ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1982, p. 224–230
0066-4804/82/020224-07$02.00/0

Vol. 21, No. 2

Pharmacokinetics of Fosmidomycin, a New Phosphonic Acid Antibiotic

TAKEO MURAKAWA, HIROSHI SAKAMOTO, SHIGEMI FUKADA, TAKAO KONISHI, AND MINORU NISHIDA*

Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan

Received 6 July 1981/Accepted 29 October 1981

The pharmacokinetics of fosmidomycin was investigated in animals and humans after parenteral and oral dosing. In dogs the serum concentration was 54.8 μg/ml at 0.25 h after an intravenous dose of 20 mg/kg, and the half-life was 1.14 h. Peak concentration was 41.4 μg/ml after an intramuscular dose of 20 mg/kg and 16.6 μg/ml after an oral dose of 40 mg/kg. In volunteers, the serum concentration 0.25 h after dosing was 157 μg/ml after an intravenous dose of 30 mg/kg, 12.3 μg/ml after an intramuscular dose of 7.5 mg/kg, and 2.45 μg/ml after an oral dose of 500 mg. More than 90% of the given dose was excreted in the 24-h urine in rats and dogs after parenteral dosing with 20 mg/kg. The 24-h urinary recovery was 45.8% of the given dose in rats after oral dosing with 100 mg/kg and 37.8% in dogs after oral dosing with 40 mg/kg. In volunteers 85.5% of the intravenous dose (30 mg/kg), 66.4% of the intramuscular dose (7.5 mg/kg), and 26.0% of the oral dose (500 mg) were excreted unchanged in the 24-h urine. In the multiple-dose study, there was no accumulation of fosmidomycin in the serum even after 21 consecutive intramuscular dosings of 1 g every 6 h or 29 consecutive 0.5-h drip infusions of 2 g every 6 h. Biliary excretion was extremely low in rats. Fosmidomycin was well distributed to the tissues of rats after parenteral and oral dosing. The lymph concentrations in dogs were nearly the same as serum concentrations. Serum protein binding was low (4% or less) to mouse, rat, dog, and human serum.

Fosmidomycin (FR 31564) is a new antibiotic containing phosphonic acid in the molecule (Fig. 1). Fosmidomycin is active in vitro against most gram-negative bacteria, including *Pseudomonas aeruginosa*, but is not active against gram-positive bacteria or certain gram-negative species such as *Proteus morganii* and *Serratia marcescens*. When administered parenterally and orally, the drug is superior to fosfomycin in therapeutic efficacy against infections due to gram-negative bacteria in mice (6). Fosmidomycin is incorporated into bacterial cells by the active transport system in the same way as fosfomycin (4). This paper presents data on the pharmacokinetics of fosmidomycin in animals and healthy volunteers.

MATERIALS AND METHODS

Antibiotic. Fosmidomycin (FR 31564) was synthesized in the Research Laboratories of Fujisawa Pharmaceutical Co., Ltd.

Bioassay. The drug concentrations were determined by the disk plate technique with *Enterobacter cloacae* ATCC 29893 as the test organism. The test medium was prepared from a hydrated base of nutrient broth (Difco Laboratories, Detroit, Mich.) by the addition of agar powder to give a final concentration of 1% (wt/vol). The overnight culture in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) was inoculated into the medium to give a final concentration of 0.5% (vol/vol). Ten milliliters of the inoculated agar medium was poured into a 9-cm petri dish and incubated at 37°C for 18 to 20 h. The zones of inhibition were measured and compared with similarly prepared standards.

Animal study. (i) Animals. The following animals were used: 6-week-old male JCl:SD rats, male beagle dogs weighing 8.6 to 14.0 kg, and mongrel dogs weighing 10 to 16 kg.

(ii) Parenteral dosing. The antibiotic solutions for injection were prepared in 0.9% saline. The drug was given in single doses of 20 mg/kg into the hind leg muscle (intramuscular route [i.m.]) and tail vein (intravenous route [i.v.]) of rats (5 ml of drug solution per kg of body weight), and into the hind leg muscle (i.m.) and foreleg vein (i.v.) of dogs (0.5 ml/kg of body weight).

