Cationic Technetium-99m Complexes of *N*-Substituted Pyridoxal Derivatives as Renal Function Agents

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New cationic technetium-chelating agents containing a pyridinium group have been synthesized and evaluated as potential renal radiopharmaceuticals. **Methods:** The pyridinium compounds used in the study are *N*-methyl pyridoxal chloride, *N*-ethyl pyridoxal chloride, *N*-propyl pyridoxal chloride, 1-methyl-3-hydroxy-4-formylpyridinium chloride, 1-methyl-2-formyl-3-hydroxypyridinium chloride and the Schiff's bases of *N*-methyl pyridoxal chloride with amino acid, amino acid ester and amino acid amide. Complexes of these chelating agents with ^{99m}Tc were prepared using a $Na_2S_2O_4$ or a $SnCl_2$ solution as a reducing agent. The purity of the ^{99m}Tc complexes was determined by paper electrophoresis in 0.1 *M* tris buffer. **Results:** Electrophoresis indicates slightly positive-charged species. The log P values of these complexes showed a hydrophilic nature. Urinary excretion of the ^{99m}Tc *N*-alkylated pyridoxal derivatives, ^{99m}Tc-diethylenetriaminepentaacetic acid, ^{99m}Tc-mercaptoacetylglycylglycylglycine (MAG3) and ^{131}I-o-iodohippurate were determined in mice and rats at different time intervals. In a rat model, the pyridoxal-derived ^{99m}Tc complexes are rapidly excreted in urine and provide clear renal scintigrams. Hepatobiliary excretion was negligible, reducing scan interference from the intestines. Total clearances were lower than that of ^{131}I-hippurate and ^{99m}Tc-MAG3. **Conclusions:** The rate of urinary clearance of the new tracers was not significantly faster than ^{99m}Tc diethylenetriaminepentaacetic acid and the inhibitor N^1-methylnicotinamide had only a minimal effect on the renal behavior. Though the new tracers have cationic properties, the pyridinium group did not contribute largely to the excretion of active transport.

Key Words: technetium-99m; *N*-alkyl pyridoxal; renal agents; radiopharmaceutical

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The development of improved radiopharmaceuticals to assess the urinary system has attracted much interest. Iodine-131-*o*-iodohippurate (OIH)(*1*) ^{99m}Tc-diethylenetriaminepentaacetic acid (DTPA) (*2*) and ^{99m}Tc-mercaptoacetylglycylglycylglycine (MAG3) (*3*) are used routinely as renal function imaging agents. Iodine-131-OIH has the disadvantage of imparting a relatively high radiation absorbed dose to the patient at diagnostic doses. Although, ^{123}I-OIH (*4*) lowers the radiation dose, it is not available at reasonable cost for routine use. Varying amounts of free radioiodine in OIH preparations pose problems for accurate quantifiable assessment of function.

The advantages of ^{99m}Tc, with its short half-life and ideal scanning properties, have led to the development of several ^{99m}Tc complexes that are excreted into the bladder and may be useful for urinary studies. Technetium-99m-DTPA is widely used in clinical nuclear medicine for GFR measurements and is excreted solely through glomerular filtration, but the slow rate of excretion, relative to compounds that are actively excreted, is a disadvantage. Technetium-99m-MAG3 has rapid renal excretion consistent with tubular excretion by the weak acid mechanism (*5*). The renal excretion of ^{99m}Tc-MAG3 is greater than that of ^{99m}Tc-DTPA but lower than that of OIH.

Numerous, structurally diverse organic molecules are known to undergo efficient tubular secretion. N^1-methylnicotinamide (NMN) and tetraethylammonium ions are actively transported endogenous organic cations (*6–9*). These compounds, however, do not bind ^{99m}Tc.

We selected pyridoxal derivatives as a compound having a pyridine nitrogen and coordination ability (*10*). It has been known that ^{99m}Tc-pyridoxylidene glutamate (*11,12*) and ^{99m}Tc-pyridoxylidene aminates (*13*) are rapidly cleared by the liver and excreted directly through the bile duct into intestine. *N*-alkylated pyridoxal derivatives were cleared from the kidney into the bladder with a high degree of specificity and were easy to coordinate with technetium. This paper describes the preparation and potential use of ^{99m}Tc *N*-alkylated pyridoxal derivatives in evaluating renal function and urinary obstruction.

