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Evaluation of ^{99m}Tc-Labeled Amino Acids as Radiopharmaceuticals. VI.[1] *N*-Pyridoxylidenehydrazine-*N'*,*N'*-diacetic Acid

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^{99m}Tc labeled *N*-pyridoxylidenehydrazine-*N'*,*N'*-diacetic acid and related hydrazones were evaluated as hepatobiliary imaging agents. Hydrazones used in the study were *N*-pyridoxylidenehydrazine-*N'*,*N'*-diacetic acid (PLHzDA), *N*-(3-hydroxy-4-pyridylmethylene)hydrazine-*N'*,*N'*-diacetic acid (FHPHzDA), *N*-(4-pyridylmethylene)hydrazine-*N'*,*N'*-diacetic acid (INHzDA), and *N*-pyridoxylidene-*N'*,*N'*-dimethylhydrazine (PLDMHz). The hydrazones were labeled with ^{99m}Tc by the $SnCl_2$ method and the ^{99m}Tc labeling was examined by thin-layer chromatography and high-performance liquid chromatography. ^{99m}Tc labeled hydrazones were administered to golden hamsters, and the distribution indicated that clearance occurred through the hepatobiliary system. Scintigraphic studies in rabbits indicated that ^{99m}Tc-labeled PLHzDA and FHPHzDA are useful hepatobiliary radiotracers.

Keywords——radiopharmaceutical; hepatobiliary scintigraphy; ^{99m}Tc; hydrazone; pyridoxal; 4-formyl-3-hydroxypyridine; hydrazine-*N*,*N*-diacetic acid

A number of ^{99m}Tc-labeled compounds have been used as radiopharmaceuticals for scintigraphy. The ^{99m}Tc-labeled compounds are prepared from a ligand and ^{99m}Tc-pertechnetate in the presence of a reducing agent such as $SnCl_2$. Therefore the chemical forms of the ^{99m}Tc-labeled compounds are supposed to be the Tc chelates of ligands, although in most cases the details of the structure of the chelates are not known.

Several ^{99m}Tc complexes have been proposed as radiopharmaceuticals for hepatobiliary scintigraphy. Two series of ligands are currently used. One consists of iminodiacetic acid (IDA) derivatives,[2] for example *N*-(2,6-diethylphenylcarbamoylmethyl)iminodiacetic acid (E-HIDA),[3] which is one of the most widely used hepatobiliary radiopharmaceuticals. Recently, we reported that *N*-(*p*-toluenesulfonyl)ethylenediamine-*N'*,*N'*-diacetic acid and related compounds can be used for this purpose.[4] The other series of ligands consists of *N*-pyridoxylideneamino acids, which are Schiff bases formed from pyridoxal (PL) and amino acids.[5] Recently, reduced forms of the Schiff bases, *N*-pyridoxylamino acids, were reported as a new series of ligands for the same purpose.[6]

In the IDA derivatives, Tc should be chelated by the imino and carboxylate groups. In the pyridoxal Schiff bases, the Tc-chelating sites are supposed to be the azomethine N and phenolate O in the pyridoxal moiety and the carboxylate group of the amino acid moiety, though no definite evidence is available.

The title compound, *N*-pyridoxylidenehydrazine-*N'*,*N'*-diacetic acid (PLHzDA), is a

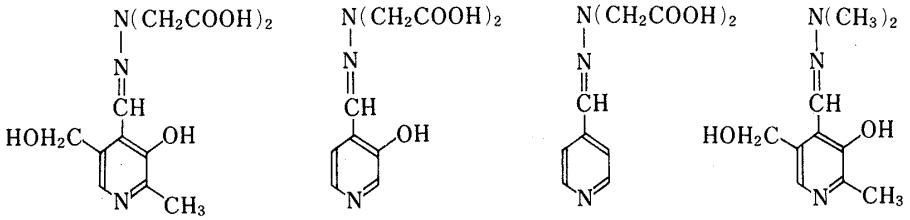

Chart 1

hydrazone of hydrazine-*N*,*N*-diacetic acid (HzDA) with PL. It has two sets of chelating groups, IDA and Schiff base, in the molecule. Therefore, the compound is a good model for the study of Tc chelation, and its ^{99m}Tc complex is expected to be a useful tracer for hepatobiliary imaging.

The present paper describes the results of studies of the Tc chelation and the *in vivo* behavior of ^{99m}Tc complexes of PLHzDA and related hydrazones. The other hydrazones studied are those of HzDA with 4-formyl-3-hydroxypyridine (FHP) and isonicotinealdehyde (IN) and the 1,1-dimethylhydrazone of PL. FHP is a chemical analog of PL, and has no vitamin B_6 activity.[7] The ^{99m}Tc complex of a Schiff base of FHP and amino acid was found to be a good hepatobiliary tracer.[8] These results indicate that the vitamin B_6 activity of PL is unrelated to its usefulness as a hepatobiliary tracer.