(iii) Oral dosing. The drug solutions for oral dosing were prepared in a 0.5% methyl cellulose solution. The test animals were starved overnight before dosing with

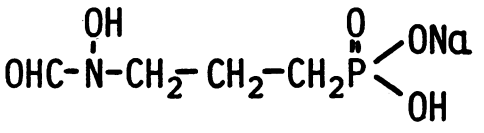


FIG. 1. Chemical structure of fosmidomycin.

100 mg/kg (10 ml/kg of body weight) to rats and 40 mg/kg (2 ml/kg of body weight) to dogs.

(iv) Concentrations in serum. Blood samples were collected by heart puncture from rats and from the antecubital vein of dogs. The concentrations of drug in serum were bioassayed; results were compared with those of standard solutions prepared with control serum of the respective species.

(v) Urinary excretion. Urine samples were collected at 0 to 3, 3 to 6, and 6 to 24 h after dosing from rats confined to a metabolism cage and from dogs in a metabolism cage or through a urinary catheter (or both). Urine samples were diluted with 0.067 M Tris-hydrochloride buffer (pH 7.0) to give suitable concentrations for bioassay. Standard solutions for the bioassay were prepared with 0.067 M Tris-hydrochloride buffer (pH 7.0).

(vi) Biliary excretion. For cannulation, rats in groups of 10 were anesthetized with 20 mg of pentobarbital per kg by the intraperitoneal route. The rats were confined in a supine position while a polyethylene cannula was inserted into the bile duct. Bile samples were collected at 0 to 3, 3 to 6, and 6 to 24 h after dosing. The drug concentrations in the bile were bioassayed with standard solutions prepared with Tris-hydrochloride buffer (pH 7.0).

(vii) Thin-layer chromatography-bioautography of urine and bile samples. Urine samples of rats and dogs and bile samples of rats were examined by thin-layer chromatography (7) by two development systems; (i) Eastman chromagram no. 6061 and a *n*-propanol–water (7:3) solvent system; and (ii) silicon-oil-pretreated Eastman chromagram no. 6061 with 0.5% silicon oil in ether and a 0.05 M Tris-hydrochloride buffer (pH 7.0) solvent system. Bioautography was then performed with *E. cloacae* ATCC 29893 as the test organism.

(viii) Tissue concentrations. Rats in groups of three were exsanguinated at specified intervals after dosing. The liver, kidneys, lungs, and heart were removed, washed with 0.9% saline, and blotted dry with filter paper. The organs from each group were pooled and homogenized with a Polytron homogenizer after the addition of 2 ml of 99% ethanol per g of tissue weight. The homogenates were centrifuged at 10,000 × *g* for 10 min. The drug concentrations in each supernatant were bioassayed with standard solutions prepared with aqueous ethanol solution (ethanol-water, 2:1). The extraction and analysis were performed in triplicate, and the values were averaged.

(ix) Peripheral lymph concentrations. The technique of Smith et al. (10) was used for sampling peripheral lymph. Lymph and blood were collected under anesthesia (20 mg of pentobarbital intraperitoneally per kg) at 0.25, 0.5, 1, 2, 3, 4, and 6 h after i.v. dosing. The drug concentrations in the lymph samples were bioassayed with standard solutions prepared from the control lymph.

(x) Exudate concentrations. For determination of exudate concentrations, rats in groups of 10 were used 6 days after the formation of granuloma pouches (described in a previous paper [8]). The drug concentrations in the samples of exudate were bioassayed and compared with standard solutions prepared from control exudates.

(xi) Determination of serum and tissue binding. A sample (0.5 ml) of the drug solution (300 μg/ml) in 0.05 M Tris-hydrochloride buffer (pH 7.0) was added to 4.5 ml of fresh serum and incubated at 37°C for 1 h. This mixture was placed in a Visking tube (size 8/32; Visking Co.) and centrifuged at 1,000 × *g* for 30 to 40 min to obtain about 0.3 ml of ultrafiltrate. The drug concentrations in the ultrafiltrates were bioassayed, and the degree of binding was calculated (9). Rat kidneys and liver were homogenized individually with a Polytron homogenizer to form a paste. A sample (0.5 ml) of drug solution (300 μg/ml) was added to 4.5 g of the paste and stirred well. After incubation for 1 h at 37°C, the mixture was treated in the same manner as the serum mixtures.

Volunteer study. Administration of fosmidomycin to healthy male volunteers and sampling of blood and urine were performed in phase I studies by N. Shephard, London, England and J. Arnold, Kansas City, Mo.