MATERIALS AND METHODS

Isotopes

Technetium-99m-pertechnetate was eluted from a sterile ^{99}Mo-^{99m}Tc shielded generator (Dai-ichi Radioisotope Laboratories, Tokyo, Japan) with isotonic saline. Iodine-131-OIH was obtained from Dai-ichi Radioisotope Laboratories. *N*-alkylated pyridoxal chloride and related compounds were synthesized by the method

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described previously (*14,15*). Other materials were purchased from commercial sources.

Technetium-99m Complex Formation

Pyridoxal Derivatives. A solution (pH 7.0–8.0) containing 0.1 *M* of *N*-alkylated pyridoxal analog in 1 ml was prepared by adding water. Freshly prepared 0.1 ml of a 50 mg/ml solution of $Na_2S_2O_4$ or 0.1 ml of a 2.0 mg/ml solution of $SnCl_2$ was added and the final pH was adjusted to 7.4. The resulting solution was passed through a 0.22-μm membrane filter (Millipore, Bedford, MA) into a sealed vial. Pertechnetate ($^{99m}TcO_4^-$) (37 MBq, 1.5–2.0 ml) was added with mixing. The mixture was allowed to stand for 15 min at room temperature.

Schiff's Base Derivatives. A solution (pH 8.5–9.5) of Schiff's base derived from 0.1 *M* of *N*-methyl pyridoxal chloride and 0.2 *M* of amino acid or amino acid derivatives in 1 ml was prepared. Freshly prepared 0.1 ml of a 2.0-mg/ml solution of $SnCl_2$ was added. The solution was passed through a 0.22-μm membrane filter (Millipore) into a sealed vial. A saline solution of $^{99m}TcO_4^-$ (37 MBq, 1.5–2.0 ml) was added to the vial. The mixture was allowed to stand for 10 min at room temperature.

Chemical Studies and Physical Characteristics of ^{99m}Tc Complex

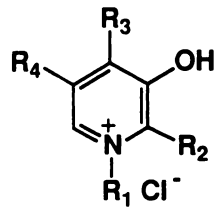
The efficiency of complexation with ^{99m}Tc was evaluated chromatographically using a 0.25-mm Silica-gel 60 F254 plate (E. Merck, Darmstadt, Germany) developed with an acetonitrile-to-water (7:3) solvent system. The purity and charge sign of ^{99m}Tc complexes were determined by paper electrophoresis. Paper strips were run at a constant voltage of 600 V for 30 min using 0.1 *M* tris buffer, pH 7.4. The TLC plates and paper strips were counted by images in a gamma camera equipped with a high-resolution collimator with a digital computer (VP-450). Movement was determined relative to anionic markers, ^{99m}Tc-DTPA (movement = +4.6 cm) and ^{99m}Tc-pertechnetate (movement = +9.0 cm).

The partition coefficient was measured by mixing the ^{99m}Tc complexes with 1 ml each of 1-octanol and 0.1 *M* phosphate buffer (pH 7.0) in a glass tube. This tube was shaken for 20 min at 25°C and then centrifuged 1500 rpm for 10 min. Two precisely measured samples (0.1 ml each) from the 1-octanol and buffer layers were counted in a well-type gamma counter (Packard COBRA II, Meridien, CT). The partition coefficients were determined by calculating the ratio of the radioactivity of the octanol layer to that of the buffer layer. This measurement was generally repeated three times.

