

Characterization of Technetium-99m-L,L-ECD for Brain Perfusion Imaging, Part 1: Pharmacology of Technetium-99m ECD in Nonhuman Primates

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Technetium-99m ethyl cysteinyl dimer (^{99m}Tc]ECD) is a neutral, lipophilic complex which rapidly crosses the blood-brain barrier. Brain retention and tissue metabolism of ^{99m}Tc]ECD is dependent upon the stereochemical configuration of the complex. While both L,L and D,D enantiomers are extracted by the brain, only the L,L but not the D,D form, is metabolized and retained in the monkey brain (4.7% injected dose initially, $T_{1/2} > 24$ hr). Dynamic single photon emission computed tomography imaging studies in one monkey indicates ^{99m}Tc -L,L-ECD to be distributed in a pattern consistent with regional cerebral blood flow for up to 16 hr postinjection. Dual-labeled ^{99m}Tc -L,L-ECD and ^{14}C]iodoantipyrine autoradiography studies performed 1 hr after administration show cortical gray to white matter ratios of both isotopes to be equivalent (~4–5:1). These data suggest that ^{99m}Tc -L,L-ECD will be useful for the scintigraphic assessment of cerebral perfusion in humans.

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Alterations in regional blood flow (rCBF) are observed in cerebrovascular disease and other neurologic disorders. A diagnostic and prognostic role for the measurement of rCBF using xenon-133 (^{133}Xe) in these patients is developing (1–6). Unfortunately, rCBF studies with xenon gas are technically difficult to perform and require specialized instrumentation which is not readily available. In contrast, less quantitative imaging of brain perfusion is readily available. These studies are being performed routinely using iodine-123 (^{123}I) labeled amines, [^{123}I]iodoamphetamine (IMP), and ^{123}I -N,N,N'-trimethyl-N'-(2-hydroxyl-3-methyl-5-iodobenzyl)-1,3-propane-diamine. IMP is commercially available and has in many studies demonstrated its clinical utility as an adjunct in diagnosis of various neurologic diseases (7,8). However, the physical properties of ^{123}I and the brain pharmacokinetic properties of IMP makes this agent suboptimal for high resolution

brain perfusion imaging (7,8). This has led to substantial research toward the development of technetium-99m- (^{99m}Tc) labeled radiopharmaceuticals to image perfusion patterns in the brain with conventional nuclear medicine instrumentation (7–10).

With the exception of ^{99m}Tc -hexamethylpropyleneamine oxime (HM-PAO) (11) which has been validated as a marker of rCBF in humans (12), brain retention of new ^{99m}Tc agents is insufficient for routine imaging and/or they do not distribute in accordance with rCBF (9,10,13–15). Although ^{99m}Tc]HM-PAO is a major advancement for the routine assessment of brain perfusion, the technetium (Tc) complex decomposes in vitro after reconstitution which necessitates its usage within 30 min of preparation (11). This precludes its preparation in a central radiopharmacy and complicates its ready use in certain situations such as ictal imaging in epileptics.

The objective of the investigation reported here was to identify a complex of Tc which would be highly extracted and retained in the brain, could be prepared readily in the clinic at a high level of radiochemical purity, and which would be radiochemically stable for

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routine use. Since diamine dithiol (N_2S_2) chelates form pure, stable, and neutral lipophilic complexes with ^{99m}Tc (15), many derivatives based on this chelate system were synthesized and evaluated as potential perfusion imaging agents (13,14). The chemistry and preclinical pharmacologic characterization of one of these complexes, technetium-99m ethyl cysteinylate dimer ($[^{99m}Tc]ECD$), is reported here.

METHODS

Chemistry and Kit Formulation of $[^{99m}Tc]ECD$

N,N' -1,2-ethanedithiolbis-L and D-cysteine diethylester dihydrochloride were prepared by a published procedure (16) and purified by extraction from a basic solution with diethyl ether. These extracts were reacidified and crystallized from hot ethanol/water containing excess 12N hydrochloric acid. The $[^{99m}Tc]ECD$ complexes (Fig. 1) were prepared by room temperature exchange labeling with $[^{99m}Tc]glucoheptonate$, prepared as a Glucoscan Kit (E.I. Du Pont de Nemours Co., Inc., No. Billerica, MA) reconstituted with $[^{99m}Tc]O_4$ from a commercial molybdenum-99 (^{99}Mo)/ ^{99m}Tc generator (Du Pont Company No. Billerica, MA). The ^{99m}Tc complexes were purified by high performance liquid chromatography (HPLC) on a Brownlee RP-8 Spheri 5 column using a 0.05M ammonium acetate/methanol gradient. Radiochemical purity was >90%. Octanol/water partition coefficients were determined for both ^{99m}Tc -L,L- and D,D-ECD was found to be 48 ± 3 ($n = 4 \pm s.d.$), and 51 ± 1 ($n = 3 \pm s.d.$), respectively.

A "kit" formulation was also developed for the L,L isomer of ECD. The kit consisted of two serum-capped, 5-ml vials: Vial A was freeze-dried under a N_2 headspace and contained 0.90 mg L,L-ECD $\cdot 2HCl$, 72 μg $SnCl_2 \cdot 2H_2O$, 360 μg $Na_2EDTA \cdot 2H_2O$ and 24 mg mannitol. Vial B contained 1 ml of pH 7.5 phosphate buffer (0.0187M) under an air headspace. To form the ^{99m}Tc -L,L-ECD complex, saline (1.2 ml) was injected into Vial A and the vial was shaken to dissolve the contents. One milliliter of the solution was withdrawn by syringe and injected into Vial B. Technetium-99m generator eluate (25–50 mCi) then was injected into Vial B and reacted for at least 15 min.