(i) Single-dose study. Fosmidomycin was injected into the forearm vein (i.v.) for 5 min at a dose of 30 mg/kg to 10 volunteers (mean body weight, 73.5 kg) or into the hip muscle (i.m.) at a dose of 7.5 mg/kg to 10 volunteers (mean body weight, 73.5 kg). Blood samples were collected from the forearm vein 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h after administration. In the oral study fosmidomycin was given in a single dose of 500 mg to five volunteers fasted overnight, and blood samples were collected 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after administration. In the i.v. and i.m. studies urine samples were taken 0 to 3, 3 to 6, and 6 to 24 h after dosing, in the oral study urine samples were taken at 0 to 2, 2 to 4, 4 to 6, 6 to 8, and 8 to 24 h after dosing.

(ii) Multiple-dose study. Fosmidomycin was injected in doses of 1 g into the hip muscle (i.m.) of six volunteers every 6 h for 21 consecutive injections. For the i.v. drip infusion study the drug was given to six volunteers in doses of 2 g every 6 h for 29 consecutive 0.5-h infusions. Serum samples were taken at the times shown in Fig. 5a and b, and urine was collected 0 to 6, 6 to 12, and 12 to 24 h after the first and last dosings. The samples were stored at −20°C and airfreighted frozen with dry ice to our research laboratories. The drug concentrations were bioassayed with standard solutions prepared with control human serum (Consera; Nissui, Tokyo) for serum samples and with 0.05 M Tris-hydrochloride buffer (pH 7.0) for urine samples. The active substance in the urine was examined by the method described above. The serum concentration-time data from volunteers and dogs were fitted to one- or two-compartment open models by using a NONLIN program with the aid of a FACOM 230/38 digital computer (Fujitsu Co., Ltd., Tokyo).

RESULTS

Serum concentrations. The concentrations of fosmidomycin in the serum of dogs are shown in Fig. 2. The mean drug concentration 0.25 h after i.v. dosing with 20 mg/kg was 54.8 μg/ml, and the mean half-life was 1.14 h. After i.m. dosing with 20 mg/kg and oral dosing with 40 mg/kg, the concentrations peaked at 41.4 μg/ml (0.25 h) and 16.6 μg/ml (2 h), respectively. The mean half-life was 1.01 h for i.m. injection and 1.99 h for oral dosing. The concentration-time equations for regression curves were as follows: i.v. injection,

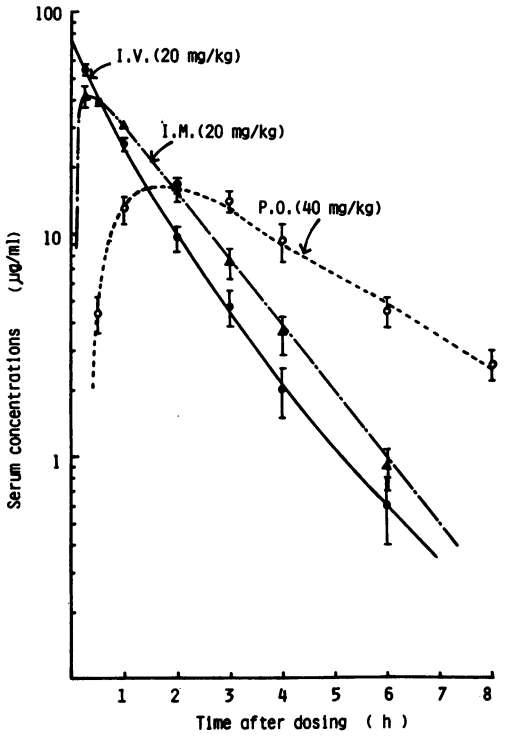


FIG. 2. Serum concentrations of fosmidomycin in dogs (male Beagles [n = 5], 8.6 to 14.0 kg).

$C = 50.9 \cdot e^{-1.39t} + 22.0 \cdot e^{-0.606t}$; i.m. injection, $C = 59.7(e^{-0.685t} - e^{-7.47t})$; oral dosing, $C = 34.9(e^{-0.348t} - e^{-1.36t})$. The areas under the serum level curves after i.v. and i.m. dosing (20 mg/kg) were similar, i.e., 72.9 and 79.1 μg.h/ml, whereas the area under the curve after oral dosing with 40 mg/kg was 74.7 μg.h/ml. These results indicate that the bioavailability of the drug in dogs after oral dosing was about half that after i.v. and i.m. dosing. The pharmacokinetic parameters are shown in Table 1.

