RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Technetium-99m-Labeled *P*-Aminohippuric Acid Analog: A New Renal Agent: Concise Communication

L. Rao Chervu, B. M. Sundoro, and M. Donald Blaufox

Albert Einstein College of Medicine, Bronx, New York

A renal agent labeled with Tc-99m and quantitatively secreted by the tubules has been sought for many years. To meet this need, a PAH analog (PAHIDA), has been synthesized by the coupling of *p*-aminohippuric acid with nitrilotriacetic acid anhydride, using Sn(II) reduction. This yields a stable complex with Tc-99m at a pH of 5.8. The purity and stability of the Tc-99m complex have been established by ITLC and high performance liquid chromatography (HPLC). Urinary excretions of the Tc-99m PAHIDA and I-131 hippurate, determined in mice and rats at different time intervals, are similar. The new compound is clearly excreted but its total clearance is lower than that of hippurate as a result of high protein binding. Rat urine analysis by ITLC and HPLC suggests that the agent excreted is similar to the complex administered to the animals. The Tc-99m-labeled agent is rapidly excreted in urine with no significant extrarenal pathway, thus providing excellent renal scintigrams in a rabbit model. The Tc-99m PAHIDA contains the R-CO-NH-CH_2-COOH grouping, analogous to that in hippurate, and consequently may provide the substrate specificity for renal excretion of this new class of agents labeled with technetium-99m.

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Radionuclidic techniques may provide assessment of glomerular filtration rate (GFR), effective renal plasma flow (ERPF), or individual renal function (*1*,*2*). Several agents are currently available for determination of these parameters but the formulation of a suitable Tc-99m-labeled agent for the determination of ERPF has eluded investigators. The tracer of choice for the clinical evaluation of renal tubular function is *o*-[^{131}I]iodohippurate (OIH). It has the disadvantage of imparting a relatively high absorbed radiation dose to the patient at low diagnostic doses. The radiation dose at 3 hr after intravenous administration of 500 μCi of I-131 OIH is 47 mrad to a normal kidney and 1.7 rad to the bladder wall. Renal absorbed doses of 3 rad or higher may be encountered in patients with impaired renal function (*2*,*3*). Although, I-123-labeled OIH lowers the radiation dose, it is not available at a reasonable cost for routine use. The presence of varying amounts of free radioiodine in OIH preparations often poses problems for accurate quantifiable assessment of function.

Technetium-99m has ideal physical properties for many applications in nuclear medicine, by virtue of its short half-life and favorable radiation characteristics. The low radiation dose permits the administration of large amounts of activity within short time intervals for serial measurements. Several Tc-99m agents have been reported for use in renal imaging and perfusion studies (*2*). Tc-99m DTPA is widely used in clinical nuclear medicine for GFR measurements. EDTA or DTPA complexes of Tc-99m are excreted solely through the slower process of glomerular filtration, and their slow rate of excretion, relative to that of compounds that are actively excreted, is a serious disadvantage. Tc-99m-labeled agents that are cleared rapidly by active tubular

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For reprints contact: L. Rao Chervu, PhD, Dept. of Nuclear Medicine, Albert Einstein College of Medicine, Bronx, NY 10461.

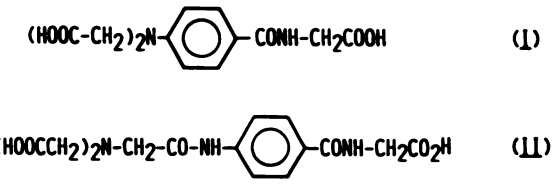

FIG. 1. Chemical structures of Compound 1 and Compound 2.

excretion, with minimal or preferably no reabsorption from the tubular lumen, would provide significant advantages over the agents mentioned above. They would be excreted within a short time interval, yielding a high target-to-background ratio and minimal radiation dose.