The radiochemical purity of $[^{99m}Tc]ECD$ was determined by reverse-phase thin layer chromatography (TLC) (Whatman MKC18 plates, developed with acetone: 0.5M, pH 7.0 ammonium acetate [60:40]). Radioactivity profiles were quantitated using a linear scanner fitted with a sodium iodide detector (17). The ^{99m}Tc -L,L- and D,D-ECD (retention factors $[R_f] = 0.35 \pm 0.06$) complexes formed were >90% radiochemical purity within 15 min at room temperature and showed no sign of decomposition over 24 hr.

Several parameters (with ranges investigated) were shown

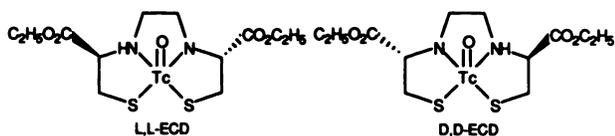


FIGURE 1
Structure of ^{99m}Tc -L,L and D,E-ECD.

to have no influence on radiolabeling performance: total activity at constant volume (25–200 mCi at 2 ml), final volume at constant activity (1–5 ml at 25 mCi), time since previous generator elution (24–72 hr at 25 mCi), and Tc generator eluate age (0.2–24 hr at 25 mCi).

Animal Preparation for Brain Extraction Studies

The brain first-pass extraction fraction of ^{99m}Tc -L,L-ECD was determined in one young mature, male rhesus monkey and in 12 rats. The extraction fraction study in the monkey was determined using a modification of an external counting technique (18). The monkey was food-deprived for 24 hr prior to induction of anesthesia with an i.m. injection of a mixture of ketamine hydrochloride (10 mg/kg) and acepromazine maleate (1 mg/kg). Anesthesia was maintained with subsequent constant intravenous (i.v.) infusion of 1.5 mg/ml sodium thiamylal (Park Davis, Div. of Warner Lambert Co., Morris Plains, NJ) at a rate averaging <0.5 ml/min. The external carotid artery was ligated at its origin from the common carotid artery and ^{99m}Tc -L,L-ECD was injected in a rapid bolus (60 mCi in 1 ml) through a 25-gauge butterfly cannula positioned in the common carotid artery. Immediately after administration, dynamic planar brain imaging was performed (3 sec/view) for 5 min. In order to maintain patency, the cannula was pretreated and repeatedly rinsed with sodium heparin (100 units/ml). The monkey's rectal temperature, arterial blood gases and pH were monitored during the studies (ranges temperature 33–37°C, pCO₂ 35–42, pO₂ 130–142, pH = 7.38–7.46).

The estimated fraction of ^{99m}Tc -L,L-ECD extracted by the brain during a single capillary transit (E) was calculated by analysis of residuals (JANA-Statistical Consults Inc., Lexington, KY) and by extrapolating the relatively slow clearance of ^{99m}Tc -L,L-ECD from brain tissue (A) to the maximum of the perfusion peak (P). The estimated extraction fraction was calculated as $E = A/P$.

Brain uptake index (BUI) studies were performed using a modification of a previously described procedure (19). Adult, male Sprague-Dawley rats were anesthetized using sodium pentobarbital (35 mg/kg, i.p.) and their right common carotid arteries isolated. Approximately 10 μCi of ECD and 10 μCi of iodine-125 iodoantipyrine ($[^{125}I]IAP$), (Du Pont Company, No. Billerica, MA) were injected into the artery using a 1-ml syringe with a 23-gauge needle. Ten seconds after injection, the animals were killed and their brains removed. The right forebrain was weighed and assayed for radioactivity in a gamma scintillation counter (Packard Instrument Co., Sterling, VA). The BUI was defined as being the ratio of activity in the brain of L,L- or D,D-ECD to that of $[^{125}I]IAP$:

$$BUI = \frac{\mu Ci^{99m}Tc \text{ in Right Forebrain} / \mu Ci^{125}I \text{ in Right Forebrain}}{\mu Ci^{99m}Tc \text{ Injected} / \mu Ci^{125}I \text{ Injected}} \times 100.$$

From these results, the vascular permeability of the $[^{99m}Tc]$ ECD complexes were compared with that of iodoantipyrine.

Monkey Imaging Studies

Young, mature (1–3 yr old) rhesus or cynomolgus monkeys were food-deprived for 24 hr prior to induction of anesthesia (as previously specified). Immediately prior to imaging, 18–22 mCi of the ^{99m}Tc complex was administered through a saphenous vein.

Standard planar imaging studies were performed using a Picker International Digital Dyna Camera (Picker International, Inc., Highland Hts., OH) or a Nuclear Chicago (Medex Update, Medex Inc., Palatine, IL) gamma camera equipped with high resolution, low-energy collimators. In general, a dynamic series of 1-min frames was collected for at least 1 hr following i.v. injection. The percent of the injected dose (% ID) in the organs of one cynomolgus and three rhesus monkeys was determined by comparison of decay-corrected count rates with those of a standard phantom. Anterior and posterior images (500k counts per view) were acquired at 10, 30, 60, 120, 360, and 1440 min postinjection. Clearance rates of the radioisotope were calculated based on the decay-corrected, time-activity curves obtained from a background subtracted isocontour, whole brain region of interest or in the case of the biodistribution study, a geometric mean of the anterior/posterior count density. In one monkey, urine and feces were collected to determine total recovery.

Venous blood samples were drawn from a peripheral vein catheter other than the injection site. The % ID in the blood was estimated by multiplying the activity per ml in the venous sample by an estimated total vascular volume (8.5% body weight).

Tomographic imaging was performed using a SME 810 head imaging system (Strichman, Medfield, MA) which is a single slice system with a resolution of ~1 cm full width half maximum. Dynamic single photon emission computed tomography (SPECT) images (5 min per frame) of a 1-cm transverse slice at a mid-cortical level were obtained for 2 hr postadministration of [^{99m}Tc]ECD. Serial images from the cerebellum through the cortex also were obtained at 2 and 16 hr postinjection.