Biodistribution

The time course of organ distribution was determined in four ICR mice. Each was injected with 0.15 ml (3.7 MBq) of the ^{99m}Tc complex solution through the tail vein. For comparison purposes, 3.7 MBq of ^{99m}Tc-DTPA, 3.7 MBq of ^{99m}Tc-MAG3 and 1.85 MBq of ^{131}I-OIH were also injected intravenously. The effect of NMN was investigated on the distribution of ^{99m}Tc complexes 15 min after injection in mice pretreated with NMN. NMN dose was 70 mg/kg (*6,7*) given 5 min intravenously before injection of the ^{99m}Tc complex. The animals were killed with blood collection in the heart at 5, 15, 30 and 60 min after injection. The organs or tissues were removed, weighed and counted by images in a gamma camera (Ohio Nuclear Co.) equipped with a high-resolution collimator with a digital computer (VP-450). The %dose/organ was determined by comparison of tissue radioactivity levels with the total radioactivity.

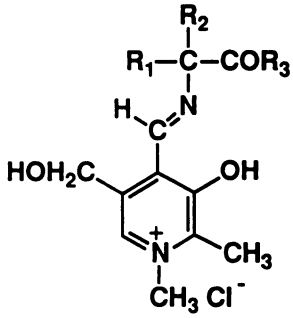
A



Ligand	R_1	R_2	R_3	R_4
PalNMe	CH_3	CH_3	CHO	CH_2OH
PalNEt	C_2H_5	CH_3	CHO	CH_2OH
PalNPr	C_3H_7	CH_3	CHO	CH_2OH
MHFP	CH_3	H	CHO	H
MFHP	CH_3	CHO	H	H

PalNMe=*N*-Methyl pyridoxal chloride
PalNEt=*N*-Ethyl pyridoxal chloride
PalNPr=*N*-Propyl pyridoxal chloride
MHFP=1-Methyl-3-hydroxy-4-formyl pyridinium chloride
MFHP=1-Methyl-2-formyl-3-hydroxy pyridinium chloride

B



Ligand	R_1	R_2	R_3
SB-Gly	H	H	OH
SB-Ala	CH_3	H	OH
SB-2AB	CH_3	CH_3	OH
SB-Leu	$(CH_3)_2CHCH_2$	H	OH
SB-GlyOMe	H	H	OCH_3
SB-GlyOEt	H	H	OC_2H_5
SB-AlaOEt	CH_3	H	OC_2H_5
SB-GlyNH2	H	H	NH_2
SB-AlaNH2	CH_3	H	NH_2

SB=Schiff's base derived from *N*-Methyl pyridoxal chloride and amino acid or amino acid derivatives
Gly=Glycine
Ala=Alanine
2AB=2-aminoisobutyric acid
Leu=Leucine
GlyOMe=Glycine methyl ester
GlyOEt=Glycine ethyl ester
AlaOEt=Alanine ethyl ester
$GlyNH_2$=Glycine amide
$AlaNH_2$=Alanine amide

FIGURE 1. (A) Structures of *N*-methyl pyridoxal and its related compound. (B) Structures of Schiff's base derived from *N*-methylpyridoxal chloride and amino acid or amino acid derivatives.

TABLE 1
Paper Electrophoresis, Partition Coefficients and % Excreted in Urine after 30 Minutes of ^{99m}Tc Complexes

Ligand	Electrophoresis migration (cm)	Octanol/ H_2O log P	Excreted in urine after 30 min (%)
PalNMe	−1.1	−3.6	73.0
PalNEt	−1.1	−2.8	75.5
PalNPr	−1.1	−3.0	70.5
MHFP	−1.4	−2.4	68.7
MFHP	−1.3	−2.3	62.2
SB-Gly	−0.7	−4.3	77.8
SB-Ala	−0.8	−4.0	60.6
SB-2AB	−0.8	−4.0	55.9
SB-Leu	−0.9	−3.4	55.6
SB-GlyOMe	−2.4	−2.3	78.3
SB-GlyOEt	−2.7	−2.2	76.8
SB-AlaOEt	−2.9	−2.2	71.7
SB-GlyNH2	−2.7	−2.2	67.7
SB-AlaNH2	−2.2	−2.8	−61.0
DTPA	+4.6	—	83.6
[^{99m}Tc]pertechnetate	+9.0	—	—
MAG3	—	—	86.3
[^{131}I] OIH	—	—	96.9