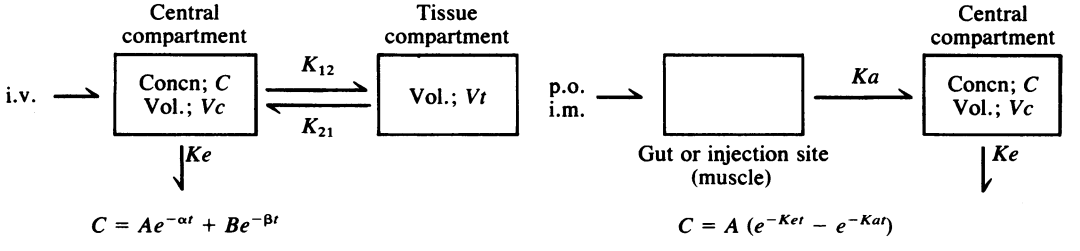
Urinary and biliary excretion. Urinary excretion of fosmidomycin in dogs after i.v., i.m., and oral dosing is shown in Fig. 3. More than 90% of the given dose was excreted in the urine in 24 h, and most of it was recovered in the first 3 h after parenteral dosing with 20 mg/kg. The recovery in the 24-h urine was 37.8% after a 40-mg/kg oral dose. The urinary recovery of the drug was well sustained, i.e., 11 to 13% of the given dose was recovered at each of the sampling times of 0 to 3 h, 3 to 6 h, and 6 to 24 h. In rats, the 24-h urinary recoveries of fosmidomycin after i.v. and oral dosing were 95.4 and 45.8%, respectively, of the given dose.

The recovery of fosmidomycin in the 24-h bile was extremely low, i.e., 0.1 and 0.07%, respectively, after i.v. (20 mg/kg) and oral (100 mg/kg) dosing.

Serum concentrations and urinary recovery in volunteers. (i) Single-dose study. After a single i.v. dose of 30 mg/kg (about 2 g per volunteer), the mean plasma concentrations were 157 μg/ml at 0.25 h, 65.2 μg/ml at 1 h, 28.3 μg/ml at 2 h, and 9.2 μg/ml at 4 h (Fig. 4). After a single i.m. dose of 7.5 mg/kg (about 500 mg per volunteer), the mean concentration peaked at 12.3 μg/ml 1 h after injection and gradually decreased to 4.4 μg/ml at 4 h and 0.8 μg/ml at 8 h (Fig. 4). After a single oral dose of 500 mg, the drug was moderately absorbed, and mean plasma concentrations peaked at 2.45 μg/ml 2 h after dosing and were well sustained (Fig. 4). These plasma concentration-time data were fitted to either a one- or two-compartment open model (Table 1). The serum half-life was 1.65 h by i.v. dosing, 1.58 h by i.m. dosing, and 1.87 h by oral dosing. The areas under the serum level curves were 210, 42.2, and 14.0 μg.h/ml after i.v. dosing with 30 mg/kg, i.m. dosing with 7.5 mg/kg, and oral dosing with 500 mg, respectively. Bioavailability by i.m. and oral dosing was 80.3 and 30%, respectively, of that by i.v. dosing on the basis of the area under the serum level curve.

Recovery of fosmidomycin in the 24-h urine was 85.5% for i.v. dosing, 66.4% for i.m. dosing, and 26.0% for oral dosing. The major portion of the drug was recovered in the first 3 h after i.v. dosing and in the first 6 h after i.m. dosing (Table 2).

No antimicrobial substances except fosmidomycin were detected in the urine samples by thin-layer chromatography-bioautography. The



above results indicate that the given drug was mainly excreted unchanged in the urine.