Brain Subcellular Distribution Studies

The subcellular distribution of [^{99m}Tc]ECD in the brain was determined in cortical gray matter tissue 1 hr postadministration of [^{99m}Tc]ECD. Monkeys were killed with T61-euthanasia solution (American Hoechst Corp., Somerville, NJ). The brain tissue was immediately homogenized in ice cold isotonic Ringer's buffer (pH = 7.4). Fractions of the homogenate (20% w/v) were obtained by differential centrifugation using a modification of the procedure of Otto (20). Total recovery (>95%) was determined based on the amount of activity in the tube prior to centrifugation. The homogenates were centrifuged twice at 650 *g* for 10 min to obtain the nuclear and plasma membrane fraction. The supernatants were combined and re-centrifuged at 20,000 *g* for 15 min to obtain the mitochondrial fraction. Microsomes were obtained following centrifugation of the resultant supernatant at 100,000 *g* for 60 min.

Metabolism of ECD

To determine whether D,D or ^{99m}Tc-L,L-ECD complexes could be metabolized enzymatically in monkey brain tissue, the complexes (~1 × 10⁻⁸ mM) were incubated at 37°C with rhesus or cynomolgus monkey brain homogenate (1:20 w/v dilution in phosphate buffer pH 7.4) for 1 hr. After incubation, the tubes were centrifuged, (650 *g* for 10 min) and an aliquot of the supernatant was analyzed by TLC (see chemistry methods). At times, quantitation of activity using the TLC method was not possible due to the low levels of activity on the plate. These plates were scraped and the activity was counted using

a gamma counter. Control incubations were performed with ^{99m}Tc complexes in buffer alone.

To determine the presence of metabolites in vivo, blood and cerebrospinal fluid (CSF) samples were withdrawn from monkeys injected with D,D or ^{99m}Tc-L,L-ECD. In one monkey injected with 80 mCi of ^{99m}Tc-L,L-ECD, venous blood and CSF samples were obtained at 2, 5, and 122 min postinjection. The CSF samples were obtained via a cerebellomedullary cannula positioned under fluoroscopy. Blood samples (5 ml) were centrifuged and plasma and red blood cells (RBCs) were separated and treated with 1 ml 50% ethanol to denature blood proteins. The RBCs were centrifuged and the supernatant used for analysis. All samples were analyzed by HPLC on a Spheri 5 μm C-8 column (20 ml/min gradient phase of acetonitrile/0.075M aqueous ammonium sulfate) and radioactive species were detected by a sodium iodide probe and a NICO ratemeter.

The amount of ^{99m}Tc-L,L- or D,D-ECD available for brain extraction was estimated by determining the amount of unmetabolized parent compound in the venous blood. At various times postadministration of ^{99m}Tc-L,L- or D,D-ECD blood samples were obtained from three anesthetized male rhesus monkeys via an external saphenous vein catheter. Blood (0.5 ml) was drawn into a heparinized syringe (100 units/ml blood), acidified immediately by adding 0.025 ml of 1.0 N HCl and transferred to a tube containing 500 μl of ethyl acetate and 500 μl of 1-octanol. The extraction tubes were vortexed for 15–20 sec, centrifuged for 10 min at 650 *g*. The upper, clear organic layer was removed. Previous studies have determined that the organic layer contains only the parent compound and that no degradation of ^{99m}Tc-L,L-ECD occurs in the ethyl acetate 1-octanol layer. The remaining pellet was resuspended and the procedure was repeated. The amount of activity in each tube was determined in a gamma counter and the percent of compound extracted (unmetabolized complex) was calculated.

Dual Labeled Autoradiography

Male monkeys for autoradiography studies were anesthetized as described previously. Rectal temperature, pH, and blood gases were maintained (body temperature 33–36°C, pH = 7.38–7.46, pCO₂ 35–42, pO₂ 130–142). Technetium-99 L,L-ECD (50–80 mCi) was administered as a bolus via a femoral vein or carotid artery. One hour later, 250 μCi of carbon-14 (¹⁴C) iodoantipyrine was infused at a constant rate over 72 to 90 sec into the femoral vein. Immediately following the iodoantipyrine infusion, 5 ml of T61-euthanasia solution was injected; and the brain was removed via circular craniotomy, rinsed in ice cold saline and quick frozen in 2-methylbutane at -60°C. Frozen sagittal sections (22 μ) were cut on a cryostatic microtome (Hacker Instruments Inc., Fairfield, NJ). These sections were affixed to plastic tape (Scotch Type 800, 3M St. Paul, MN), mounted on cardboard, packed within prechilled cassettes and apposed to SB5 film (Kodak, Rochester, NY) for 22 hr at -60°C. Commercial [¹⁴C]methyl methacrylate standards (Amersham Corp., Arlington Heights, IL) and were packed similarly to the test samples. Custom ^{99m}Tc brain tissue standards were prepared by adding known activities of ^{99m}Tc to gray and white matter monkey brain paste. The paste was frozen and sectioned (20 μ) to produce autoradiographic standards and a portion counted by gamma spectroscopy to give the equivalent activity per gram.

A standard curve for ^{99m}Tc activity was determined by cross-calibration of the ^{14}C standards to ^{99m}Tc equivalents (correlation coefficient = 0.98) using a video-based camera system (Loats Assoc. Inc., Westminster, MD). This indirect quantitative method (21) was shown not to vary with different batches of film. No differences in the amount of ^{99m}Tc attenuation in white and gray matter were observed.

Three film exposures of the brain sections, ^{14}C and ^{99m}Tc standards were necessary to obtain quantitative ^{14}C and ^{99m}Tc images. Initially tissue and standards were apposed to the SB-5 film for 22 hr (^{99m}Tc and ^{14}C image). A minimum of 4 days elapsed before tissues were repacked to ensure total ^{99m}Tc decay. After this time the tissues and standards were again apposed to SB-5 film for 22 hr to generate the ^{14}C contribution to a 22-hr image. The resulting 22-hr ^{14}C images were very faint ($\leq 5\%$ of the optical density generated by the first 22-hr image). These images were then subtracted from the first 22-hr image. The images were subtracted by image overlay to generate a pure ^{99m}Tc image. A pure ^{14}C image was then obtained when the tissue was apposed to the SB-5 film for 6–10 days. The optical density values of the pure ^{14}C and ^{99m}Tc images were then converted to nCi/g tissue equivalents.

