4 ^{**}
V ₁	(l) [†]	6.4 ± 0.7	6.8 ± 0.6 [‡]	4.5 ± 0.8 [§]
V ₂	(l) [†]	5.8 ± 1.3	5.6 ± 1.2 [‡]	3.5 ± 0.9 [§]
V _{TOT}	(l) [†]	12.2 ± 1.6	12.3 ± 1.5 [‡]	8.0 ± 1.4 [§]
	(% BW)	19.0 ± 3.1	19.2 ± 2.8 [‡]	12.5 ± 2.5 [§]

*Mean ± s.d. Values between brackets are ranges.

[†]Related to 1.73 m² body surface.

Paired t-test: ^{99m}Tc-L,L-EC vs. ^{99m}Tc-MAG3, [‡]p < 0.0001 [¶]p < 0.05; ^{99m}Tc-MAG3 vs. ¹²⁵I-OIH, [§]p < 0.0001 ^{**}p < 0.01; ^{99m}Tc-L,L-EC vs. ¹²⁵I-OIH, statistical tests similar to those given in Table 1 in 60 patients.

BW = body weight.

For explanation of symbols, see Appendix.

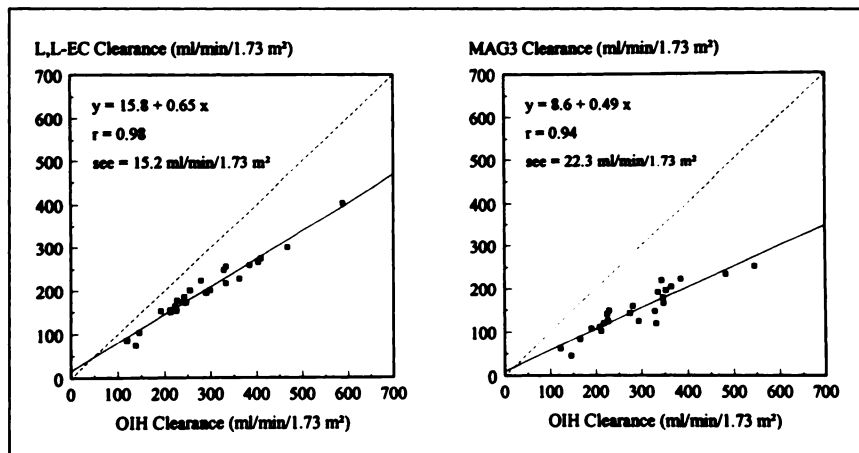


FIGURE 3. Correlation between the ^{125}I -OIH clearance and the $^{99\text{m}}\text{Tc}$ -L,L-EC clearance (left panel) and the $^{99\text{m}}\text{Tc}$ -MAG3 clearance (right panel) in the 25 patients of Group B. The dashed line indicates the line of identity.

neys and the urinary tract and minimal activity over the liver (Fig. 6).

Safety Study

No adverse reaction occurred after administration of $^{99\text{m}}\text{Tc}$ -L,L-EC. A slight, although statistically significant diminution of the systolic blood pressure was observed 30 min after injection (146 ± 19 versus 143 ± 17 mmHg, $p < 0.05$); however, this fall was very small and never exceeded 20 mmHg. No significant changes were observed in biochemical tests ($n = 23$).

DISCUSSION

Technetium-99m-L,L-EC has recently been described as a potential alternative radiopharmaceutical to $^{99\text{m}}\text{Tc}$ -MAG3 for isotopic renal studies. Animal (10,11) and preliminary human data in normal volunteers (13) and renal transplant patients (12) obtained with HPLC-purified, ligand-free $^{99\text{m}}\text{Tc}$ -L,L-EC showed that the image quality was similar to that obtained with $^{99\text{m}}\text{Tc}$ -MAG3. Moreover, the behavior of the new tracer was closer to that of OIH than is the case with $^{99\text{m}}\text{Tc}$ -MAG3. This study was per-

formed with $^{99\text{m}}\text{Tc}$ -L,L-EC under "routine conditions," which means using kit-formulated L,L-EC, without HPLC purification, and was designed to evaluate the performance of the new tracer in a large, nonselected population of renal transplant recipients.

