0.03). The larger C_{max} and shorter t_{max} with the capsules *versus* tablets indicate a more rapid absorption rate of the capsule dosage form. Our findings also confirm the results of previous comparative studies, which demonstrate the equivalence of bioavailability of the 0.2-mg capsule and 0.25-mg tablet doses (14, 15).

While no significant differences in the amount of digoxin absorbed were noted based on CUE_{48}, it is interesting to note from Fig. 3 that a smaller *CV* is seen with the capsule than the tablet for both the 240 mL (9.5 *versus* 13.7%) and the 30 mL (13.7 *versus* 17.0%) volumes of water.

Digoxin bioavailability studies (15–20) have used different coadministered volumes of water (100–240 mL). If the results of our study had indicated a difference in bioavailability with different volumes of water, some of the findings of these studies could be questioned. However, since we found no difference in bioavailability with a small *versus* a large amount of fluid, this variable does not have an important influence on digoxin absorption in normal individuals.

A review of drug solubility and volume-related variability in drug absorption is interesting. Table V presents a comparison of the solubilities and doses of drugs that have been administered in previous studies with designs similar to the present study. These data show that concerns over alterations in bioavailability of a drug due to coadministered fluids should not only take into account the solubility of the drug, but also the dose of the respective drug administered. A case in point is digoxin with a solubility of 0.095 mg/mL, one of the lowest values listed. However, because the therapeutic dose is small, only a small volume of water is theoretically required to solubilize the dose in the GI tract.

The results of this study indicate that, in normal volunteers, there are no differences in total digoxin absorption (for either the tablet or capsule forms), when digoxin is administered with relatively small or large volumes of fluid. Such pharmaceutical information is useful in identifying drugs or drug preparations which may or may not be influenced by differences in coadministered fluid volume.

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Pharmacokinetics of Fostedil, a New Calcium Antagonist, in Beagle Dogs Following Oral and Intravenous Administration

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Received August 8, 1983, from the *Drug Metabolism Department, Pharmaceutical Products Division, Abbott Laboratories, North Chicago, IL 60064.* Accepted for publication November 28, 1983.

Abstract □ Twelve adult beagle dogs received both an oral and intravenous dose (12 mg/kg) of fostedil in a cross-over design. The plasma levels and urinary excretion of intact fostedil were measured, and the pharmacokinetic parameters of the drug were defined. The results indicate that fostedil was at least 68% bioavailable after an oral dose given as a suspension or solution. The terminal half-life was about 7–8 h. The *in vitro* protein binding, at concentrations of 0.1–100 μg/mL, ranged from 95 to 97%. The binding was not concentration dependent, and saturation of the binding sites was not apparent at concentrations up to 100 μg/mL. Excretion of unchanged drug from the kidneys accounted for only a small percentage of drug clearance.

Keyphrases □ Fostedil—pharmacokinetics, dogs □ Pharmacokinetics—fostedil, dogs □ Blocking agent—calcium entry, fostedil, pharmacokinetics, dogs

Fostedil (I) (diethyl{[4-(2-benzothiazolyl)phenyl]methyl}-phosphonate) is a new calcium entry blocking compound[1] (1–4). This study was designed to determine the pharmacokinetics of fostedil in beagle dogs after intravenous and oral administration.

[1] This compound is being studied under a joint license agreement between Abbott Laboratories, North Chicago, Ill., and Kanebo, Ltd., Japan, and has been reported in the literature as KB-944.

EXPERIMENTAL SECTION

Animals—Five male and seven female adult beagle dogs (8.5–13.0 kg) were randomly divided into three groups of four dogs per group. The groups were dosed at 12 mg/kg, once with an oral solution, an oral suspension, an oral

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Table I—Pharmacokinetics of Fostedil in Beagle Dogs Following Intravenous Administration (12 mg/kg)

Dog No.	K_{21}, h^{-1}	K_{12}, h^{-1}	K_{net}, h^{-1}	Beta, h^{-1}	Half-life, h	Vd_1, L/kg	AUC, μg·h/mL	CL_t, L/h/kg
81121	0.291	0.267	0.412	0.145	4.78	1.28	22.80	0.53
81122	0.455	0.597	0.224	0.086	8.06	3.47	15.44	0.78
81128	0.193	0.381	0.386	0.085	8.15	0.76	40.92	0.29
81147	0.454	1.139	0.617	0.135	5.13	0.62	31.26	0.38
81167	0.252	0.519	0.308	0.078	8.88	1.04	37.42	0.32
81170	0.224	0.296	0.430	0.115	6.03	1.17	23.82	0.82
81174	0.302	0.697	0.420	0.096	7.22	1.57	18.24	0.66
81176	0.281	2.332	1.978	0.125	5.54	0.18	34.53	0.36
81179	0.306	0.454	0.501	0.136	5.10	1.13	21.31	0.57
81184	0.214	0.339	0.539	0.118	5.87	0.99	22.54	0.53
82505	0.174	0.213	0.307	0.088	7.88	1.82	21.52	0.56
82506	0.125	0.110	0.370	0.090	7.70	1.56	20.79	0.58
Mean	0.273	0.612	0.541	0.108	6.42[a]	1.30	25.88	0.53
SD	0.101	0.606	0.456	0.024		0.82	8.10	0.17

[a] Calculated from the mean beta rate constant.