Anatomic structures of interest were identified by using a rhesus monkey brain atlas (22). One densitometric reading was taken of each brain region from six autoradiograms (ROI range 8–80 pixels, 27 pixel/cm). Identical regions of interest (ROIs) were drawn simultaneously on both the ^{14}C and ^{99m}Tc images using a specialized "dual-labeled" software package (Loats Associates, Inc., Westminster, MD). In all animals, the tissue activity (nCi/g) of the isotopes was normalized to activity in the corpus callosum.

Toxicology

The acute and 14 day repeated-dose i.v. toxicity of the [^{99m}Tc]ECD formulation was evaluated in male rats (Charles River, Portage, MI) and male beagle dogs (Hazleton Laboratories, Madison, WI). Acute i.v. toxicity also was studied in male, wild-caught cynomolgus monkeys (Hazleton Laboratories, Madison, WI). Doses were calculated based on the kit contents on ECD dihydrochloride and expressed in relation to the anticipated maximum human dose (MHD) of 0.011 mg/kg. The potential for the formulation to be irritating following i.v. or inadvertent perivascular administration was assessed against saline in male New Zealand white rabbits (Charles River). The compatibility of the formulation with dog and human blood, plasma and serum was assessed by mixing equal volumes of it with the various blood components. Hemolysis was measured colorimetrically and serum and plasma reactions were assessed visually.

RESULTS

Rat and Monkey Brain Extraction Studies

The BUI for $^{99m}\text{Tc-L,L-ECD}$ is 71 ± 1 ($n = 12 \pm$ s.e.m.) and for $^{99m}\text{Tc-D,D-ECD}$ is 68 ± 3 ($n = 4 \pm$ s.e.m.) suggesting equivalent permeability of both compounds in the brain vasculature. The time-activity curve from the monkey extraction study of $^{99m}\text{Tc-L,L-ECD}$ was fit to a bi-exponential curve (correlation coefficient = 0.992) from the peak brain activity. The intercept and

slope of the fast component were 23.1% and $7.8 \times 10^{-4} \text{ min}^{-1}$. Those of the low component were 76.9% and $5.5 \times 10^{-2} \text{ min}^{-1}$. The estimated extraction fraction of 77 determined by these parameter estimates is in good agreement with the BUI value of 71.

Planar Imaging in Monkeys

Both the D,D and L,L form of the ^{99m}Tc complex appear to be equally well extracted by the brain as indicated by the rapid acquisition (3 sec/view) dynamic planar imaging studies (Fig. 2). The uptake phase of both compounds is rapid, reaching 95% peak brain activity within 20 sec postinjection. However, only the L,L isomeric form is retained in the brain (Fig. 3). After the first few minutes, little additional $^{99m}\text{Tc-L,L-ECD}$ reaches the brain because of its rapid metabolism and clearance from the blood (Fig. 4).

Biodistribution studies show the selective retention of $^{99m}\text{Tc-L,L-ECD}$ in the brain (Table 1). No differences in pharmacokinetics were noted between the two species of monkey. In contrast to its retention in the brain, $^{99m}\text{Tc-L,L-ECD}$ clearance is rapid from background regions, facial regions, lungs and blood (Figs. 3 and 4). Peak blood activity of $9.7 \pm 4.1\%$ ID was observed 2 min postinjection. Blood clearance is biphasic with an initial $T_{1/2}$ of 10 min for $\sim 70\%$ of the activity and a terminal $T_{1/2}$ of ~ 2.8 hr for 30% (Table 1, Fig. 4). Transient heart uptake is observed with peak activity of 2.4% ID occurring 10 min postinjection. For the first 6 hr postinjection, liver/gallbladder activity accounts for 15–20% of the injected dose. The rapid metabolism of $^{99m}\text{Tc-L,L-ECD}$ in the blood to a more polar product probably accounts for the high renal excretion of [^{99m}Tc] ECD activity (68% in 24 hr in the one monkey studied).

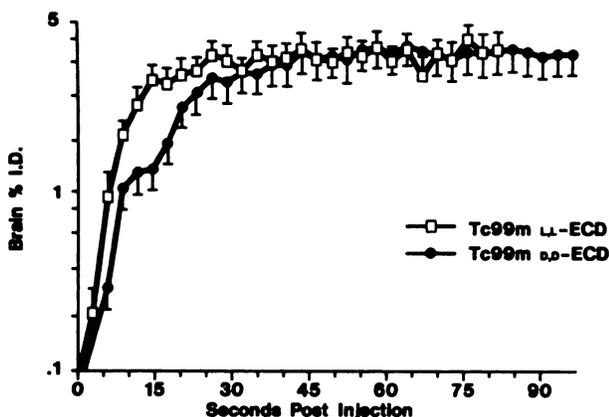


FIGURE 2 Brain uptake curves for $^{99m}\text{Tc-L,L-ECD}$ and $^{99m}\text{Tc-D,D-ECD}$ in anesthetized monkeys. Data represents % ID versus time. Brain % ID data was obtained from dynamic planar imaging studies (3 sec/view). The maximal % ID in the brain for both $^{99m}\text{Tc-L,L-ECD}$ and $^{99m}\text{Tc-D,D-ECD}$ is very similar, 4.7 and 4.6% ID. All values are mean \pm s.e.m. for three monkeys.

