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Pipemidic Acid: Absorption, Distribution, and Excretion

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Pipemidic acid was absorbed well by the oral route. Its peak levels in plasma ranged from 4 to 12 μg/ml at an oral dose of about 50 mg/kg in mice, rats, dogs, monkeys, and men. The protein binding of pipemidic acid was about 20% in dog plasma and about 30% in human serum. Pipemidic acid was distributed to most of the organs and tissues tested at the concentrations comparable to or higher than the plasma level. Its concentrations in bile and urine were much higher than the plasma level. About 25 to 88% of orally administered pipemidic acid was excreted into urine in a bacteriologically active form, the percentage depending on the animals and doses employed. The remainder was excreted into feces in men. The main active principle in vivo was unchanged pipemidic acid itself. The mean lethal dose of pipemidic acid after a single oral dose was more than 16,000 mg/kg in mice. No abnormalities were observed in mice orally receiving pipemidic acid once a day for 4 weeks at doses of 1,000, 2,000, and 4,000 mg/kg per day, and in rats orally receiving the drug once a day for 2 weeks at doses of 400 and 1,600 mg/kg per day.

Pipemidic acid is a new synthetic antibacterial agent structurally related to piromidic acid and nalidixic acid (unpublished data). It has some advantageous points over the latter two drugs, such as activity against *Pseudomonas aeruginosa*, activity against bacteria resistant to piromidic acid and nalidixic acid, the good distribution to organs and tissues, and the stability to metabolic inactivation. The present report is concerned with the pharmacological properties of pipemidic acid, together with some toxicological data.

MATERIALS AND METHODS

Drugs. Pipemidic acid trihydrate synthesized in this laboratory (unpublished data) was used for administration. The dose or concentration of the drug was always expressed as that of anhydrous compound.

Pharmacological study. Animals used were female ddy-s mice weighing 30 to 46 g, male Wister rats weighing 191 to 262 g, male and female beagle dogs weighing 9.4 to 13.2 kg, male and female monkeys (Macaca mulatta) weighing 3.8 to 9.3 kg, and male healthy human volunteers (34 to 49 years old) weighing 47 to 70 kg. Pipemidic acid was orally administered to mice, rats, and monkeys as suspensions in 0.2% carboxymethyl cellulose, and to dogs and human volunteers as 250-mg tablets. Blood was withdrawn at the indicated times from mice and rats by cardiac puncture, and from dogs, monkeys, and men by venipuncture which was centrifuged to separate plasma. Rat bile was taken as described previously (5). Urine was pooled from 0 to 3, 3 to 6, and 6 to 24 h in mice and rats, from 0 to 24 h in monkeys, and from 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 12, and 12 to 24 h in men. Organs, tissues, and body fluids were harvested 2 h after the last dosing. The appropriate amount of the organs and tissues was weighed, homogenized with two times the weight of water with a Polytron homogenizer (Kinematica G.m.b.H.), heated at 80 C for 15 min, cooled, and centrifuged to separate the supernatant. Human feces were pooled from 0 to 24 and 24 to 48 h, the small amount of which was weighed, mixed with nine times the weight of water, heated in boiling water for 10 min, cooled, and diluted appropriately with water. Plasma and spinal fluids were assayed without any dilution, whereas bile, urine, and hot water extracts from organs, tissues, and feces were appropriately diluted with 0.067 M phosphate buffer, pH 7.5, before assay if necessary. The assay method employed was the thin-layer, cup-plate method using *Escherichia coli* Kp (5) with a modification of medium pH to 7.5. Standard calibration lines were made in plasma for the assay of plasma and spinal fluid levels, and in 0.067 M phosphate buffer, pH 7.5, for those of urine, bile, feces, organ, and tissue levels.