A new class of chelating agents, Tc-99m DADS [*N,N'*-bis(mercaptoacetamido)ethylene diamine and its propionic acid derivative), has been reported to have rapid renal excretion consistent with tubular excretion (*4,5*). The renal excretion of DADS is greater than that of Tc-99m DTPA but lower than that of OIH in rodents, but its significant biliary excretion in rats and humans represents a major limitation to its use (*4,6*). A DADS propionic acid derivative has been shown to undergo less biliary excretion without significant diminution of urinary excretion (*5,7*). Nevertheless, the complicated preparatory procedure for this agent, requiring heating and a high performance liquid chromatography (HPLC) purification step to separate the component that shows optimal renal excretion kinetics, precludes its application in a routine clinical setting.

The current study was undertaken in an effort to overcome these problems. This report describes biodistribution studies, and scintigrams in rabbits, of a new Tc-99m complex formed with PAH analogs.

MATERIALS AND METHODS

Synthesis of the PAH analogs. Two analogs were prepared with the following chemical structures (Fig. 1).

By refluxing *p*-aminohippuric acid in aqueous alkali with excess chloroacetic acid (1:5) *p*-(Bis-carboxymethylamino)-hippuric acid, **1**, was prepared under low heating for 8 hr, during which time the pH of the solution was kept between 9 and 10. After cooling, the product was separated by addition of hydrochloric acid to pH 3.0. Recrystallization from methanol and water yielded white crystals (30–45% yield). *MP:* 135°C (slow decomposition); *NMR* (D_2O/NaOD) δ 7.03 (4, center of AB quartet, J = 9Hz, aromatic H), 3.98 (4H, S, H_2OCH_2N), 3.80 (2, S, H_2OCH_2N), *MS* (M + 1) as methy ester, 353 (100%).

By a modified procedure of Burns et al. (*8*), *p*-[(Bis-carboxymethyl)-aminomethyl carboxyamino] hippuric acid (PAHIDA), **2**, was prepared with a *p*-aminohippuric acid reaction with freshly prepared nitrilotriacetic acid (NTAA) in dry DMF (1:1) at 100°C for 2 hr (Fig. 2). After crystallization from acetone/water (50:50), the compound was isolated as white crystals with a 60% yield: *MP:* 222–223°C. *NMR* (DMSO d_6/TMS) δ 10.52 (H1, brS, COOH), 8.72 (1H, brS, aromatic H), 7.78 (4H, center of AB quartet), 3.91 (2H, d, J = 5Hz, CH_2NH), 3.57 (4H, S, $OCCH_2N$), 3.52 (2H, S, $OCCH_2N$); *MS* (M + 1), 368 (100%). Anal. calc. for $C_{15}H_{17}N_2O_8$ (theoretical: C, 49.05, H 4.67, N, 11.44; Found: C, 48.91, H, 4.59; N, 11.20).

Radiolabeling. A solution of 10 mg of the PAH analog in 0.5 ml of 0.1 *N* NaOH was adjusted to pH 7.0 with 0.05 *N* HCl and purged with nitrogen for several minutes. Stannous chloride dihydrate, 0.25 mg in 10 μl volume (250 mg/ml in 6*N* HCl), was then added and the pH was readjusted to 5.7. The mixture was filtered through a 0.22 μ Millipore filter and lyophilized to yield a solid pellet. This pellet was reconstituted with 3 ml of $^{99m}TcO_4^-$ in saline and used for the following studies.

Determination of radiochemical purity. Two ITLC solvent systems were developed to determine radiochemical purity: Solvent A, CH_3CN/H_2O (7:3), and Solvent B, $CHCl_3$/EtOH (3:1). The strips (ITLC-SG) were spotted with 1 μl of sample and immediately de-