PalNMe = *N*-methyl pyridoxal chloride; PalNEt = *N*-ethyl pyridoxal chloride; PalNPr = *N*-propyl pyridoxal chloride; MHFP = 1-methyl-3-hydroxy-4-formyl pyridinium chloride; and MFHP = 1-methyl-2-formyl-3-hydroxy pyridinium chloride. SB = Schiff's base derived from *N*-methyl pyridoxal chloride and amino acid or amino acid derivatives; Gly = Glycine; Ala = Alanine; 2AB = 2-aminoisobuytric acid; Leu = Leucine; GlyOMe = Glycine methyl ester; GlyOEt = Glycine ethyl ester; AlaOEt = Alanine ethyl ester; $GlyNH_2$ = glycine amide; $AlaNH_2$ = alanine amide; DTPA = diethylene-triaminepentaacetic acid; MAG_3 = mercaptoacetylglycylglycylglycine; and OIH = o-iodohippuric acid.

Blood Disappearance Rates and Renal Excretion

Male Wistar rats weighing 350 ± 20 g were used in the experiment. The animals were anesthetized with 25 mg/kg of sodium pentobarbital intraperitoneally. A catheter with three necks was placed in a femoral vein for injection of the ^{99m}Tc complex and isotonic saline or NMN infusion. They were placed under a gamma camera (Ohio Nuclear Co.) provided with digital storage (VP-450). Following infusion of isotonic saline at the rate of 20 μl/min, 90-min, digital images were acquired in the anterior projection simultaneously with bolus injection of ^{99m}Tc-*N*-alkylated pyridoxal derivatives. Rapid (10 sec) serial digital images were acquired for 21 min and were used to quantitatively estimate blood clearance by the disappearance of radioactivity in the heart. After infusion of 70 mg/kg/hr of NMN at the rate of 20 μl/min for 90 min in the same animal, ^{99m}Tc-*N*-alkylated pyridoxal derivative was injected and measured as above.

Toxicity Study

The acute toxicity of *N*-methyl pyridoxal was determined using five male ICR mice weighing 30 ± 2 g each. *N*-methyl pyridoxal was dissolved in saline and the pH of the solution was adjusted to 7.4 with aqueous NaOH. A dose of 1 g/kg *N*-methyl pyridoxal was administered intravenously to each mouse. The mice were followed for 30 days with normal animal care.

RESULTS

Chemical Studies

The structure of ligands used in the present study are shown in Figures 1A, B. In thin-layer chromatography, the ^{99m}Tc complexes of *N*-alkylated pyridoxal derivatives remained at the origin with 70% acetonitrile/water on silica gel. In this solvent system pertechnetate gave an Rf of 0.98, and reduced hydrolyzed ^{99m}Tc remained at the origin.

The electrophoresis patterns in 0.1 *M* tris buffer are shown in Table 1. Electrophoresis of ^{99m}Tc-*N*-methyl pyridoxal on paper strips showed cationic species that migrated about 1.1 cm toward the cathode, indicating a slightly positive-charged species. A control of hydrolyzed ^{99m}Tc showed no migration under the conditions of the experiment. Under the same conditions, other related ^{99m}Tc complexes, ^{99m}Tc-DTPA and pertechnetate were also investigated (Table 1).

The logarithms of the octanol/water partition coefficients of the ^{99m}Tc complexes are used to predict the relative urinary/hepatobiliary clearance of the complex (*16*). However, the data presented in this study do not support it (Table 1). Comparing the log P (the radioactivity of octanol to the radioactivity of water) of these ^{99m}Tc complexes to renal excretion at 30 min in mice, there was no correlation. There may be a threshold effect below which there is no additional change in renal excretion. Such a phenomenon is observed with membrane permeability (*17,18*).