Experiment on protein binding. The protein binding of pipemidic acid was examined by rapid ultrafiltration. [^{14}C]pipemidic acid labeled at the piperazine ring (specific activity; 11.6 μCi/mg) was dissolved in fresh dog plasma, pH 7.0, or human serum, pH 7.2 (Flow Laboratories Inc.), which was appropriately diluted with each ultrafiltrate. Filtration was made at 37 C with a PM-30 membrane-loaded filtration cell (Amicon, model 12) under the nitrogen gas pressure of about 2 kg/cm^2 with magnetic stirring. Fifty microliters of the plasma or serum inside the filtration cell was taken before and after filtration, dissolved in a mixture of 0.5 ml of Soluene 100 (Packard) and 10 ml of toluene scintillator (3), acidified with two drops of acetic acid, and assayed for radioactivity. Ultrafil-

trates were fractionated every 500 μl, 50 μl of which was dissolved in the 10-ml dioxane scintillator (1) and assayed. Radioactivity was counted by a Tri-Carb liquid scintillation spectrometer (Packard, model 544). Radioactivity in ultrafiltrates was regarded as pipemidic acid unbound to proteins. As the rate of protein binding was affected by protein concentrations, the rate of unbound pipemidic acid was calculated when the total volume of ultrafiltrates taken came to that of the ultrafiltrate initially added for dilution.

Bioautographic study. An active principle in vivo after the ingestion of pipemidic acid was examined by bioautography of the thin-layer chromatogram of plasma and urine. Plasma was deproteinized through an Amberlite XAD-2 (Rohm and Haas Co.) column on which an active substance in plasma was all absorbed while plasma protein was washed out with water. About 75% of the active substance was eluted from the column with 50% methanol in water and was concentrated under reduced pressure. An appropriate amount of the deproteinized plasma and urine was spotted on a sheet of Eastman Chromagram no. 6060 and developed in a solvent mixture (*n*-butanol/acetic acid/water; 3:1:1). The sheet was overlaid with seeded agar, which was prepared as described previously (5), in a thickness of about 2 mm and incubated at 37 C overnight. Inhibitory zones were observed after the staining with a triphenyltetrazolium chloride solution (1 mg/ml in a saturated Na_2HPO_4 solution).

Toxicological study. Pipemidic acid was dissolved in an NaOH solution for parenteral administration and suspended in 0.2% carboxymethyl cellulose for an oral one. Acute toxicity was determined using female ddy-f mice weighing 22 to 29 g and subacute toxicity using female ICR mice weighing 19 to 23 g and male SD rats weighing 110 to 134 g.

RESULTS

The plasma levels of pipemidic acid after a single oral dose are shown in Fig. 1. When administered to mice and rats at doses of 50, 100, and 200 mg/kg, the peaks of average plasma levels were seen within 1 h after administration, which were 4.1, 7.6, and 10.3 μg/ml, respectively, in mice and 4.5, 6.5, and 9.2 μg/ml, respectively, in rats. The time when the peak plasma levels were observed delayed with increasing dose. In dogs receiving a 500-mg dose (42.7 mg/kg on the average) and monkeys given