The labeling procedure appeared easy and rapid and consistently resulted in a product of high purity. Labeling at room temperature is convenient and the need for a neutralization step before injection does not hamper the simplicity of the procedure. Moreover, HPLC demonstrated the presence of a single-labeled species, and the kit-formulated tracer has been proved to have a shelf-life of at least 8 hr (10). These labeling conditions make $^{99\text{m}}\text{Tc}$ -L,L-EC convenient and reliable, with the special advantage over $^{99\text{m}}\text{Tc}$ -MAG3 that it does not have to be boiled for labeling.

The absence of any significant side effect after intravenous injection of $^{99\text{m}}\text{Tc}$ -L,L-EC was not surprising, although it is worth noting. Indeed, no toxicity was observed in mice with doses up to 0.5 mg of EC (13). As in this

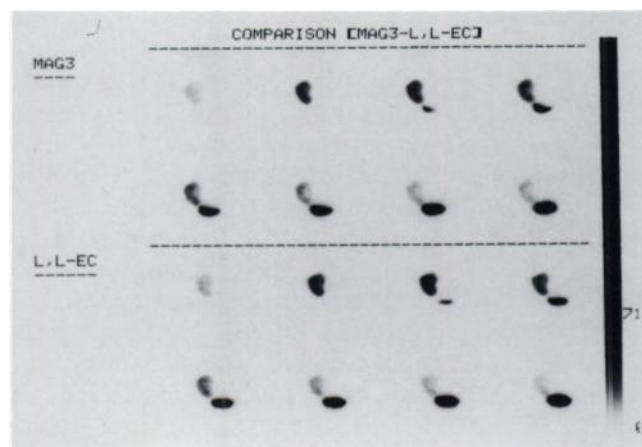


FIGURE 4. Comparison between 60-sec images obtained 1, 3, 5, 10, 15, 20, 25 and 30 min after injection of $^{99\text{m}}\text{Tc}$ -MAG3 and $^{99\text{m}}\text{Tc}$ -L,L-EC 48 hr apart in the same transplant recipient.

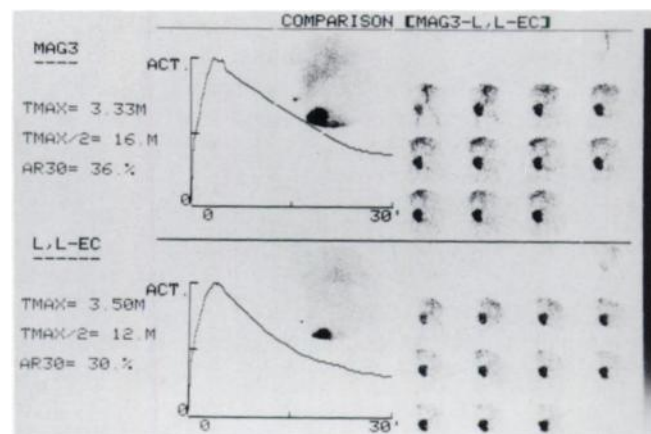


FIGURE 5. Comparison between the perfusion studies (4-sec frames, right panel), the time-activity curves (left panel) and the 2-min images of the upper abdomen obtained 30 min (insert) after injection of $^{99\text{m}}\text{Tc}$ -MAG3 (top) and $^{99\text{m}}\text{Tc}$ -L,L-EC 48 hr apart in the same transplant recipient.

TABLE 4
Scintigraphic Data for Technetium-99m-L,L-EC and Technetium-99m-MAG3 in 25 Patients (Group B)

	^{99m} Tc-L,L-EC	^{99m} Tc-MAG3	
Perfusion index	+++	+++	n = 20
	++	++	n = 2
	+++	++	n = 2
	++	+	n = 1
T _{max} (min)	4.9 ± 0.9	6.0 ± 3.4	
T _{M/2} (min)	27.8 ± 23.8	27.8 ± 25.0	
RA-30 (%)	45.5 ± 16.0	45.6 ± 16.7	

RA-30 = residual activity at 30 min.

Wilcoxon's nonparametric test for perfusion index: nonsignificant difference.

Paired Student's t-test for T_{max}, T_{M/2}, RA-30: nonsignificant difference.

study, a high specific activity was used, the amount of injected ligand was below 15 µg, even without further HPLC purification. On the other hand, ^{99m}Tc-L,L-EC is a predominant metabolite of ^{99m}Tc-L,L-ECD, and no toxicity has been reported with this tracer (18,19).