(ii) Multiple-dose study. The serum concentrations and urinary recovery were investigated in volunteers after 21 consecutive i.m. doses of 1 g every 6 h and 29 consecutive 0.5-h drip infusions of 2 g every 6 h. In the multiple-i.m. dose study, the mean serum concentrations peaked at 34.0 μg/ml after the first dose and at 35.5 μg/ml after the 21st dose. No differences in the serum level curves and in the serum half-lives were noted after the first and last dosings (Fig. 5a). The serum half-life was about 1.3 h. The recovery was 920 mg in the 6-h urine after the first dose and 979 mg in the 6-h urine after the last dose. In the multiple-drip infusion study, the serum concentration curves were almost the same after the first and last dosings. The mean concentration at the end of infusion was 125 μg/ml, and the serum half-life was 1.8 h after both doses (Fig. 5b).

The recoveries were, respectively, 1,720 and 2,080 mg in the 6-h urine after the first and last dosings. In the 24-h urine after the last dosing, 2,400 mg of the drug was recovered.

Fosmidomycin did not accumulate in the serum or urine of volunteers.

Tissue concentrations in rats. The tissue distribution of fosmidomycin was investigated in rats after i.v. (20 mg/kg) and oral (100 mg/kg) dosing (Fig. 6). After i.v. injection, fosmidomycin was well distributed to all of the tissue tested. The concentration 0.25 h after injection was the highest in the kidneys (406 μg/g), followed by the lungs (15.6 μg/g), heart (8.3 μg/g), liver (7.5 μg/g), and spleen (4.3 μg/g). The serum concentrations during the first 1 h were higher than all of the tissue concentrations except the kidneys. The elimination rate of the drug in the tissues was similar to that in the serum, except the liver and spleen, in which the drug concentrations were sustained for a longer time than in the serum. A similar order of antibiotic levels in the tissues and serum was seen after oral dosing. In the first 2 h after dosing the drug concentrations in the tissues, except the kidneys, were lower than in the serum, and the kidney concentration was about 10 times higher than the serum concentration. Tissue concentrations were sustained longer after oral dosing than after i.v. dosing.

Peripheral lymph and exudate concentrations. The concentrations of fosmidomycin in the peripheral lymph in dogs after i.v. dosing with 50 mg/kg are shown in Fig. 7. Peripheral lymph concentrations of fosmidomycin were 112 μg/ml at 0.25 h, 63.1 μg/ml at 1 h, 15.9 μg/ml at 3 h, and 5.4 μg/ml at 6 h. In the first 2 h after injection, the lymph concentrations were higher than the serum concentrations, and thereafter both concentrations were almost the same (Fig. 7a). The

TABLE 1. Pharmacokinetic parameters of fosmidomycin[a]

Species	Route of injection	A (μg/ml)	B (μg/ml)	α (h^{-1})	β (h^{-1})	Half-life (h)	AUC (μg.h/ml)	K_{12} (h^{-1})	K_{21} (h^{-1})	Ke (h^{-1})	$K\alpha$ (h^{-1})	Vc (ml/kg or body)	Vf (ml/kg)	Body clearance (ml/min)	Peak concn (μg/ml)	Peak concn h
Dogs	i.v.	50.9	22.0	1.39	0.606	1.14	72.9	0.154	0.843	1.00		274	50.0	274		
	i.m.	59.7				1.01	79.1			0.685	7.47	369			42.6	0.352
	Oral	34.9				1.99	74.7			0.348	1.36	1,540			16.3	1.35
Humans	i.v.	146	47.2	1.50	0.42	1.65	210	0.315	0.684	0.921		155	71.4	175		
	i.m.	26.1				1.58	42.2			0.438	1.50	406			11.1	1.16
	Oral	14.5				1.87	14.0			0.370	0.575	96,700			2.33	2.36

[a] Column headings refer to the following diagram:

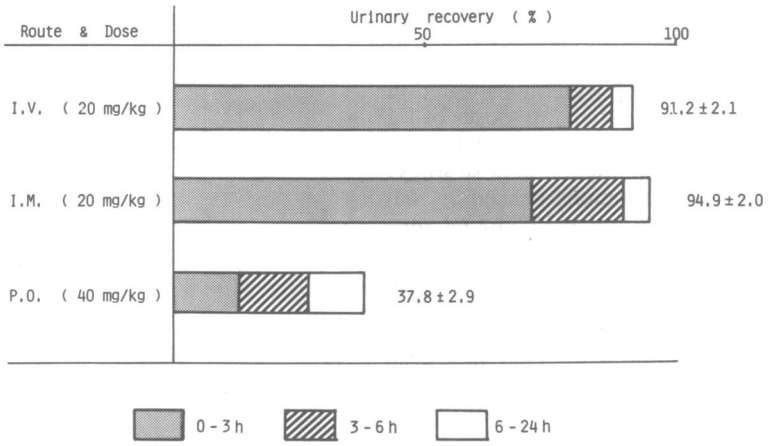


FIG. 3. Urinary recovery of fosmidomycin in dogs (male Beagles [n = 5], 8.6 to 14.0 kg) after parenteral and oral dosing.