In Vivo Distribution Studies

The organ distribution of ^{99m}Tc complexes from *N*-alkyl pyridoxal and its related compound in mice at 30 min after injection is shown in Table 2. Most (75%) of the dose was accounted for in the urine with less than 5% found in the

TABLE 2
Biodistribution Data of ^{99m}Tc Complexes of *N*-Alkyl Pyridoxal and Its Related Compound in Mice*

Organ	PalNMe	PalNEt	PalNPr	MHFP	MFHP
Urine	73.00 ± 6.08	75.52 ± 1.13	70.45 ± 3.46	68.70 ± 6.56	62.24 ± 3.93
Kidneys	1.22 ± 0.26	1.33 ± 0.22	1.58 ± 0.36	1.48 ± 0.45	4.39 ± 0.17
Blood†	3.40 ± 0.92	3.38 ± 0.38	3.51 ± 0.88	3.94 ± 0.89	4.39 ± 0.17
Liver	1.43 ± 0.40	2.84 ± 0.25	2.78 ± 0.27	3.38 ± 0.55	3.46 ± 0.25
Intestines	0.64 ± 0.15	0.60 ± 0.15	1.37 ± 0.38	0.57 ± 0.16	3.36 ± 0.13
Stomach	0.48 ± 0.09	0.55 ± 0.06	1.42 ± 0.12	0.62 ± 0.23	0.89 ± 0.17

	SB-Gly	SB-Ala	SB-2AB	SB-Leu
Urine	77.79 ± 0.83	60.58 ± 4.19	55.93 ± 6.21	55.57 ± 1.80
Kidneys	1.57 ± 0.82	2.43 ± 0.47	4.33 ± 3.09	5.97 ± 2.51
Blood†	3.39 ± 0.22	5.69 ± 0.73	5.30 ± 0.20	4.55 ± 0.89
Liver	2.75 ± 0.19	3.33 ± 0.80	6.74 ± 1.43	6.87 ± 0.35
Intestines	0.89 ± 0.02	6.10 ± 0.56	5.76 ± 0.65	3.76 ± 0.55
Stomach	0.23 ± 0.03	0.38 ± 0.08	0.29 ± 0.05	0.39 ± 0.03

	SB-GlyOEt	SB-GlyOEt	SB-AlaOEt	SB-GlyNH2	SB-AlaNH2
Urine	78.25 ± 1.75	76.82 ± 2.84	71.68 ± 0.56	67.72 ± 9.78	60.99 ± 6.09
Kidneys	1.18 ± 0.20	1.43 ± 0.11	1.29 ± 0.24	2.58 ± 0.94	3.53 ± 1.82
Blood†	2.48 ± 0.15	3.23 ± 0.63	4.23 ± 0.39	4.22 ± 0.64	4.57 ± 0.09
Liver	6.04 ± 1.02	2.05 ± 0.24	3.28 ± 0.01	1.98 ± 0.52	6.86 ± 0.87
Intestines	1.06 ± 0.14	1.19 ± 0.07	1.39 ± 0.01	1.24 ± 0.16	1.48 ± 0.29
Stomach	0.20 ± 0.00	0.24 ± 0.00	0.26 ± 0.00	0.32 ± 0.08	0.30 ± 0.01

*Values are percent injected dose, mean ± s.d. for four mice at 30 min after injection.
†Blood was assumed to account for 7.78% of total body mass (*21*).
Abbreviations the same as Table 1.

liver or intestine. The distribution was unaffected by the difference of the *N*-alkyl group. The ^{99m}Tc complexes of 1-methyl-3-hydroxy-4-formyl pyridinium chloride and 1-methyl-2-formyl-3-hydroxy pyridinium chloride without the methyl group at 2 position and hydroxymethyl group at 5 position have a lipophilic character and reduced urinary excretion.

Typical scintigrams (Fig. 2) from mice revealed that the ^{99m}Tc complex was rapidly excreted in urine and provided excellent renal images. In mice and rats, brain blood flow and the kidneys are visualized immediately after intravenous injection, the bladder after 1–2 min. Technetium-99m-*N*-alkylated pyridoxal derivatives have excellent renal excretion characteristics, as confirmed by the organ distribution studies in animals.

