Evaluation of the Renal Clearance of Technetium-99m PAHIDA in Dogs

David A. Summerville, Alan B. Packard, Barbara Bartynski, K.S. Lim, L. Rao Chervu, and S.T. Treves

Division of Nuclear Medicine, Department of Radiology, The Children's Hospital and Harvard Medical School, Boston, Massachusetts; and Department of Nuclear Medicine, Albert Einstein College of Medicine, Bronx, New York

The renal clearance of the technetium-99m complex of para[(biscarboxylmethyl)-aminomethylcarboxyamino]hippuric acid ([^{99m}Tc]PAHIDA), has been previously studied in rodents and falls between that of [^{99m}Tc]DTPA (diethylenetriaminepentaacetic acid) and iodine-131 (^{131}I) orthoiodohippuran (OIH). To investigate the effect of species variation, the plasma clearance of [^{99m}Tc]PAHIDA was investigated in dogs. The plasma disappearance of the renal agent approached that of [^{99m}Tc]DTPA and was significantly less than that of OIH. Despite the structural similarities of the PAHIDA ligand and aminohippurate, the [^{99m}Tc] PAHIDA complex undergoes little, if any, tubular secretion in the canine kidney.

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There have been a number of attempts to prepare a technetium-99m (^{99m}Tc) renal agent that would be cleared by the kidneys with an extraction efficiency approaching that of aminohippuran. Recent attempts have centered on a series of technetium chelates containing $Tc(V)ON_2S_2$ (*1,2*) and $Tc(V)ON_3S$ (*3,4*) cores. Although early studies with chelates containing $Tc(V)ON_2S_2$ were promising, the necessity for HPLC purification of the complexes limits the potential clinical utility. A recently reported complex having the $Tc(V)ON_3S$ core, [^{99m}Tc]MAG_3, appears more promising but is not yet commercially available (*3,4*).

Recently, Chervu et al. (*5*), taking an alternate approach, reported the preparation of a complex formed by the stannous reduction of $^{99m}TcO_4^-$ in the presence of the ligand para-[(biscarboxymethyl)-aminomethylcarboxyamino]hippuric acid (PAHIDA; Fig. 1). This ligand was prepared by the formation of an amide linkage between the paraamino group of aminohippuran (PAH) and a carboxyl group of nitrilotriacetic acid. The resulting PAHIDA molecule consists of an iminodiacetic acid functional group attached to a PAH moiety. Administration of the [^{99m}Tc]PAHIDA complex to rats showed the compound to be cleared from the body exclusively by the kidneys with a clearance rate between that of [^{131}I]orthoiodohippuran (OIH) and that expected for an agent excreted exclusively by glomerular filtration. Because the renal handling of various compounds can show wide interspecies variation, we have evaluated the plasma clearance of the [^{99m}Tc] PAHIDA in dogs.

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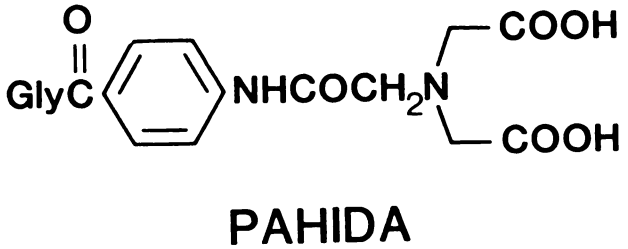
For reprints contact: David A. Summerville, Division of Nuclear Medicine, Department of Radiology, The Childrens Hospital and Harvard Medical School, 300 Longwood Ave., Boston, MA 02115.

MATERIALS AND METHODS

Technetium-99m PAHIDA was prepared as previously described (*5*). OIH was purified by HPLC using a C-18 reverse phase column (4.5 × 250 mm). The starting solvent was 1% acetic acid with a gradient of 0%–20% ethanol in 20 min with a flow rate of 1 ml/min.

The plasma clearances of [^{99m}Tc]PAHIDA, OIH, and [^{99m}Tc]DTPA, were measured in two adult beagle dogs (12 kg) under light pentobarbital anesthesia. In most cases, both a ^{99m}Tc containing agent (either the DTPA or PAHIDA complex, 1.0 mCi) and OIH (0.1 mCi) were injected simultaneously. After intravenous administration of the radiopharmaceuticals, 15 blood samples (~4 ml) were obtained between 1 and 120 min after injection from the limb opposite that used for injection. Duplicate 0.5-ml aliquots of whole blood and plasma were counted in a well counter and the activity in the ^{99m}Tc channel was corrected for activity from ^{131}I. The plasma (or whole blood) activity in cpm per mCi injected dose was obtained by comparison of the samples with a standard solution. Whole blood disappearance curves were plotted as percent injected dose remaining in the blood, assuming a blood volume equal to 8% body weight (*6*). Plasma clearances were



PAHIDA

FIGURE 1
The PAHIDA ligand.

calculated using the method of Hall et al (*7*), from the plasma disappearance curves through 60 min.

RESULTS

Whole blood clearance curves for OIH, [^{99m}Tc]DTPA, and [^{99m}Tc]PAHIDA are shown in Figure 2. The blood clearance curves of OIH and [^{99m}Tc]DTPA are similar to those reported by McAfee et al. (*8*). The percent injected dose of [^{99m}Tc]PAHIDA remaining in the blood differs little from that of [^{99m}Tc]DTPA; both are cleared much more slowly than OIH.