Experimental

Materials——FHP was synthesized by the method of Heinert and Martell.[7] HzDA was prepared from hydrazine hydrate and monochloroacetic acid according to Bailey and Read.[9] Pyridoxal hydrochloride, isonicotinealdehyde hydrochloride, E-HIDA, 1,1-dimethylhydrazine (DMHz), and other chemicals were obtained from commercial sources.

Hydrazones were prepared from the appropriate aldehyde and hydrazine. The pyridinealdehydes (PL, FHP, IN) were allowed to react with an equimolar amount of HzDA in an aqueous or aqueous alcoholic solution at room temperature. The hydrazone of IN was prepared in an atmosphere of N_2. The pale yellow products thus obtained were recrystallized from water. Yield 80—90%.

N-Pyridoxylidenehydrazine-*N'*,*N'*-diacetic Acid (PLHzDA): mp 218—219 °C (dec.). *Anal.* Calcd for $C_{12}H_{15}N_3O_6 \cdot 1/2H_2O$: C, 47.06; H, 5.27; N, 13.72. Found: C, 47.00; H, 5.39; N, 13.71. IR ν_{max}^{Nujol} cm^{-1}: 3500 (OH), 1750 (COOH). ^{1}H-NMR (pyridine-d_5 + D_2O) δ: 2.60 (3H, s, CH_3), 4.68 (4H, s, CH_2COOH), 4.96 (2H, s, PL-CH_2), 7.98 (1H, s, aromatic H), 8.20 (1H, s, azomethine H).

N-(3-Hydroxy-4-pyridylmethylene)hydrazine-*N'*,*N'*-diacetic Acid (FHPHzDA): mp 231 °C (dec.). *Anal.* Calcd for $C_{10}H_{11}N_3O_5 \cdot H_2O$: C, 44.28; H, 4.83; N, 15.49. Found: C, 44.30; H, 4.96; N, 15.49. IR ν_{max}^{Nujol} cm^{-1}: 3500—3200 (OH), 1740 (COOH). ^{1}H-NMR (pyridine-d_5 + D_2O) δ: 4.60 (4H, s, CH_2COOH), 7.18 (1H, d, J=5 Hz, 5-H), 7.52 (1H, s, 2-H), 8.06 (1H, d, J=5 Hz, 6-H), 8.28 (1H, s, azomethine-H).

N-(4-Pyridylmethylene)hydrazine-*N'*,*N'*-diacetic Acid (INHzDA): mp 217—218 °C (dec.). *Anal.* Calcd for $C_{10}H_{11}N_3O_4 \cdot H_2O$: C, 47.06, H, 5.13, N, 16.46. Found: C, 46.94; H, 5.24; N, 16.28. IR ν_{max}^{Nujol} cm^{-1}: 1740 (COOH).

N-Pyridoxylidene-*N'*,*N'*-dimethylhydrazine (PLDMHz): An aqueous solution of PL and an ethanol solution of an equimolar amount of DMHz were mixed and the mixture was made slightly acidic by addition of AcOH, then refluxed for 1 h. After removal of ethanol, the mixture was made alkaline with $NaHCO_3$, and the pale yellow precipitate was recrystallized from benzene. Pale yellow needles. Yield 79%. mp 114—116 °C. *Anal.* Calcd for $C_9H_{13}N_3$: C, 57.40; H, 7.23; N, 20.08. Found: C, 57.04; H, 7.33; N, 19.89. ^{1}H-NMR (CD_3OD) δ: 2.40 (3H, s, 2-CH_3), 3.08 (6H, s, $N(CH_3)_2$), 4.68 (2H, s, PL-CH_2), 7.62 (1H, s, 6-H), 7.78 (1H, s, azomethine H).

A JEOL PS-100 nuclear magnetic resonance (NMR) spectrometer and a JASCO DS-701 infrared (IR) grating spectrophotometer were used throughout the present study.

^{99m}Tc Labeling——$SnCl_2$ in aqueous HCl was added to 1.0 ml of aqueous solution containing 10—40 mg of ligand. The amount of $SnCl_2$ was varied in the range of 5—100 μg. The pH of the mixture was adjusted to a predetermined value with 0.1 M NaOH. The solution was allowed to stand at room temperature for 15 min and passed through a 0.2-μm Millipore filter into a sealed vial. ^{99m}Tc-pertechnetate (5—20 mCi) in saline was added to the vial. The mixture was shaken gently and allowed to stand for 30 min.