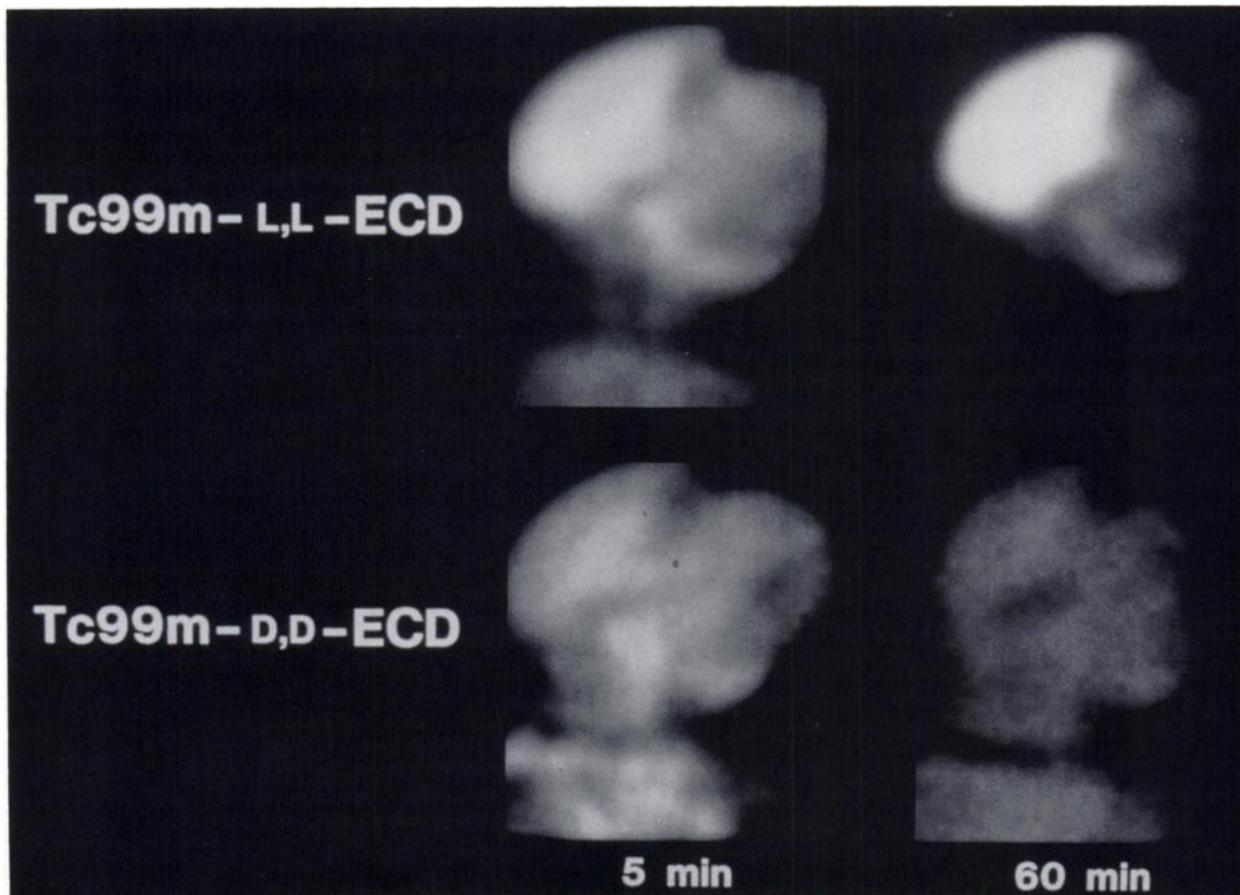


FIGURE 3

One-minute lateral planar anesthetized monkey images taken 5 and 60 min postadministration of 20 mCi of $^{99m}\text{Tc-L,L-ECD}$ or $^{99m}\text{Tc-D,D-ECD}$ to the same monkey on two different days. Brain retention is only observed for the L,L-complex. In contrast, both complexes show rapid clearance from background and facial activity.

Dynamic SPECT Imaging

Qualitatively, the SPECT images are typical of those which would be anticipated from a tracer which distributes according to rCBF with relatively higher concentrations seen in the occipital and frontal cortices and thalamus as compared with adjacent white matter areas. The initial dynamic SPECT and the 2 hr to 16 hr serial SPECT images indicate no change in distribution of [^{99m}Tc]ECD over time and a close correspondence between known gray matter areas and high ^{99m}Tc activity (Fig. 5).

Metabolism

The metabolic fate of [^{99m}Tc]ECD is similar in vitro and in vivo. Only the L,L form of the complex is metabolized (Fig. 6). Radio-TLC of the supernatant from monkey brain homogenate after in vitro incubation with either $^{99m}\text{Tc-L,L-ECD}$ or $^{99m}\text{Tc-D,D-ECD}$ shows $^{99m}\text{Tc-L,L-ECD}$, but not $^{99m}\text{Tc-D,D-ECD}$ to be metabolized to a single more polar product ($R_f = 0.70$). Stereospecific metabolism of the L,L form also was observed in blood plasma extraction studies performed 10 min postinjection of

L,L or D,D-ECD in three monkeys (major metabolite at $R_f = 0.70$).

In order to determine if the metabolite(s) formed in vivo in the brain and periphery had similar chemical properties, HPLC analysis of plasma, RBC and CSF samples taken from a monkey 2, 5, and 120 min postinjection of $^{99m}\text{Tc-L,L-ECD}$ was performed. The results were similar in all samples. Over time there was an increase in the appearance of two polar metabolites, the less polar of which appeared to be transient. The HPLC chromatograms of these samples had similar R_f for both metabolites.

The structure of the metabolite(s) was not determined. However, the similarity in the R_f of the metabolites observed in the HPLC and TLC chromatograms suggest that the metabolic species formed in the brain is the same as that formed in the rest of the body. It was demonstrated in both TLC and HPLC studies the polar product is not $^{99m}\text{TcO}_4^-$.