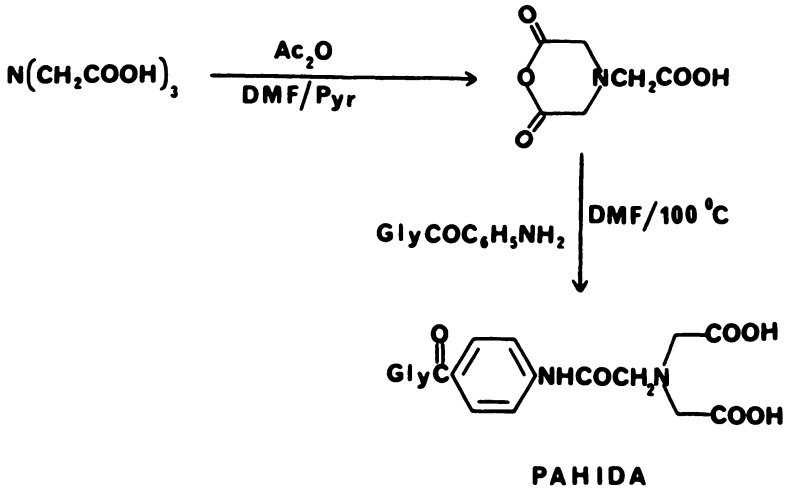

FIG. 2. Scheme of synthesis of *p*-[(bis carboxymethyl)-aminomethyl carbamino] hippuric acid (PAHIDA), 2.

TABLE 1. STABILITY STUDIES OF Tc-99m PAHIDA AT 25°C

Time	% Complex	% Complex*
1 hr	99	99
2 hr	99	99
4 hr	98	98
24 hr	89	84

* Tc-99m complex was exposed to air.

veloped for approximately 9 cm, then air-dried and 1-cm cuts were counted in a well counter to determine the complex, free pertechnetate, and reduced-hydrolyzed technetium. A reverse-phase HPLC system, consisting of a 250- × 4.1-mm Zorbax C-8 column eluted with 0.02 *M* phosphate buffer (pH 3.0) and acetonitrile as modifier, was used for quality control. The system was run in an isocratic mode at 8% aqueous CH_3CN, with a flow rate of 2.0 ml/min, using an uv detection system at 254 nm for PAHIDA. An aliquot (2–25 μl), containing 1 μCi of the preparation of Tc-99m PAHIDA, was injected; 1-ml eluate fractions were collected and the activity in each fraction was measured in a gamma counter. Rat urine aliquots (5–10 μl) containing the excreted activity were also similarly analyzed by HPLC.

Animal biodistribution. Initial biodistribution studies were performed in mice (25–30 g) after injection of a 100-μl volume of the preparation. Subsequent studies were performed in Sprague-Dawley male rats (160–200 g) under light ether anesthesia. Injections were into the tail vein. Rats were injected with 0.2 ml of Tc-99m PAHIDA preparation (100 μg of the agent). The animals were killed at different time intervals (n = 6 for each interval) and the tissues of interest were excised, weighed, and counted in a NaI(Tl) detector. The % dose/organ was determined by comparison of tissue radioactivity with suitably diluted aliquots of the injected dose.

Imaging studies were performed with New Zealand White male rabbits (3–4 kg), injected intravenously under light pentobarbital anesthesia with 400 μCi of Tc-99m PAHIDA. Immediately after injection of the labeled product, serial images were obtained up to 6 hr with a gamma camera, using an ultrafine, low-energy collimator. A total of 50k counts was obtained for each image at each time interval.

Single-injection clearances. These were obtained for the Tc-99m PAHIDA and I-131 OIH simultaneously in 200-g rats following a protocol previously described (*9*). About 50 μCi of Tc-99m activity and 10 μCi of I-131 activity were administered by indwelling catheter into the femoral vein, and blood samples (100 μl) were drawn at different times after injection into heparinized capillary tubes. The blood was centrifuged and plasma was pipetted into counting tubes (20-μl aliquots) for measurement of the activities.