Figure 3 shows the time activity curves for the kidneys

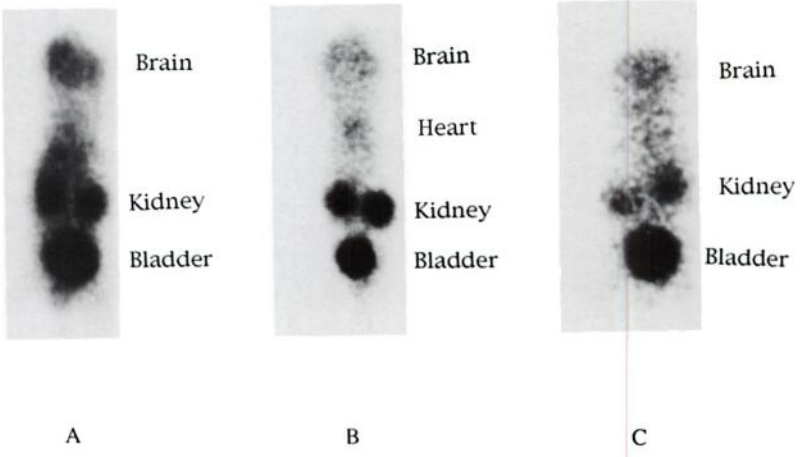


FIGURE 2. Scintigrams obtained with ^{99m}Tc complexes after the administration to mice (posterior projection). (A) ^{99m}Tc-*N*-methyl pyridoxal at 10 min; (B) ^{99m}Tc-1-methyl-3-hydroxy-4-formylpyridinium at 5 min; and (C) ^{99m}Tc complex of the Schiff's base derived from *N*-methyl pyridoxal and glycine at 5 min.

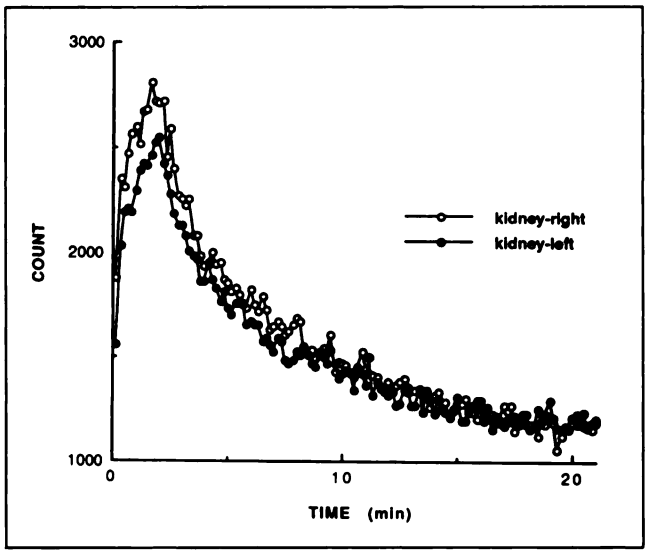


FIGURE 3. Time-activity curves for the kidneys in a rat administered ^{99m}Tc-*N*-ethyl pyridoxal.

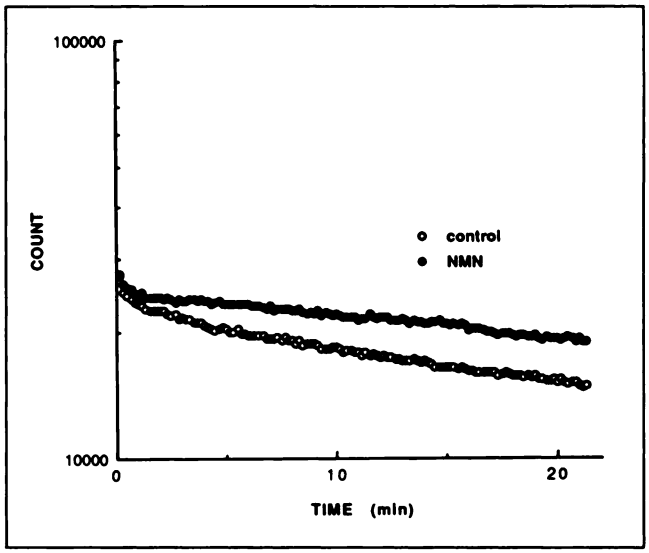


FIGURE 4. Time-activity curves for the heart in rat administered ^{99m}Tc-N-ethyl pyridoxal (NMN = N^1-methyl nicotinamide).

in rats administered ^{99m}Tc-*N*-ethyl pyridoxal. Scintigraphic studies of ^{99m}Tc-*N*-alkylated pyridoxal derivatives suggest its potential utility for assessment of the renal system. These results are satisfactory for renal functional agent.