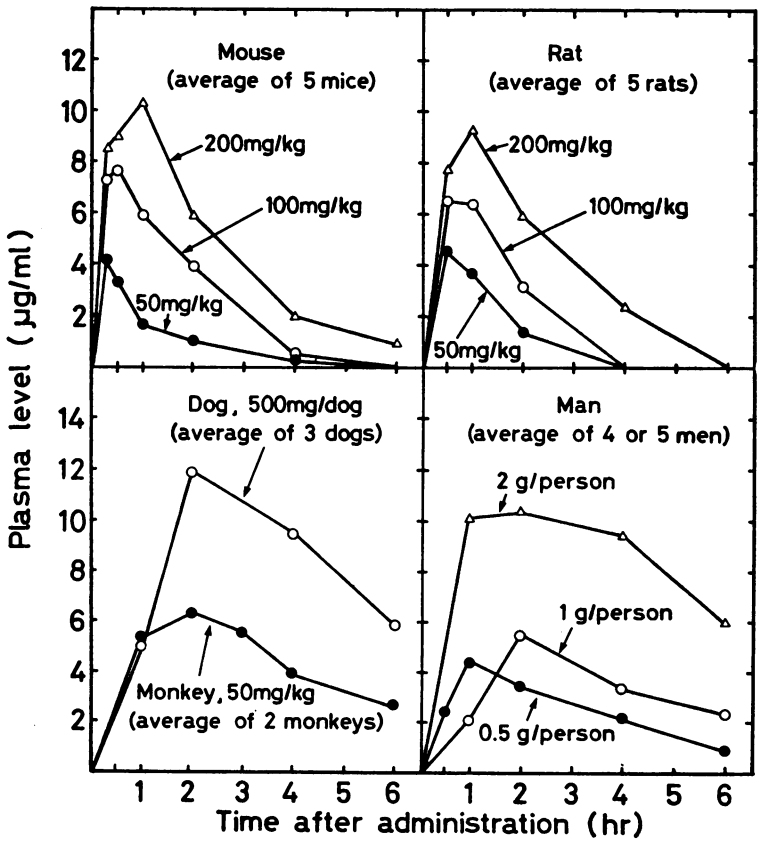

FIG. 1. *Concentrations of pipemidic acid in the plasma of mice, rats, dogs, monkeys, and men receiving a single oral dose.*

a 50-mg/kg dose, average plasma levels rose up to maxima of 11.9 and 6.3 μg/ml, respectively, at 2 h and relatively high levels of 5.8 and 2.7 μg/ml were seen even at 6 h. In men ingesting the drug at doses of 0.5, 1, and 2 g (8.3, 16.9, and 33.6 mg/kg on the average), average plasma levels attained peaks of 4.4, 5.4, and 10.2 μg/ml, respectively, at 1 or 2 h and dropped to 0.9, 2.3, and 5.9 μg/ml at 6 h. Compared with mice and rats, dogs, monkeys, and men showed relatively high concentrations and relatively long duration of pipemidic acid in plasma.

The protein binding of pipemidic acid is shown in Fig. 2. The rate of unbound pipemidic acid was about 80% in dog plasma and about 70% in human serum, almost independent of the drug concentrations used. The protein binding of pipemidic acid was reversible and rapidly broken down by dilution.

The concentrations of pipemidic acid in organs, tissues, and body fluids are shown in Table 1. In dogs receiving a single oral dose of 500 mg, the average concentration of pipemidic acid in plasma was 7.8 μg/ml; the drug was detected in all of the body parts tested except for the brain and spinal fluid. The drug concentrations in the organs and tissues tested were comparable to or higher than the plasma level. The concentrations in bile and urine were much higher than the plasma level. Similar results were obtained in dogs given four oral doses of 500 mg at intervals of 8 h and in monkeys given 50-mg/kg oral doses twice a day for 1 month. It was noted that the levels of pipemidic acid in most organs and tissues were higher than the plasma levels.

Biliary excretion of pipemidic acid was tested in rats receiving a single oral dose of 50 mg/kg. An average bile level attained a peak of 48 μg/ml at 2 h and dropped to 14 μg/ml in 7 h (Fig. 3). The recovery for 24 h was 1.35% on the average.

Urinary excretion of pipemidic acid after oral

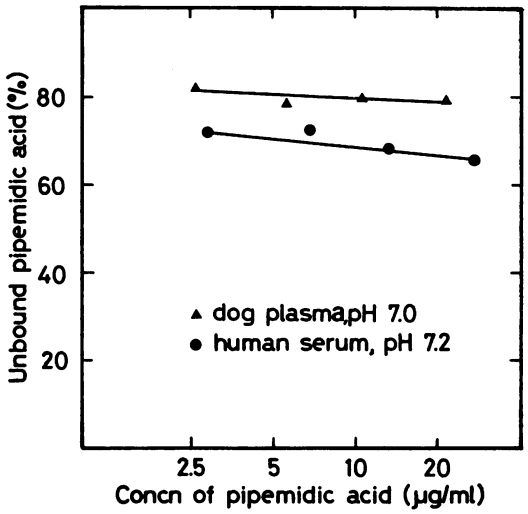

FIG. 2. *Protein binding of pipemidic acid.*

TABLE 1. *Concentrations of pipemidic acid in organs, tissues, and body fluids of dogs and monkeys*