RESULTS

The Tc-99m PAHIDA complex (Compound **2**) was stable when formulated at pH 5.8 under the conditions described. ITLC with Solvent A (CH_3CN/H_2O, 3:1) gave an R_f value of 1 for the complex and for pertechnetate, and $R_f = 0$ for the hydrolyzed form. In the Solvent B system ($CHCl_3$/EtOH, 3:1), R_f values of 0 and 1.0 are seen, respectively, for the Tc-99m-labeled product and free pertechnetate. HPLC indicated that PAHIDA appeared at t_R = 2.1 min, the free pertechnetate at t_R = 2–3 min, and the Tc-99m complex at between 6 and 7 min. The stability of the complex was followed for 24 hr at room temperature, and the purity of the complex was >89% even over prolonged periods (Table 1) after reconstitution of the vial. Labeling of Compound **1** with Tc-99m resulted in a purity of the complex that was no greater than 83%, as shown by ITLC using Solvents A and B.

The percent administered dose present at various time intervals in different organs is given in Table 2 and 3 for mice and rats, respectively. The rat data were compared

TABLE 2. ORGAN DISTRIBUTION DATA IN MICE FOR Tc-99m PAHIDA % ADMINISTERED DOSE* (MEAN VALUE ± 1 s.d.)

	15 min	30 min	60 min	120 min	240 min
Blood	3.3 ± 0.5	1.3 ± 0.4	0.6 ± 0.2	0.6 ± 0.2	0.3 ± 0.1
Urine	58.5 ± 10.0	79.4 ± 6.0	87.0 ± 8.6	84.8 ± 6.4	90.0 ± 7.3
Kidney	2.1 ± 0.2	1.4 ± 0.4	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
Liver	0.9 ± 0.2	0.7 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.3 ± 0.1
Stomach	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	
Intestine	1.2 ± 0.4	1.7 ± 0.2	1.6 ± 0.1	1.1 ± 0.1	1.6 ± 0.4

* Six animals for each time interval.

TABLE 3. ORGAN DISTRIBUTION DATA IN RATS FOR Tc-99m PAHIDA*

Organ	% Administered dose†			
	15 min	30 min	60 min	240 min
Blood	9.2 ± 1.1	6.3 ± 1.0	2.5 ± 0.6	1.2 ± 0.2
	(3.7 ± 0.4)	(1.2 ± 0.2)	(0.5 ± 0.1)	(0.2 ± 0.0)
Urine	42.0 ± 7.5	61.3 ± 12.1	73.1 ± 8.9	76.5 ± 5.2
	(54.5 ± 7.0)	(64.8 ± 2.0)	(69.7 ± 3.0)	(79.0 ± 7.0)
Liver	1.8 ± 0.4	1.2 ± 0.3	0.7 ± 0.1	0.4 ± 0.1
	(1.8 ± 0.2)	(0.9 ± 0.3)	(0.6 ± 0.3)	(0.1 ± 0.0)
Kidney	4.1 ± 0.4	4.4 ± 0.7	3.2 ± 0.4	3.5 ± 0.4
	(3.7 ± 0.4)	(0.9 ± 0.2)	(0.6 ± 0.2)	(0.1 ± 0.0)

* I-131 OIH values are shown in parentheses.

† Six animals for each time interval; mean value ±1 s.d.

with I-131 OIH biodistribution performed in an identical strain of animals. These data show that the urinary excretion was almost as rapid as that of OIH at all time intervals, though the kidney retention values are slightly higher. Gastrointestinal elimination of the compound was less than 2%. A comparison of rat urine analysis by ITLC and HPLC showed identical retention volumes and chromatograms, respectively. Single-injection renal clearance values for both Tc-99m PAHIDA and OIH are given in Table 4.

Scintigrams from rabbits (Fig. 3) revealed that the Tc-99m complex was rapidly excreted in urine, and provided excellent renal images with no significant extrarenal background.

DISCUSSION

Though the Tc-99m CO_2-DADS-A agent has been reported as superior to I-131 OIH for renal imaging (*7*), its biological properties in renal failure and its complicated mode of preparation are disadvantages for its widespread use as a substitute for OIH.