Chromatography——The ^{99m}Tc labeled ligands were analyzed by means of thin layer chromatography (TLC)

and high-performance liquid chromatography (HPLC).

For TLC, 0.5-mm Silica gel 60 F_{254} plate (E. Merck) were used; the solvent systems were (a) ethanol–water (7 : 3) and (b) 1-butanol–acetic acid–water (4 : 1 : 1). The spots were detected with an ultraviolet (UV) lamp and the radiochromatogram was obtained with an Aloka 101 TLC scanner.

The HPLC system consisted of a Waters M-6000A solvent delivery system, a U6K universal injector, a model 660 solvent programmer, and a radial compression separation system (10 × 0.8 cm Radial Bondapak C_{18} cartridge, RCM-100 module). Ultraviolet detection was done with a Waters 440 absorption detector at 254 nm and radioactivity detection, with a Berthold LB 503 radioactivity monitor.

***In Vivo* Behavior**——For the *in vivo* distribution study, golden hamsters (male; body weight, 150 ± 10 g) were used. A saline solution of ^{99m}Tc-labeled ligand (100 μCi, 0.1 ml) was injected into the heart. The animals were sacrificed 1 h after the injection. The organs and the blood were removed, weighed, and measured for radioactivity with a Packard 5360 autogamma scintillation spectrophotometer.

For the scintigraphic study, a rabbit (male; body weight, 3.5 kg) was used. A solution of ^{99m}Tc-labeled ligand (3 mCi, 1.5 ml) was injected intravenously at the ear. Sequential scintigrams were made at predetermined intervals using a Toshiba GCA 202 scintillation camera.

Results and Discussion

The hydrazones were labeled with ^{99m}Tc by the procedure described in the experimental section. The amount of $SnCl_2$ and the pH of the solution were varied so as to achieve the maximum labeling yield. The labeling yields were estimated by TLC. In the TLC-radioscannogram of PLHzDA, two peaks were observed at the origin and at *Rf* 0.76 (0.56). The *Rf* value was obtained in solvent system (a) described in the experimental section, and that in parentheses was obtained in system (b).

The peak at the origin can be attributed to polymeric or colloidal forms of ^{99m}Tc and the other peak to the ^{99m}Tc complex of PLHzDA (^{99m}Tc PLHzDA). The TLC spot of PLHzDA was detected at *Rf* 0.62 (0.10) by UV irradiation. The labeling yield estimated from the area was roughly 70%. Contamination by ^{99m}Tc-pertechnetate cannot be excluded, since the *Rf* value of this form is 0.85 (0.64). A similar chromatogram was obtained for ^{99m}Tc FHPHzDA.

The chromatogram of PLDMHz also had two radioactive peaks, at the origin and at *Rf* 0.71 (0.61). The latter was broad and may be formed by an overlap of ^{99m}Tc PLDMHz and ^{99m}Tc-pertechnetate. The chromatogram of INHzDA indicated that INHzDA was not labeled under the conditions used.

Since the labeling yields were unexpectedly low, ^{99m}Tc labeled solutions were analyzed by HPLC. Figure 1 shows a chromatogram of ^{99m}Tc-labeled PLHzDA. The mobile phase was water until 10 min, when it was replaced by tetrahydrofuran–water (6 : 4). The UV peak at 22 min is the solvent front. The UV peak at 7 min can be assigned to Sn complex or uncomplexed forms of PLHzDA. The injection of PLHzDA solution gave a single peak at the same retention time.

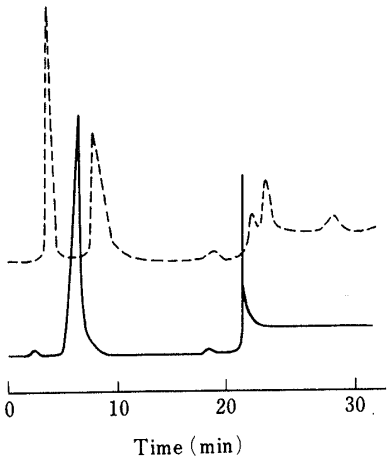

Fig. 1. High-Performance Liquid Chromatogram of ^{99m}Tc-Labeled PLHzDA

Solid line, UV monitor; dotted line, radioactivity monitor.

TABLE I. Distribution of Radioactivity in Golden Hamsters 1 h after Administration of ^{99m}Tc-Labeled Compounds[a)]

Organ	^{99m}Tc-Labeled compounds			
	PLHzDA	FHPHzDA	HzDA[b)]	E-HIDA
Blood	1.97	2.97	5.35	0.074
Liver	3.01	2.48	1.28	0.070
Pancreas	0.41	0.54	1.69	0.022
Spleen	0.97	1.72	0.95	0.016
Stomach	0.31	0.28	0.42	0.009
Kidney	2.71	2.72	13.73	0.256
Small intestine	1.00	1.00	1.00	1.000

a) Values are ratios of radioactivity per g of organ (ml of blood) to that in the small intestine. Mean of 3 animals.
b) Y. Karube, T. Maeda, M. Ohya, S. Sugata, A. Kono, and Y. Matsushima, *J. Radiat. Res.*, **23**, 234 (1982).