Body part	Concn (μg/g or ml)[a]		
	Dog		Monkey twice a day for 1 month[d]
	Single dose[b]	4 doses[c]	
Plasma	7.8 (4.0-14.6)	14.2 (12.5-16.8)	8.6 (5.2-14.8)
Brain	ND (all ND)	ND (all ND)	ND (all ND)
Heart	13.8 (8.0-24.6)	24.5 (20.4-30.9)	25.9 (16.2-43.8)
Trachea	6.1 (3.4-10.7)	13.6 (11.6-15.9)	12.4 (6.8-18.0)
Lung	7.4 (3.6-14.7)	14.2 (12.2-17.1)	11.3 (7.2-16.8)
Liver	18.9 (10.7-32.1)	26.7 (21.3-33.0)	41.7 (25.8-72.0)
Kidney	28.4 (14.0-50.4)	45.8 (33.0-63.6)	142 (111-198)
Spleen	11.2 (7.3-18.6)	23.3 (17.1-27.3)	12.9 (10.2-17.4)
Pancreas	11.7 (4.8-23.7)	21.3 (18.0-26.7)	8.5 (4.1-17.2)
Muscle	11.5 (5.6-21.3)	25.0 (22.2-30.0)	19.2 (13.7-26.4)
Stomach	13.1 (5.2-27.6)	17.7 (15.3-22.5)	44.2 (12.9-67.5)
Small intestines	7.5 (4.0-14.1)	15.6 (14.4-18.9)	32.4 (15.6-51.0)
Large intestines	12.9 (4.7-19.2)	24.0 (21.3-28.8)	41.3 (36.8-48.5)
Spinal fluid	ND (all ND)	ND (all ND)	
Bile in gallbladder	45.2 (17.5-75.0)	183 (115-227)	217 (187-235)
Urine in urinary bladder	471 (183-690)	2,620 (1,710-3,150)	1,616 (1,200-1,900)

[a] Average of three animals (range). ND, Not detectable.
[b] A single oral dose of 500 mg (42.7 mg/kg on the average).
[c] Four oral doses of 500 mg (42.0 mg/kg per dose on the average) at intervals of 8 h.
[d] Twice a day for 1 month at an oral dose of 50 mg/kg per dose. Animals were sacrificed 2 h after the last dose.

dosing is summarized in Table 2. In mice given a single dose, average urine levels were the highest in 0- to 3-h urine, the concentrations of which were 134, 310, and 532 μg/ml at doses of 20, 50, and 100 mg/kg, respectively. The average recoveries for 24 h ranged 24.5 to 40.0%. In rats receiving a single dose, average concentrations reached peaks of 364, 876, and 794 μg/ml in 3- to 6-h urine at doses of 20, 50, and 100 mg/kg, respectively. The average recoveries for 24 h were 26.5 to 36.2%. In monkeys receiving two doses of 50 mg/kg at an interval of 8 h, the average level of urine pooled for 24 h was 1,029 μg/ml and the average recovery for 24 h was 45.2%. In men given a single dose, average urine levels were the highest in 2- to 4-h or 4- to 6-h urine, the concentrations of which were 1,116, 2,441, and 1,949 μg/ml at doses of 0.5, 1, and 2 g, respectively. High concentrations above 100 μg/ml were detected even in 12- to 24-h urine at doses above 1 g. The average recoveries for 24 h ranged 68.3 to 87.8%.

Fecal excretion was examined in men ingesting a single oral dose of 1 g. As shown in Table 3, an average concentration was 1,361 μg/g in feces taken within 24 h and 622 μg/g in feces taken between 24 and 48 h. The average recovery from feces for 48 h was 36.2%. As the average recovery from urine measured at the same time was 62.6%, the recovery from feces and urine totaled 98.8%.