The ^{99m}Tc complexation of *N*-alkylated pyridoxal analogs by a stannous reduction technique to replace $Na_2S_2O_4$ yield ^{99m}Tc colloids. As a result, some radioactivity remained at the center in electrophoresis and in the liver imaged in scintigrams.

Comparison with ^{99m}Tc-MAG3 suggests that ^{99m}Tc-*N*-alkylated pyridoxal derivatives are cleared more slowly (Table 3). The ^{99m}Tc-*N*-alkylated pyridoxal derivative's complexes localized in the kidney and were excreted into the bladder with times like those of the DTPA complex. In addition, the kinetics of the ^{99m}Tc-*N*-alkylated pyridoxal derivative's complexes also resemble that of the DTPA complex in that 80% of the dose after 30 min is excreted by the kidneys.

Disappearance of ^{99m}Tc radioactivity from the blood is almost the same as that of ^{99m}Tc-DTPA and lower than ^{99m}Tc-MAG3 and ^{131}I-OIH. The rate of blood clearance in the rat is slightly affected by the administration of NMN used as a test for tubular secretion by the weak base mechanism (Fig. 4). However, the effect of NMN was negligible on the distribution of ^{99m}Tc-*N*-methyl pyridoxal and Schiff's base derived from *N*-methyl pyridoxal and glycine at 15 min after injection in mice pretreated with NMN (Table 4).

Toxicity

In the toxicity study, no animal died during the test period, even at doses 800 times that applicable to human patients. No significant differences in body weight were observed between the tested animals and the controls 30 days after administration. The organs of all tested animals excised at 30 days after the administration revealed no histologic differences from those of controls.

DISCUSSION

The objective of the present work was to evaluate the ^{99m}Tc complexes of pyridinium compounds as potential renal radiopharmaceuticals.

N-alkylated pyridoxal may be present predominantly as

TABLE 3
Biodistribution Data of ^{99m}Tc-MAG3, ^{99m}Tc-DTPA and Corresponding OIH Percentages in Mice*

Organ	MAG3	DTPA	OIH
Urine	86.26 ± 2.50	83.56 ± 3.43	96.85 ± 2.21
Kidneys	1.91 ± 0.37	1.00 ± 0.65	0.75 ± 0.23
Blood†	0.68 ± 0.05	1.74 ± 0.63	0.47 ± 0.27
Liver	2.95 ± 0.40	0.75 ± 0.14	0.43 ± 0.24
Intestines	2.62 ± 0.58	0.33 ± 0.08	0.36 ± 0.19
Stomach	0.26 ± 0.04	0.09 ± 0.03	0.24 ± 0.09

*Values are percent injected dose, mean ± s.d. for four mice at 30 min after injection.
†Blood was assumed to account for 7.78% of total body mass (*21*).

TABLE 4
Effect of N^1-Methyl Nicotinamide on the Biodistribution of ^{99m}Tc Complexes in Mice*

Organ	PalNMe	NMN-PalNMe	SB-Gly	NMN-SB-Gly
Urine	53.00 ± 2.16	53.30 ± 3.14	62.30 ± 3.54	63.68 ± 2.56
Kidneys	4.22 ± 0.85	4.17 ± 0.76	3.17 ± 0.58	3.36 ± 0.52
Blood†	7.40 ± 1.15	7.61 ± 0.85	6.61 ± 0.85	6.85 ± 0.95
Liver	3.43 ± 0.56	2.35 ± 0.66	2.15 ± 0.16	1.67 ± 0.22
Intestines	1.64 ± 0.37	1.84 ± 0.32	0.84 ± 0.02	1.07 ± 0.10
Stomach	0.48 ± 0.14	0.44 ± 0.13	0.34 ± 0.03	0.36 ± 0.04