The $^{99m}\text{Tc-L,L-ECD}$ metabolite complex, isolated from monkey brain tissue homogenate ($R_f = 0.70$), was administered i.v. to a rhesus monkey. Dynamic planar

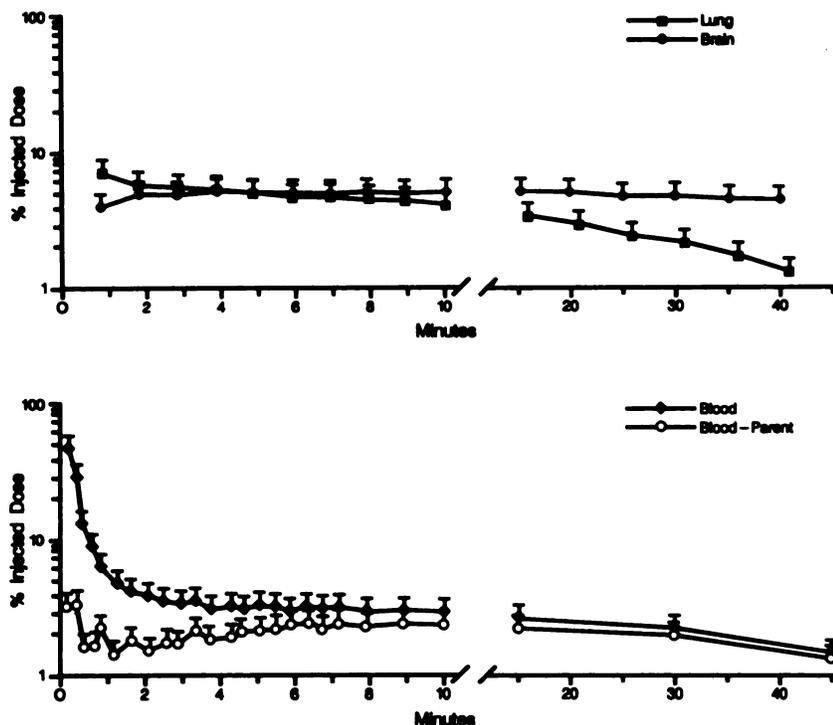


FIGURE 4
Pharmacokinetic of $^{99m}\text{Tc-L,L-ECD}$ in anesthetized monkeys. Percent injected dose data for the brain and lungs were obtained from dynamic planar imaging studies (20 sec-1 min/view). Blood % ID was determined from venous blood samples. The percent of activity in the blood existing in the form of the $^{99m}\text{Tc-L,L-ECD}$ (blood-parent) by performing ethyl acetate/1-octanol extraction of the venous blood samples. It was demonstrated that only the parent is extracted into the ethyl acetate 1-octanol. All values are mean for two animals or mean \pm s.e.m. for three animals. This graph demonstrates that after the first few minutes little $^{99m}\text{Tc-L,L-ECD}$ reaches the brain because of its rapid clearance from the blood and metabolism to a polar substance.

imaging showed that the labeled product is not taken up by the brain, suggesting that the polar metabolite(s) in the blood can not cross the blood-brain barrier (BBB).

Subcellular Distribution

The cytosolic fraction of monkey cortical gray matter contained greater than 70% of the activity in the tissue sample (Table 2). The activity is in the form of at least one metabolite with the majority of the activity having a TLC- R_f of 0.65–0.72. Therefore, at 1 hr postadministration of [^{99m}Tc]ECD, the radioactive species retained in the brain is in the form of a metabolite(s) which has the same R_f as the metabolite isolated from blood plasma and brain homogenate in vitro.

Monkey Dual-Labeled Autoradiography

Qualitatively, there is a close correspondence between the distribution pattern of ^{99m}Tc and [^{14}C]IAP in

monkeys killed 1 hr postadministration of $^{99m}\text{Tc-L,L-ECD}$ (Fig. 7).

The ^{99m}Tc images show good delineation between high flow gray matter areas and low flow white matter areas in all parts of the brain. No significant differences were noted in the ratios between ^{14}C and ^{99m}Tc for any of the brain regions investigated (paired t-test). The highest concentrations of both ^{99m}Tc and ^{14}C were in the dentate nucleus and in the intraparietal and superior frontal cortices. White matter areas showed a four- to fivefold lower concentration, consistent with relative blood flow in these areas (Fig. 8).

Toxicology

Acute toxicity studies showed no adverse reactions in any animal at doses up to $56 \times$ MHD. No histopathologic alterations were seen in organs from any animals. Repeat-dose toxicity studies show no adverse reactions

TABLE 1
Technetium-99m-L,L-ECD Biodistribution in Monkeys

Region	Minutes Postadministration $^{99m}\text{Tc-L,L-ECD}$					
	10	30	60	120	360	1440
Brain	4.8 ± 0.3	4.6 ± 0.3	4.7 ± 0.5	4.6 ± 0.6	3.9 ± 0.6	3.1 ± 0.5
Bladder	13.8 ± 1.0	22.4 ± 1.8	31.7 ± 3.0	37.8 ± 4.4	1.4 ± 0.3	0.3 ± 0.3
Heart	2.4 ± 0.8	1.3 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0	0
Kidney	4.7 ± 0.9	3.7 ± 1.2	2.6 ± 0.9	1.6 ± 0.8	0.8 ± 0.6	0.5 ± 0.6
Liver/Gallbladder	17.4 ± 3.0	18.0 ± 2.8	18.6 ± 5.0	18.4 ± 5.4	16.9 ± 5.9	8.8 ± 2.9
Blood	6.0 ± 2.7	3.6 ± 1.6	2.7	1.7 ± 0.6	0.7 ± 0.6	0.4 ± 0.4

Values are expressed as a mean percent injected dose \pm s.e.m. for four monkeys, except for blood values which are a mean \pm s.e.m. for three monkeys or mean for two monkeys.