penetration of fosmidomycin into the exudate of rat granuloma pouch was investigated after i.m. dosing with 20 mg/kg. The exudate concentrations peaked at 7.9 μg/ml 1 h after injection and were 2.9 μg/ml at 3 h (Fig. 7b). The elimination rate of the drug was slower in the exudate than in the serum. In the first 1.5 h after injection, the exudate concentrations were lower than the serum concentrations, but thereafter the exudate concentrations were more prolonged and higher than the serum concentrations. The area under the concentration curve ratio (exudate/serum) was about 50%.

Serum and tissue binding. Serum protein and tissue binding of fosmidomycin were very low. Serum protein binding was 3 to 4% to dog and mouse serum and 1% or less to human, rabbit, and rat serum. The drug was bound at a low extent of 2% or less to 90% rat liver and kidney homogenates. These binding rates did not change at concentrations of 30 to 300 μg/ml.

DISCUSSION

The results of the previous studies on a new phosphonic acid antibiotic, fosmidomycin, suggest that this antibiotic is effective in mice against infection due to organisms sensitive to

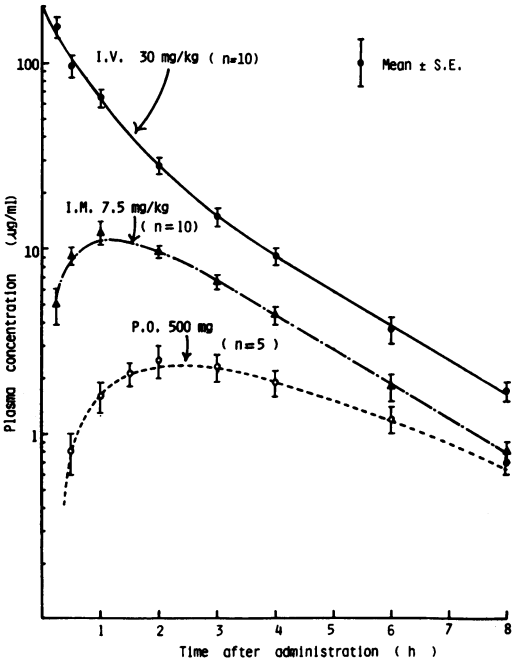


FIG. 4. Plasma concentrations of fosmidomycin in healthy volunteers after a single dose.

TABLE 2. Urinary recovery of fosmidomycin in healthy volunteers

Route of injection	Dose	No. of volunteers	Urinary recovery (mean ± SE) at the following times						
			0 to 3 h		3 to 6 h		6 to 24 h		0 to
			μg/ml	%	μg/ml	%	μg/ml	%	μg/ml
i.v.	30 mg/kg	10	3,960 ± 737	72.8 ± 3.6	980 ± 190	8.4 ± 0.9	103 ± 19.2	4.3 ± 0.4	
i.m.	7.5 mg/kg	10	526 ± 125	38.7 ± 4.2	562 ± 86.9	22.3 ± 4.1	36.7 ± 9.6	5.4 ± 1.0	
Oral	500 mg	5							83.0 ± 26.8

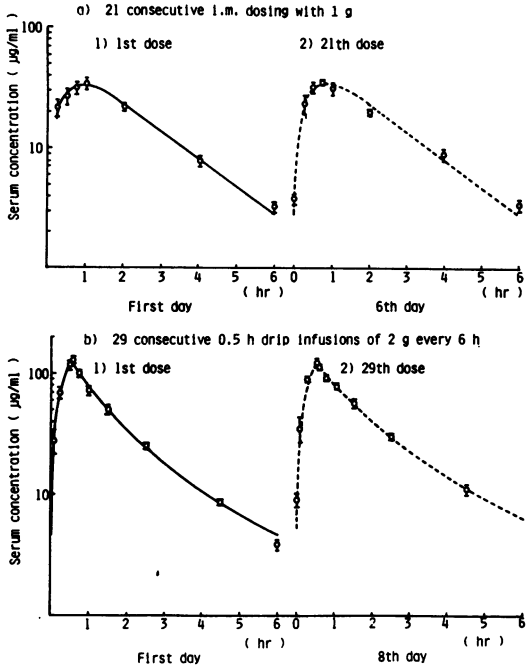


FIG. 5. Serum concentrations of fosmidomycin in healthy volunteers after multiple doses.