The plasma clearance of ^{99m}Tc-L,L-EC, measured over a wide range of renal function values, amounted to 70.9% ± 10.3% of the ¹²⁵I-OIH clearance, which is in accordance with data of other authors (12,13,20,21). The clearances of both tracers were highly correlated (r = 0.99). With ¹²⁵I-OIH as a reference for determination of ERPF, the clearance of ^{99m}Tc-L,L-EC allowed it to be determined with a standard error of 15.9 ml/min/1.73 m², which is acceptable in clinical practice. Preliminary data showed that the clearance of ^{99m}Tc-L,L-EC, measured by two samples obtained at 12.5 and 90 min, allowed the estimate of the OIH clearance with a s.e.e. of 18.4 ml/min (22). The lower clearance of ^{99m}Tc-L,L-EC is due to a lower renal extraction, as shown by the smaller value of the rate constant of the second exponential in Sapirstein et al.'s (16) model (presented in this study as the T_{50β}). Otherwise, the parameters derived from the two-compartment model were similar. The distribution volumes of ^{99m}Tc-L,L-EC and ¹²⁵I-OIH were not statistically different (approximately 20% of the body weight). This is the result of various factors. The protein binding of ^{99m}Tc-L,L-EC is lower than that of ¹²⁵I-OIH [31% (13) versus 53%–70% (3,23)]. On the other hand, the red cell binding of ^{99m}Tc-L,L-EC is lower than that of ¹²⁵I-OIH [2.0% ± 3.1%, n = 5 (preliminary data) versus 15%–30% (3,24)]. It is also likely that the extrarenal clearance of ^{99m}Tc-L,L-EC is lower than that of ¹²⁵I-OIH, which results in a reduction of its apparent volume of distribution. Indeed, in anuric patients, the clearance of ^{99m}Tc-L,L-EC ranged between 10 and 15 ml/min/1.73 m², whereas the clearance of OIH was shown to average 30 ml/min/1.73 m² (25), a value similar to that observed in this study. Although error measurements with a single-injection multiple sample plasma clearance technique could account for part of these figures, this might

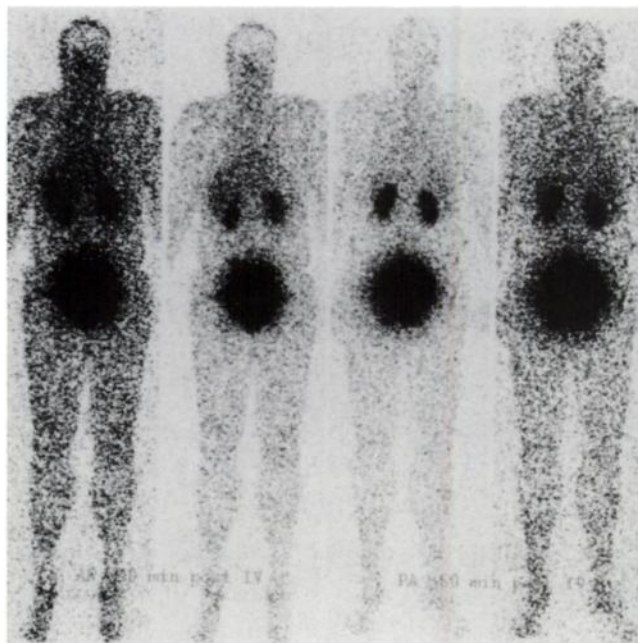


FIGURE 6. Whole-body scan of a normal volunteer 40 min after injection of ^{99m}Tc-L,L-EC (left, anterior; right, posterior).

also indicate a slightly lower biliary clearance of ^{99m}Tc-L,L-EC than OIH. Evidence for minimal extrarenal clearance was found in the low biliary activity observed in animals (10) and in the faint liver uptake detected in patients, even with severely impaired renal function. As ^{99m}Tc-L,L-EC and ¹²⁵I-OIH have similar molecular sizes (379 and 303 D, respectively), the lower protein binding of ^{99m}Tc-L,L-EC is likely to result in a higher fraction excreted by glomerular filtration. It can therefore be postulated that the lower renal clearance of ^{99m}Tc-L,L-EC compared with ¹²⁵I-OIH is likely to be due to a lower tubular extraction. Evidence for renal tubular extraction arises from the fact that ^{99m}Tc-L,L-EC clearance is more than 2.5 times higher than the glomerular filtration rate and from experiments using competition with probenecid (10). Technetium-99m-L,L-EC does not contain the carbonylglycine moiety (-CO-NH-CH₂-COOH) of OIH, which is assumed to play a major role in the interaction with the transport proteins (26). However, the new tracer has in its chelate structure an oxotechnetium-glycine sequence (TcO-NH-CH₂-COOH) that structurally resembles the carbonylglycine chain and therefore may account for an efficient fit with the tubular proteins. Whether these molecules are handled by exactly the same transport mechanism in the tubular cell and with a similar affinity is unclear and remains to be explored, e.g., using a PAH loading test (3).