To determine the main active principle in vivo, plasma and urine of rats, dogs, and men receiving pipemidic acid were developed on thin-layer chromatography followed by bioautography for the detection of antimicrobialy active spots. As seen in Fig. 4, only one active spot with the R_f value corresponding to authentic pipemidic acid was detected in all plasma and urine tested. This active substance was purified from rat urine and identified as unchanged pipemidic acid from ultraviolet, infrared, and mass spectral data.

The toxicity of pipemidic acid was preliminarily examined in mice and rats. The mean lethal dose values of pipemidic acid after a single dose to mice were 707 mg/kg intravenously, 2,244 mg/kg subcutaneously, and more than 16,000 mg/kg orally. Figure 5 shows the growth curves of mice orally receiving pipemidic

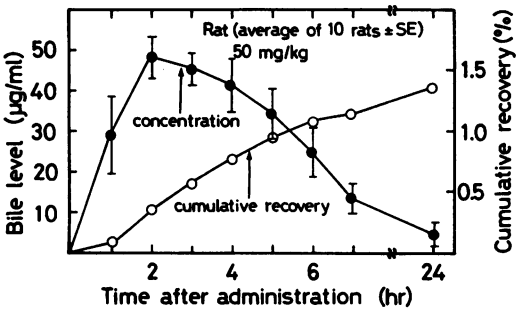

FIG. 3. *Biliary excretion of pipemidic acid in rats receiving a single oral dose of 50 mg/kg.*

TABLE 3. *Fecal and urinary excretion of pipemidic acid in men given a single oral dose of 1 g*

Sample	Time (h)	Concn[a] (μg/ml or g)	Recovery[a] (%)
Feces	0-24	1,361 (850-2,360)	21.7 (13.4-26.0)
	24-48	622 (30-1,250)	14.5 (0.3-43.8)
Urine	0-24	563 (355-1,188)	62.6 (33.7-91.1)

[a] Average of four persons (range).

TABLE 2. *Urinary excretion of pipemidic acid after oral dosing to mice, rats, monkeys, and men*

Animal	Animal no.	Dose (mg/kg)	Peak		Recovery for 24 h[a] (%)
			Time (h)	Concn[a] (μg/ml)	
Mouse	12	20	0-3	134 (117-143)	37.8 (33.5-43.9)
	12	50	0-3	310 (330-422)	40.0 (35.3-42.7)
	12	100	0-3	532 (460-725)	24.5 (18.2-33.5)
Rat	3	20	3-6	364 (270-510)	36.2 (25.8-43.5)
	6	50	3-6	876 (580-1,350)	30.7 (21.1-39.9)
	3	100	3-6	794 (590-1,060)	26.5 (20.5-31.5)
Monkey	2	50 × 2	0-24	1,029 (880-1,170)	45.2 (40.8-49.6)
Man	4	0.5[b]	2-4	1,116 (690-2,200)	87.8 (78.5-97.1)
	5	1.0[b]	2-4	2,441 (1,920-3,210)	68.3 (54.4-91.1)
	5	2.0[b]	4-6	1,949 (1,600-2,670)	69.1 (61.0-76.7)

[a] Average (range).
[b] Expressed in grams per person.

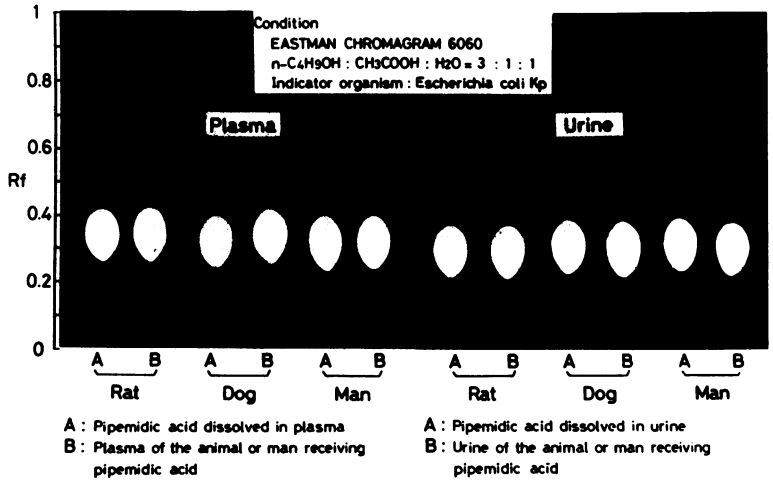

FIG. 4. *Bioautography of the thin-layer chromatograms of plasma and urine of dogs, rats, and men receiving pipemidic acid.*