the drug (6). In the present pharmacokinetic study, fosmidomycin was well absorbed orally in animals and humans. Fosmidomycin was not metabolized and was excreted unchanged in the urine as a bioactive substance. The urinary recovery in volunteers was 85% for i.v. dosing and 66% for i.m. dosing. The pharmacokinetics of two other phosphonic acid-containing antibiotics, fosfomycin and alafosfalin, have already been reported. Allen et al. (1) showed that alafosfalin was well absorbed in volunteers after both oral and i.m. dosing, but was unstable in the human body; the urinary recovery of the unchanged drug was 4%, and that of its metabolite was 51%. Kirby (3) reported that fosfomycin accumulated in small amounts in the serum of volunteers after multiple dosing. In our experiments with rats, the concentrations of fosmidomycin in the liver and spleen were sustained longer than in the serum after i.v. dosing with 20 mg/kg, and the concentrations in the serum and tissues were sustained considerably longer after oral dosing with 100 mg/kg than after i.v. dosing. These results suggest that fosmidomycin may accumulate to some extent in human serum. In our multiple-dose study of fosmidomycin in volunteers, however, the drug did not accumulate in the serum after 21 consecutive i.m. doses of 1

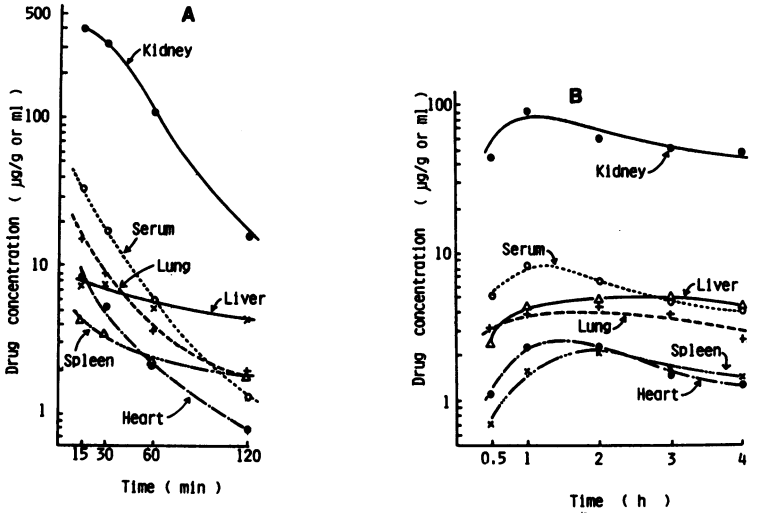


FIG. 6. Tissue distribution of fosmidomycin in rats (JCL SD strain, male, 6 weeks old, $n = 3$). (A) i.v. dosing with 20 mg/kg; (B) oral dosing with 100 mg/kg.

TABLE 2—*Continued*

2 h	2 to 4 h		4 to 6 h		6 to 8 h		8 to 24 h		Total (%)
%	μg/ml	%	μg/ml	%	μg/ml	%	μg/ml	%	
									85.5 ± 3.3
									66.4 ± 4.1
5.2 ± 1.1	312 ± 121	9.0 ± 1.6	274 ± 121	5.5 ± 1.0	92.8 ± 18.6	3.0 ± 0.6	20.5 ± 5.0	3.4 ± 0.4	26.0 ± 3.9