The comparison between ^{99m}Tc-L,L-EC and ^{99m}Tc-MAG3 disclosed two main features. The imaging properties of the new ^{99m}Tc-complex were at least as good as those of ^{99m}Tc-MAG3. The information provided by the scintigraphic images and by the renographic curves was essentially similar, except for less prominent liver activity for ^{99m}Tc-L,L-EC than for ^{99m}Tc-MAG3, as demonstrated

by a semiquantitative liver activity index, which suggests a lower liver extraction of the former. However, it has to be pointed out that a direct comparison between the two tracers was only available in stable patients at long-term follow-up. In patients with acute complications or in the early days after transplantation, it was assumed that the instability of the renal function, which can dramatically improve or deteriorate over a short period, did not allow a direct comparison at an interval of 48 hr. In the authors' experience, the scintigraphic appearances observed with $^{99m}\text{Tc-L,L-EC}$ were not significantly different from those usually observed with $^{99m}\text{Tc-MAG3}$. In particular, in acute tubular necrosis, the classic characteristics were observed, which are shown by tubular agents of a progressively rising renogram curve and not an early peak followed by a down slope, as shown by $^{99m}\text{Tc-DTPA}$. In any case, $^{99m}\text{Tc-L,L-EC}$, with its rapid renal clearance, is eminently suitable to study transplant recipients or patients with renal failure and appears a promising tracer for future urologic studies.

The plasma clearance of $^{99m}\text{Tc-L,L-EC}$ was systematically higher than the clearance of $^{99m}\text{Tc-MAG3}$ by a mean 39% and confirms previous data (12,13). Moreover, the correlation between the $^{99m}\text{Tc-L,L-EC}$ and OIH clearance was significantly better ($p < 0.0001$) than the correlation between the $^{99m}\text{Tc-MAG3}$ and OIH clearance. This is important because the clearance of $^{99m}\text{Tc-L,L-EC}$ is a better predictor of the clearance of OIH, which is considered as the reference radiopharmaceutical for ERPF measurements (27). Finally, especially in transplant recipients and in patients with renal diseases, the low protein binding of $^{99m}\text{Tc-L,L-EC}$ compared with that of $^{99m}\text{Tc-MAG3}$ [90% (3)] might be an advantage because these patients may present significant variations of plasma protein levels, leading to unsuspected and uncontrolled variations of the distribution volumes.

In conclusion, this study demonstrates that kit-formulated $^{99m}\text{Tc-L,L-EC}$ without HPLC purification is suitable for both imaging and quantitative measurement of the renal function. It behaves more closely to OIH than does $^{99m}\text{Tc-MAG3}$ and, owing to a convenient and efficient labeling at room temperature, appears to be a promising alternative to $^{99m}\text{Tc-MAG3}$ and radioiodinated OIH for renal studies.

APPENDIX

Plasma activity was plotted against time and fitted to the general formula of a biexponential function. The clearance, Cl (ml/min) was calculated from the plasma disappearance curve using the following formula:

$$\text{Cl} = \frac{D\alpha\beta}{A\beta + B\alpha},$$

where A and B are the intercept in ordinate (in counts/min/ml of plasma) of the fast and of the slow exponential, respectively; α and β are the slopes (min^{-1}) of the fast and of the slow exponential, respectively; and D equals the total injected dose (cpm).

The two slopes can also be expressed as biologic half-lives (min).

$$T_{50\alpha} = \frac{\ln 2}{\alpha} \quad \text{and} \quad T_{50\beta} = \frac{\ln 2}{\beta}.$$

The distribution volumes were calculated using Sapirstein et al.'s (16) formulas:

$$V_1 = \frac{D}{A + B}$$

$$\delta = \frac{V_1[A\alpha + B\beta]}{A + B} - \text{Cl}$$

$$V_2 = \frac{\delta \text{Cl}}{V_1\alpha\beta}$$

$$V_{\text{TOT}} = V_1 + V_2,$$

where V_1 is the initial volume of distribution (in ml), V_2 is the second volume of distribution (in ml), V_{TOT} is the total volume of distribution (in ml) and δ the intercompartmental flow (in ml per min).

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