Plasma clearances for each of the radiopharmaceuticals are shown in Table 1. The clearance rates measured for [^{99m}Tc]DTPA (4.3 and 3.5 ml/min/kg) are comparable with those reported in the literature for the glomerular filtration agent ^{14}C-labeled inulin (3.55 ± 0.14 ml/min/kg) (*9*). Further, the plasma clearance values for OIH (9.0 and 8.7 ml/min/kg) are similar to, but slightly less than, values for effective renal plasma flow measured with ^{3}H-tetraethylammonium bromide (10.51 ± 0.72). This is consistent with reports that the extraction efficiency of OIH may be considerably less than 1.0 (*8*). No significant differences were observed between the renal clearances of [^{99m}Tc]DTPA and [^{99m}Tc]PAHIDA in either dog.

To determine if the PAHIDA ligand had any effect on the rate of tubular secretion of OIH, the plasma clearance of hippuran was also determined in the absence of the PAHIDA ligand and was found to be 108 and 104 ml/min for Dogs 1 and 2, respectively. In the presence of PAHIDA, OIH clearance values of 107 and 104 ml/min, respectively, were obtained. To determine whether or not [^{99m}Tc]PAHIDA undergoes tubular secretion, the plasma clearances of [^{99m}Tc]PAHIDA and OIH were determined in the presence of the tubular blocking agent probenecid. Intravenous administration of 25 mg/kg probenecid prior to injection of [^{99m}Tc]PAHIDA and OIH in Dog 2 resulted in plasma clearances of 46 and 78 ml/min, respectively. When 50 mg/kg probenecid was administered to Dog 1, values of 55 and 82 ml/min, respectively, were obtained.

Extrarenal excretion of the radiopharmaceutical was evaluated by imaging the two dogs 2 hr after administration of [^{99m}Tc]PAHIDA. The resulting images showed no evidence of biliary excretion or extrarenal accumulation.

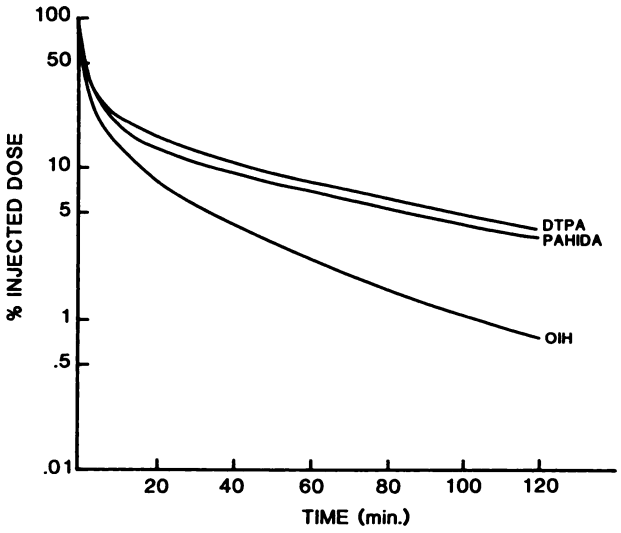


FIGURE 2
Whole blood disappearance curves for [^{99m}Tc]DTPA, [^{99m}Tc]PAHIDA, and OIH for Dog 2. Corresponding plasma clearance values were 38, 46, and 101 ml/min, respectively.

DISCUSSION

For a compound to be excreted by the kidneys with an extraction efficiency of 1.0, it is necessary that it undergo renal tubular secretion. Organic anions having the structure R-C:O-NX-(CHR')$_n$-COOH, such as hippuran and several of its derivatives, undergo renal tubular secretion by a substrate specific process that depends on the interaction of both the carbonyl and carboxylate moieties of the anion with receptors on the renal tubular cells (*10*). Despite the fact that this structure is present in the PAHIDA ligand, clearance of the [^{99m}Tc]PAHIDA complex by the kidneys occurs at a rate consistent with glomerular filtration, rather than tubular secretion. In addition, the rate of clearance is not decreased by the presence of probenecid, a known inhibitor of tubular secretion.

The PAHIDA ligand is structurally similar to the hepatobiliary agents HIDA, DISIDA, etc. The differ-

TABLE 1
Renal Clearances in Dogs*

	OIH	[^{99m}Tc]DTPA	[^{99m}Tc] PAHIDA
Dog 1	108 (9.0)	51 (4.3)	50 (4.2)
Dog 2	104 (8.7)	42 (3.5)	51 (4.3)

* In ml/min. Values in parenthesis are ml/min/kg. All values represent an average of two to three determinations.

ence between PAHIDA and the hepatobiliary agents is the substitution on the phenyl group of the ligand (Fig. 1). Hydrophobic alkyl substituents on the phenyl ring cause hepatobiliary excretion, whereas, the glycine amide substituent of PAHIDA causes the complex to be eliminated by glomerular filtration. Consistent with differences in lipophilicity of the substituents, no biliary excretion of the PAHIDA complex was observed in two dogs with normal renal function.

The structure of the [^{99m}Tc]PAHIDA complex was not determined as part of this study. It seems reasonable, however, to expect that complexation of Tc with the PAHIDA ligand occurs in a manner similar to the IDA-hepatobiliary agents (*11,12*). Costello et al. (*12*) have shown that the hepatobiliary agents occur as bis-Tc (III) complexes with the ligand bound to the metal core via the aminodiacetate group. A similar mode of complexation in the [^{99m}Tc]PAHIDA complex would leave two glycyl-benzoate amide moieties free to interact with the renal tubule receptor. The complex is not secreted via the renal tubular mechanism, as shown by the probenecid studies. One must presume, therefore, that the inability of the free glycyl amide of the [^{99m}Tc] PAHIDA complex to facilitate tubular secretion can be attributed to steric or charge considerations that make it impossible for the substrate to interact with the renal tubular receptor.

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