The agent described here (Tc-99m PAHIDA) satisfies structural requirements for renal secretion (*10*) due to the presence of the R-CONHCH$_2$COOH grouping analogous to OIH. Compound **1** does not form a stable complex with Tc-99m, probably as a result of weak chelation caused by the resonance effect, which decreases the electron density of the amino groups, making them unavailable as donors of unshared electrons. It was not found promising for further study in spite of the requisite grouping present in the compound. Unlike the other well-known hepatobiliary agents (*11*), Tc-99m PAHIDA has excellent renal excretion characteristics, as confirmed by the organ distribution studies in rats and scintigrams in rabbits. A comparison of an aliquot of rat's urine with Tc-99m PAHIDA as injected has shown the same R_f value and same retention values with ITLC and HPLC techniques, respectively. These results indicate the identity of the excreted agent with the admin-

TABLE 4. SINGLE-INJECTION CLEARANCES IN RATS FOR Tc-99m PAHIDA AND I-131 OIH

Animal #	Plasma clearance*		Plasma clearance (protein free)†	
	Tc-99m agent	I-131 OIH	Tc-99m agent	I-131 OIH
1	1.25	4.39	1.99	4.47
2	1.21	4.29	2.22	4.58
3	1.12	4.52	2.04	5.31
4	1.33	5.22	1.77	5.85
Mean	1.23	4.61	2.01	5.05

* Plasma clearance values are expressed as ml/min-100 g body weight, not corrected for protein binding of agents.

† Protein-free plasma clearance values are corrected for protein binding of the agent, which was 24%, 34%, 46%, and 56% at 5 min, 10 min, 20 min, 40 min, 60 min, and 80 min, respectively, as determined by TCA precipitation method. Protein binding of I-131 OIH is much less, but the clearance is similarly corrected.

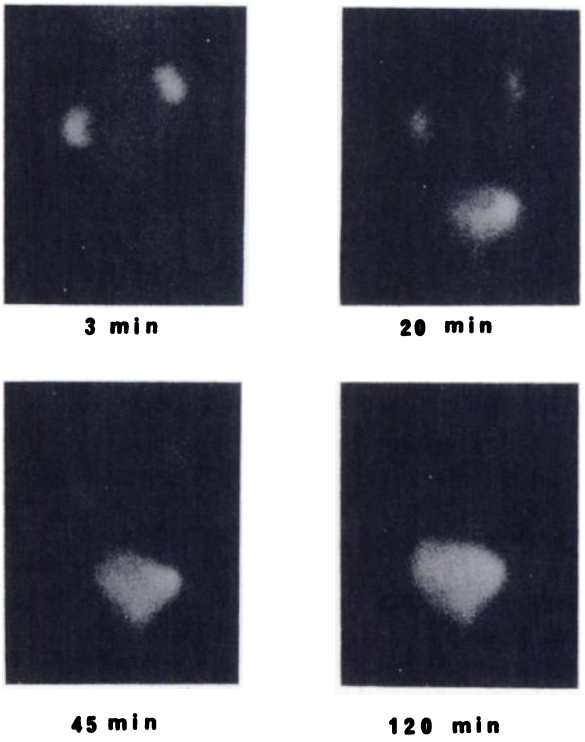

FIG. 3. Serial scintigrams in rabbit, obtained after administration of Tc-99m PAHIDA. Significant accumulation of renal activity at 3 min after injection, and absence of extrarenal activity, are promising features for this complex.

istered complex. Kidney retention of 2% of the activity occurred in rats. However, the kidneys are barely seen in the rabbit scintigrams at 2 hr. Renal clearance values (Table 4) show a slower clearance for Tc-99m PAHIDA, relative to OIH, by a factor of 2, although these values are significantly higher than for a typical GFR agent.

The present study raises the possibility of the development of complexes of Tc-99m with agents similar to Compound 2 (PAHIDA). Various substituents on the ring may lead to alteration of the lipophilicity of the agents, which would facilitate evaluation of the structure distribution requirements. The synthetic method and the resulting compound described here appear to offer a promising approach toward developing a renal agent with improved characteristics.

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