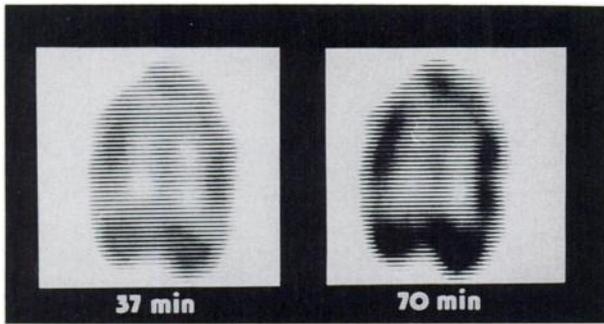


FIGURE 5
Transverse SPECT brain images of a monkey at 37 and 70 min after $^{99m}\text{Tc-L,L-ECD}$ administration. Repeat SPECT brain images at this mid-cortical level was obtained every 5 min between 30–120 min post-tracer administration. These images show the relatively higher concentration of ^{99m}Tc in high flow gray matter areas (cortical mantle and thalamus) as compared to adjacent white matter areas. No change in the distribution pattern of the tracer was observed at any time during the imaging study.

occurred in rats or dogs following 14 consecutive doses at up to $20 \times \text{MHD}$ (cumulative dose $280 \times \text{MHD}$). No test material-related effects were noted during gross or histopathologic examination in any species. The test material was found not to be irritating following i.v. or perivascular administration and was compatible with dog and human whole blood, plasma, and serum.

DISCUSSION

The results of these studies show $^{99m}\text{Tc-L,L-ECD}$ to be a stable, neutral, and lipophilic complex which readily crosses the BBB and is retained in the brain in a pattern consistent with regional cerebral perfusion. Excellent ^{99m}Tc complex stability (>6 hr) offers the poten-

TABLE 2
Cortical Gray Matter Subcellular Distribution of $^{99m}\text{Tc-L,L-ECD}$ in Monkeys 1 hr After In Vivo Administration

Nuclear and plasma membranes	Percent total activity		
	Mitochondria	Microsomes	Cytosol membrane
10 ± 1.7	13.3 ± 4.2	3.1 ± 1.2	73.6 ± 6.8

All values are mean \pm s.e.m. % recovery for four monkeys killed 1 hr postadministration of $^{99m}\text{Tc-L,L-ECD}$
* Most of activity is in the form of metabolite(s)

tial advantage of being able to provide the clinician with a compound that is readily available and can be used in the routine assessment of regional cerebral perfusion in a variety of clinical settings.

Upon intra-arterial or i.v. administration to animals, both the D,D and $^{99m}\text{Tc-L,L-ECD}$ complexes show good brain uptake as determined by rat BUI studies and monkey brain dynamic planar imaging. The brain uptake of these complexes is similar. Since the physical properties of the enantiomeric species also are similar, the extraction of $[\text{}^{99m}\text{Tc}]\text{ECD}$ likely is by a process of simple diffusion. The BUI of the $[\text{}^{99m}\text{Tc}]\text{ECD}$ complexes (D,D = 68 ± 3 and L,L = 71 ± 1) is much higher than the BUI of carrier mediated substrates which often show highly stereospecific uptake (23). Although the absolute rate of blood flow was not determined in the rats or in the monkey extraction study, a cerebral blood flow rate of between 50–60 ml/100 g/min has been determined for barbiturate anesthetized rats (24) and rhesus monkeys (25). The close agreement between the BUI of the L,L form of the complex in rats and its estimated monkey brain extraction ($E = 77\%$) is expected for a complex which crosses the BBB by diffusion.

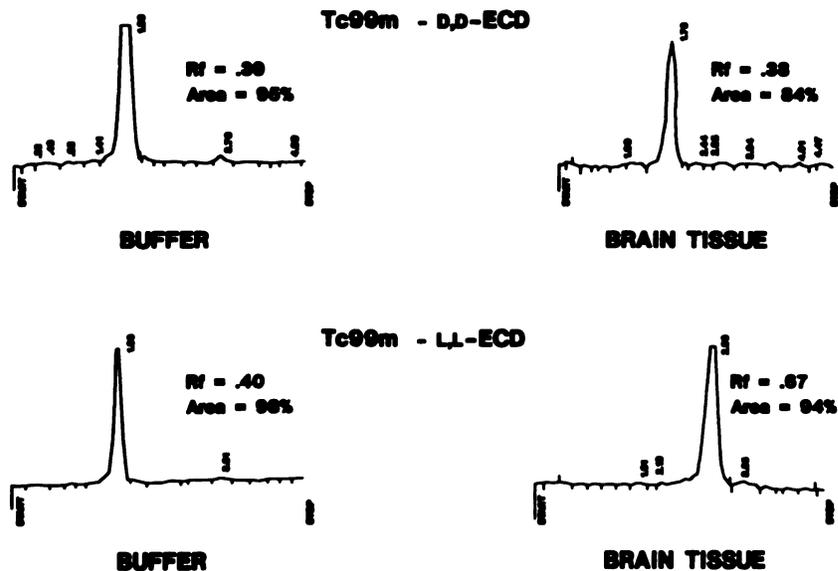
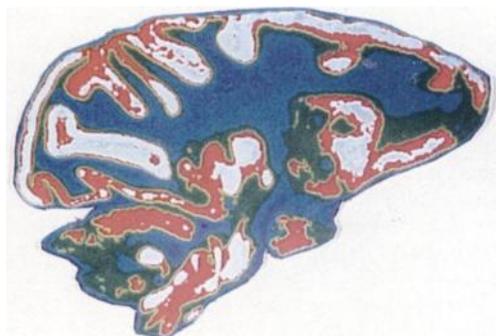
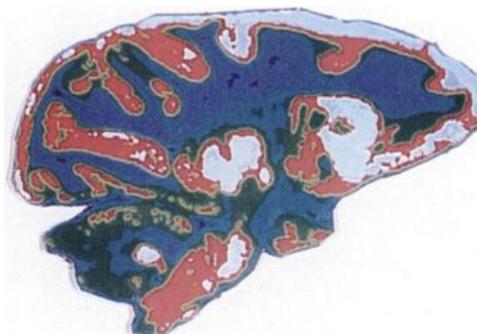