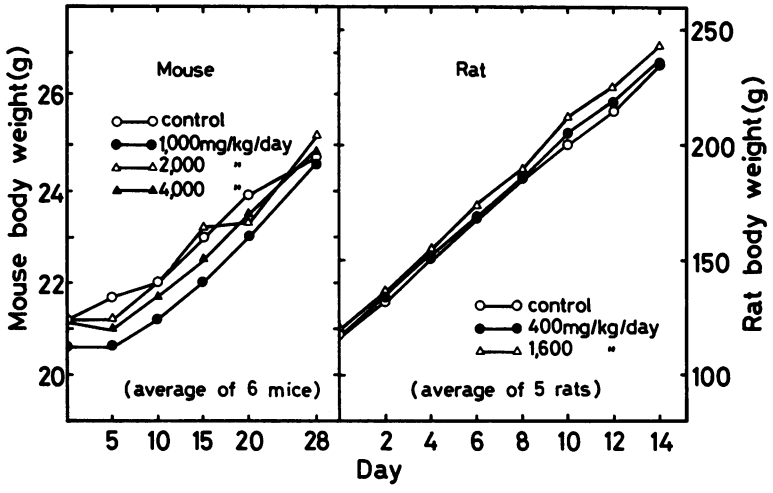

FIG. 5. *Growth curves of mice and rats serially receiving pipemidic acid by the oral route.*

acid once a day for 4 weeks at doses of 1,000, 2,000, and 4,000 mg/kg, and of rats orally receiving the drug once a day for 2 weeks at doses of 400 and 1,600 mg/kg. No abnormalities were observed on body weight gains, nor on the results of hematological, biochemical, and histological analyses. The low toxicity of orally administered pipemidic acid is also shown in subacute and chronic toxicity tests using rats, dogs, and monkeys, which will be described elsewhere.

DISCUSSION

It is evident from the present results that pipemidic acid is well absorbed by the oral route. The peak plasma levels of pipemidic acid were higher than 4 μg/ml at an oral dose of about 50 mg/kg in all the animals tested. These plasma levels seem to be sufficient to inhibit the growth of most susceptible bacteria considering that pipemidic acid undergoes little or no metabolic changes and about 70 to 80% of it remains unbound to proteins in serum or plasma. Pipemidic acid has proven to be generally more effective against the systemic infections with some gram-negative bacteria than piromidic acid, nalidixic acid, ampicillin, and cephalexin, and to be more effective on the *P. aeruginosa* infections than carbenicillin in mice (unpublished data). One of the possible reasons why pipemidic acid is so effective in vivo seems to be partly due to its good distribution to organs and tissues and its stability to metabolic inactiva-

tion as shown in this report. The levels of pipemidic acid in bile were high in rats, dogs, and monkeys, although the recovery from bile was relatively small as examined in rats. On the other hand, the levels in urine were very high in all the animals examined and the recovery from urine was large, suggesting that the main route for the excretion of the absorbed pipemidic acid was by the urinary tract. As pipemidic acid orally administered to men was recovered from urine and feces in nearly 100% in a bacteriologically active form, it was expected that the drug was neither accumulated in a body nor inactivated metabolically. In fact, it was shown that a main active principle in plasma and urine was unchanged pipemidic acid itself. Since piromidic acid, nalidixic acid, and oxolinic acid are known to be metabolized to a fairly great extent into bacteriologically inactive form in a living body (2, 4, 5), the stability of pipemidic acid to metabolic inactivation is an advantage over the former three drugs. The preliminary toxicity tests revealed that the oral toxicity of pipemidic acid was low. The safety margin of pipemidic acid seems to be broad.

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