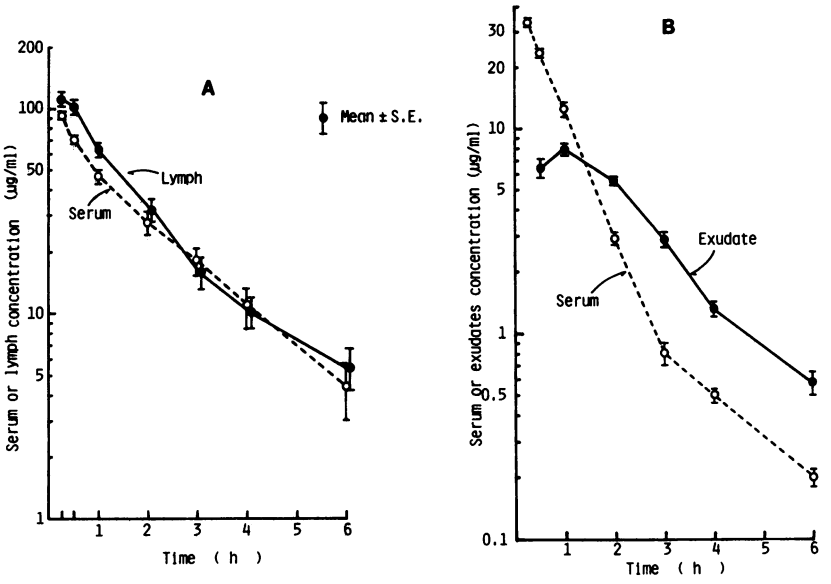


FIG. 7. Mean peripheral lymph and exudate concentrations of fosmidomycin. (A) Peripheral lymph in dogs (male Mongrels [n = 5], 10 to 16 kg). Dose, 50 mg/kg; route, i.v. (B) Granuloma pouch exudate in rats (JCL SD strain, male, 6 weeks old, n = 10). Dose, 20 mg/kg; route, i.m.

g every 6 h or 29 consecutive 0.5-h drip infusions of 2 g every 6 h. It was found that oral absorption was slow and moderate and was almost the same as that of fosfomycin. Although the clinical efficacy of fosfomycin after oral dosing was good (2, 5), it is of special interest to evaluate the therapeutic effect of fosmidomycin because its in vitro and in vivo activities are superior to those of fosfomycin.

LITERATURE CITED

1. **Allen, J. G., L. Havas, E. Leicht, I. Lenox-Smith, and L. J. Nisbet.** 1979. Phosphonopeptides as antibacterial agents: metabolism and pharmacokinetics of alafosfalin in animals and humans. Antimicrob. Agents Chemother. 16:306–313.
2. **Allona, A., J. A. D. Cabrera, and P. Manchado.** 1977. Fosfomycin in chronic urinary infections. Chemotherapy 23(Suppl. 1):267–274.
3. **Kirby, W. M.** 1977. Pharmacokinetics of fosfomycin. Chemotherapy 23(Suppl. 1):141–151.
4. **Kojo, H., Y. Shigi, and M. Nishida.** 1980. FR 31564, a new phosphonic acid antibiotic. Bacterial resistance and membrane permeability. J. Antibiot. 33:44–48.
5. **Lagunero, R. L., and M. L. Menéndez.** 1977. Urinary tract infections treated with fosfomycin. Chemotherapy 23(Suppl. 1):302
6. **Mine, Y., T. Kamimura, S. Nonoyama, M. Nishida, S. Goto, and S. Kuwahara.** 1980. In vitro and in vivo antibacterial activities of FR 31564, a new phosphonic acid antibiotic. J. Antibiot. 33:36–43.
7. **Murakawa, T., Y. Wakai, M. Nishida, R. Fujii, M. Konno, K. Okada, S. Goto, and S. Kuwahara.** 1970. Chromatographic assay of mixed penicillins, ampicillin and cloxacillin, in body fluids. J. Antibiot. 23:250–251.
8. **Nishida, M., and T. Murakawa.** 1977. Exudate levels and bactericidal activity of cefazolin in a new local infection system using rat granuloma pouches. Antimicrob. Agents Chemother. 11:1042–1048.
9. **Nishida, M., T. Murakawa, T. Kamimura, N. Okada, H. Sakamoto, S. Fukada, S. Nakamoto, Y. Yokota, and K. Miki.** 1976. In vitro and in vivo evaluation of ceftezole, a new cephalosporin derivative. Antimicrob. Agents Chemother. 10:1–13.
10. **Smith, J. R., E. F. Dunton, J. M. Protas, T. G. Blocker, Jr., R. Cooley, and S. R. Lewis.** 1959. Canulation of the lymphatics of the lower extremity. Surg. Forum 9:811–814.