The radioactive peak is delayed by the tubing volume from the UV detector to the radioactivity detector; ^{99m}Tc-pertechnetate gives a peak at 4 min. The peak at 8 min can be attributed to ^{99m}Tc complex of PLHzDA, and the four peaks at 20—30 min to polymeric forms of ^{99m}Tc. The labeling yield calculated from the HPLC peaks was much lower than that obtained by TLC, probably because of decomposition during the chromatographic procedures.

The chromatogram of FHPHzDA was quite similar to that of PLHzDA. Those of PLDMHz and INHzDA lacked the peak corresponding to the ^{99m}Tc complex.

The results of the *in vivo* distribution studies in golden hamsters 1 h after administration of ^{99m}Tc-labeled compounds are summarized in Table I. Values are ratio of radioactivity per g organ or ml blood to that in the small intestine. INHzDA and PLDMHz were not used in the distribution study, since the labeling yields were unsatisfactory. Table I also shows data on HzDA and E-HIDA for comparison.

In the case of ^{99m}Tc E-HIDA, high radioactivity was found in the small intestine, indicating clearance by the hepatobiliary system. The results for PLHzDA and FHPHzDA indicate hepatobiliary clearance, though the clearance from the blood and the liver was slower. The high accumulation of HzDA by the kidneys indicated the urinary excretion of the labeled ligand.

Sequential scintigrams of a rabbit after *i.v.* administration of ^{99m}Tc-labeled PLHzDA indicated that the radioactivity was concentrated initially in the liver and the kidneys. Within 30 and 45 min, ^{99m}Tc radioactivity was excreted into the small intestine through the hepatobiliary route, though the liver image was still clear. The thyroid gland was not visualized, indicating that ^{99m}Tc-pertechnetate was not liberated *in vivo*. Scintigrams obtained with ^{99m}Tc-labeled FHPHzDA were similar to those with PLHzDA.

In conclusion, ^{99m}Tc-labeled PLHzDA and FHPHzDA were found to be good hepatobiliary tracers. However, as hepatobiliary radiopharmaceuticals, they are not as satisfactory as E-HIDA, since considerable radioactivity was present in the liver and kidneys. This may possibly be due to the presence of polymeric forms of ^{99m}Tc. Attempts to improve ^{99m}Tc labeling and studies on metal chelation of these ligands are in progress.

References and Notes

1) Part V: Y. Karube, T. Imoto, T. Maeda, M. Ohya, S. Sugata, A. Kono, H. Mashiba, Y. Ichinose, K. Tanaka,

M. Kaku, and Y. Matsushima, *Chem. Pharm. Bull.*, **31**, 3242 (1983).
2) E. Harvey, M. Loberg, and M. Cooper, *J. Nucl. Med.*, **16**, 533 (1975); L. Rosenthall, E. A. Shaffer, and R. Losbona, *Radiology*, **126**, 467 (1978).
3) S. Pouwels, M. Steels, and L. Piret, *J. Nucl. Med.*, **19**, 783 (1978); S. P. Nielsen, J. Trap-Jensen, and J. Lindenberg, *J. Nucl. Med.*, **19**, 452 (1978); T. Maeda, H. Shiokawa, and Y. Karube, *Iryo*, **34**, 40 (1980).
4) Y. Karube, A. Kono, T. Maeda, M. Ohya, and Y. Matsushima, *J. Nucl. Med.*, **22**, 619 (1981).
5) R. J. Baker, J. C. Bellen, and P. M. Ronai, *J. Nucl. Med.*, **16**, 720 (1975); M. Kato and M. Hazue, *Jpn. J. Nucl. Med.*, **14**, 927 (1977); M. Kato-Azuma, *ibid.*, **17**, 575 (1980); K. Mitani, Y. Yumoto, and H. Nagashima, *ibid.*, **17**, 553 (1980).
6) M. Kato-Azuma, *J. Nucl. Med.*, **23**, 517 (1982).
7) D. Heinert and A. E. Martell, *Tetrahedron*, **3**, 49 (1958).
8) A. Kono, T. Maeda, Y. Karube, H. Shiokawa, and Y. Matsushima, *J. Nucl. Med.*, **20**, 39 (1979).
9) J. R. Bailey and W. T. Read, *J. Am. Chem. Soc.*, **36**, 1747 (1914).