*Values are percent injected dose, mean ± s.d. for four mice at 15 min after injection.
†Blood was assumed to account for 7.78% of total body mass (*21*). *N*-methyl nicotinamide (NMN) dose was 70 mg/kg (*6,7*) given 5 min intravenously before intravenous injection of ^{99m}Tc complex. SB-Gly = Schiff's base derived from *N*-methyl pyridoxal chloride and glycine.

the intramolecular hemiacetal species, whereas 1-methyl-3-hydroxy-4-formylpyridinium chloride and 1-methyl-2-formyl-3-hydroxypyridinium chloride are present as free-aldehyde species. Though there are differences in lipophilicity between the two pyridine aldehydes, their in vivo behaviors are almost the same. These results suggest that these complexes have a similar structure.

In spite of the impossibility to isolate the ^{99m}Tc complexes derived from *N*-methyl pyridoxal and amino acid or amino acid derivatives, we assume it to be a ^{99m}Tc chelate of the Schiff's base (aldimine). The ^{99m}Tc chelates of the Schiff's bases, electrophoresis as cationic complexes with a pyridinium group and its urinary excretion was the major route determined by scintigrams. It might be possible that these Schiff's bases undergo further chemical changes by metal chelation and that the complex is the product of the reaction, such as a transaminated Schiff's base (ketimine) and compounds where the alpha-position of the amino acid or amino acid derivatives is substituted. This possibility, however, can be ruled out because 2-aminoisobutyric acid formed the complex with urinary excretion. Urinary excretion of these ^{99m}Tc complexes was lowered by the substitution of a bulky alkyl group on the amino acid. In addition to the possibility of liver metabolism, mechanisms have been proposed for the biliary excretion of several classes of compounds, including pyridoxal derivatives. It is known that pyridoxal is oxidized by the liver aldehyde oxidase to pyridoxic acid (*19,20*). If liver metabolisms were important, free pertechnetate might be formed and subsequently localize in the stomach. The low values for stomach activity in the mice argue against this.

The physicochemical properties of the ^{99m}Tc complexes of *N*-alkylated pyridoxal derivatives suggest that the complexing involves both the phenolic hydroxyl and the formyl or azomethine nitrogen groups. It can be assumed that the technetium atom in ^{99m}Tc *N*-alkylated pyridoxal derivatives is bound between two ligand molecules, in a similar way as it has been shown for the structurally similar ^{99m}Tc-pyridoxylidene-glutamate (*11*).

Stannous ion undergoes slight hydrolysis to form Sn colloid when the formyl group exists as a chelating group. If, however, the azomethine nitrogen group is present together with the stannous ion, complex formation occurs before hydrolysis.

The lipophilicity of the complex must also be kept within certain limits to minimize the degree of hepatobiliary excretion of the complex. Technetium-99m-*N*-alkylated pyridoxal derivatives are hydrophilic complexes because of a pyridinium group. The suggestion that the ^{99m}Tc-*N*-alkylated pyridoxal and other derivatives' complexes have a pyridinium group may explain its specificity for the kidneys and lack of biliary excretion through the liver.

In rats, the renal clearances of ^{99m}Tc-*N*-methyl pyridoxal are not significantly different from that of ^{99m}Tc-DTPA and the rate of clearance is not remarkably decreased by treatment with N^1-methyl nicotinamide. It would seem that glomerular filtration is sufficient to explain the renal excretion of these complexes. Despite the presence of two pyridinium moieties, this complex is apparently so different from N^1-methyl nicotinamide that it can not interact efficiently with the renal tubular carrier mechanism. The excretion of a pyridinium compound may also change with the chelation of technetium.

N-methylation of compounds leads to marked changes in polarity and solubility, which will obviously influence distribution and excretion from the body. Though a pyridinium compound such as paraquat is pharmacologically active, *N*-alkyl pyridoxal derivatives are inactive. The resulting compounds described here appear to offer a promising approach toward developing a renal agent with improved characteristics.

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