FIGURE 6
Integrated radio-thin layer chromatograms of the supernatant from monkey brain homogenates or phosphate buffer. Data show the stereo-selective brain metabolism of only the L,L form of the $[\text{}^{99m}\text{Tc}]\text{ECD}$ complex

MONKEY BRAIN AUTORADIOGRAPHY



99mTc-ECD



14C-IAP

FIGURE 7

Technetium-99m and ^{14}C autoradiographic images of a sagittal brain section from a monkey injected intravenously with $^{99\text{m}}\text{Tc-L,L-ECD}$ (70 mCi) followed by an intravenous infusion of $[^{14}\text{C}]\text{IAP}$ (250 μCi in 72 sec) 1 hr later. Brain tissue was removed and processed for autoradiographic analysis. Each image is a computer-enhanced pseudocolor reconstruction which is thresholded to its own maximal pixel value.

The metabolism studies suggest that selective brain retention of $^{99\text{m}}\text{Tc-L,L-ECD}$ may be the result of the rapid in vivo brain metabolism to a more polar species which is trapped in the brain. While evidence for this is indirect, a number of results lead to this conclusion.

First, in monkey brain homogenates, the L,L form of the complex is completely metabolized to a polar species. Metabolites are also observed in monkey CSF and blood 5 min after injection of $^{99\text{m}}\text{Tc-L,L-ECD}$. Presumably, the metabolite(s) isolated from the CSF resulted from brain tissue metabolism since CSF contains negligible enzymatic activity. Second, imaging of a monkey injected i.v. with the metabolite of $^{99\text{m}}\text{Tc-L,L-ECD}$ demonstrates the inability of the compound to cross the BBB, suggesting that if it is formed in the brain, the metabolite cannot cross the BBB in either direction. Next, only the L,L but not the D,D is metabolized in biologic samples and only the former is retained specifically in the brain. Finally, the subcellular distribution studies in monkey brain demonstrate that most of the $^{99\text{m}}\text{Tc}$ activity retained in the brain in vivo is in the form of a metabolite(s). The enzyme system and structure of the metabolite have not yet been fully characterized. Preliminary information suggests that $^{99\text{m}}\text{Tc-L,L-ECD}$ is de-esterified (26). Whatever enzymatic system is responsible for the conversion, it seems ubiquitously distributed in the brain, since the monkey autoradiography studies show a close correspondence between the $^{99\text{m}}\text{Tc}$ distribution and that of the reference perfusion tracer $[^{14}\text{C}]\text{jodoantipyrine}$. The similarity between the $^{99\text{m}}\text{Tc}$ and ^{14}C ratios in all brain area suggests that the rate limiting step for retention is not the rate

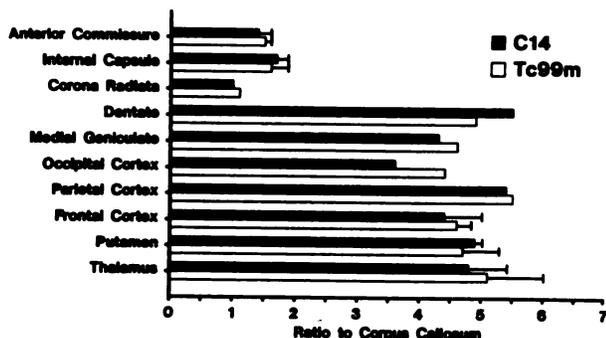


FIGURE 8

Dual labeled autoradiographic analysis of $^{99\text{m}}\text{Tc-L,L-ECD}$ brain distribution pattern. One hour after $^{99\text{m}}\text{Tc-L,L-ECD}$ was administered, the animals received an intravenous infusion of $[^{14}\text{C}]\text{jodoantipyrine}$. Values are ratios of activity for brain areas as compared to a white matter reference area (corpus callosum). Each value is a mean for two monkeys or a mean \pm s.e.m. for three monkeys. Paired t-test showed no difference between ^{14}C ratio and $^{99\text{m}}\text{Tc}$ ratios for any brain area.

of brain metabolism but the delivery of the substrate via passive diffusion across the BBB. However, in some pathologies the rate of $^{99m}\text{Tc-L,L-ECD}$ brain metabolism may be decreased to the point when it becomes the rate limiting step in brain retention.

Although the autoradiography studies were only performed in animals killed 1 hr postadministration of $^{99m}\text{Tc-L,L-ECD}$, the dynamic brain SPECT study and the slow brain washout of the compound suggest that the pattern of $^{99m}\text{Tc-L,L-ECD}$ distribution should be maintained for more than 1 hr postadministration.

In contrast to the slow brain clearance, the background facial areas, blood and lungs clear rapidly via renal excretion. A few minutes postinjection of $^{99m}\text{Tc-L,L-ECD}$, the brain imaging studies showed excellent delineation of brain structures with minimal contamination from adjacent nonbrain areas. Venous blood clearance was also rapid and indicated that the parent compound is rapidly metabolized in vivo to a nonbrain extractable polar species. Therefore, the brain input function (extractable [^{99m}Tc]ECD pool) was gone within a few minutes postinjection. The rapid brain uptake and good retention of $^{99m}\text{Tc-L,L-ECD}$ coupled with the rapid clearance of the parent compound from the blood suggest that calculation of absolute rCBF flow is possible.

The results of these studies suggest that the radiochemically stable L,L isomer of [^{99m}Tc]ECD exhibits pharmacologic properties useful for planar and SPECT imaging of the blood flow patterns in the brain. In addition, the chemical properties of the complex suggest that it will be convenient for use under a wide variety of clinical conditions.

In a companion article (27), the results of initial human studies are presented which suggest that the agent is useful for the scintigraphic assessment of regional cerebral perfusion in humans.

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