Table II—Pharmacokinetics of Fostedil in Beagle Dogs Following Oral Solution Administration (12 mg/kg)

Dog No.	K_a, h^{-1}	Beta, h^{-1}	Half-life, h	AUC, μg·h/mL	F, %	T_{max}, h	C_{max}, μg/mL
81121	4.22	0.033	21.00	21.42	94.0	0.99	1.81
81122	2.83	0.097	7.14	15.16	98.2	0.95	2.21
81128	2.22	0.054	12.83	21.20	51.8	1.10	2.41
81147	1.58	0.155	4.47	13.05	41.8	1.10	2.45
81167	0.91	0.061	11.36	22.50	60.1	1.67	2.13
81170	18.12	0.067	10.34	18.35	77.0	0.25	3.20
81174	1.27	0.075	9.24	15.03	82.4	1.00	2.06
81176	0.68	0.065	10.66	15.43	44.7	1.82	1.83
81179	1.85	0.092	7.53	20.92	98.2	0.87	3.29
81184	17.68	0.073	9.49	13.36	59.3	0.23	2.72
82505	2.12	0.113	6.13	14.20	66.0	0.64	2.14
82506	0.36	0.104	6.66	17.40	83.7	3.05	1.74
Mean	4.49	0.082	8.45[a]	17.33	71.4	1.14	2.33
SD	6.35	0.032		3.44	20.3	0.76	0.51

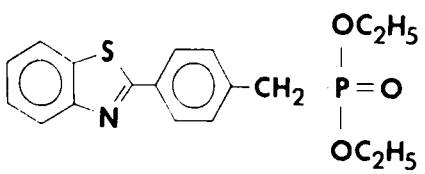
[a] Calculated from the mean beta rate constant.

capsule, and an intravenous formulation. At ~2-week intervals, the three groups were crossed-over until each group had received each treatment once. The dogs were fasted for at least 12 h prior to dosing and for at least 5 h following dosing. They were housed individually in stainless steel metabolism cages and given water *ad libitum*.

Treatment—The oral and intravenous solution formulations were prepared as 24 mg of fostedil per mL in ethanol–propylene glycol–water (10:60:30, v/v/v). The suspension formulation was prepared as 24 mg/mL in 0.2% hydroxypropyl methylcellulose–water. The oral solution and suspension were administered by gavage, 0.5 mL/kg to each animal. The intravenous solution was administered intravenously as a bolus (0.5 mL/kg) into the right cephalic vein to provide a dose of 12 mg/kg. For the capsule dosing, the drug was weighed into clear gelatin capsules[2] and administered orally with 10 mL of water.

Heparinized blood samples (2–5 mL) were collected from the jugular vein at 0, 0.1 (intravenous route only), 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, 26, 28, and 32 h postdose. The plasma was harvested and stored frozen. If possible, urine was collected over the following intervals: 0–4, 4–8, 8–12, 12–24, 24–28, and 28–32 h postdose. The volumes were measured and recorded, and a 5-mL aliquot was stored frozen.

Assay of Fostedil in Plasma and Urine—Measurement of plasma and urine concentrations of fostedil was performed by using an HPLC fluorescence assay (5). The procedure was shown to be sensitive (5 ng/mL) and specific for the intact drug.



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[2] No. 0.

Pharmacokinetic Analysis—The individual and mean plasma concentrations *versus* time data were subjected to sequential polyexponential regression analyses using STRIP, a slightly modified version of the computer program CSTRIP (6). Based on the criteria outlined by Sedman and Wagner (7), a triexponential model (*e.g.*, a two-compartment open model) was deemed more appropriate for the intravenous, suspension, and oral solution data. A biexponential model (*e.g.*, a one-compartment open model) was chosen for the capsule dosing data.

The data were fitted with NONLIN (8), using appropriate DFUNC subroutines. For the one-compartment model, the parameters on which iterations were performed included: K_a (apparent input rate constant), K_{net} (apparent net elimination rate constant), Vd (volume of distribution), and the absorption lag time. For the two-compartment model, the estimated preexponential and exponential terms were used to calculate the initial estimates of the microscopic rate constants by using the assumptions and equation described by Gibaldi and Perrier (9). Iterations by NONLIN were then performed on the following parameters: K_a, K_{net}, K_{12}, K_{21}, and Vd_1. During the fitting process, the data were weighted by reciprocal squared concentrations ($1/C^2$) (10).

Protein Binding—Fostedil, labeled with carbon-14 in the 2-position of the benzothiazolyl ring, was used in the *in vitro* protein binding studies. The binding of fostedil (concentrations, 0.1–100 μg/mL) was determined in the plasma of male and female beagle dogs by the ultrafiltration technique described by Rolinson and Sutherland (11). The ultrafiltrates and plasma were oxidized, assayed to determine levels of radioactivity, and corrected for quenching by automatic external standardization.

RESULTS AND DISCUSSION

Single-Dose Intravenous Study—A summary of the pharmacokinetic parameters as derived by NONLIN from the intravenous data is presented in Table I. The total body clearance ranged from 0.5 to 0.8 L/h/kg, with a mean value of 0.53 L/h/kg. The beta half-life ranged from 4.8 to 8.9 h, with a mean value of 6.4 h.

Single-Dose Oral Studies—The calculated pharmacokinetic parameters derived by NONLIN for the oral solution, suspension, and capsule dosing data

Table III—Pharmacokinetics of Fostedil in Beagle Dogs Following Suspension Administration (12 mg/kg)

Dog No.	K_a, h^{-1}	Beta, h^{-1}	Half-life, h	AUC, μg·h/mL	F, %	T_{max}, h	C_{max}, μg/mL
81121	2.79	0.137	5.06	13.41	58.8	1.15	1.57
81122	1.45	0.081	8.56	13.45	87.1	1.82	1.58
81128	1.16	0.031	22.35	27.00	66.0	1.18	1.42
81147	1.57	0.088	7.88	17.50	56.0	1.43	2.16
81167	0.54	0.059	11.75	25.58	68.4	2.25	2.24
81170	1.65	0.024	28.88	19.95	83.8	1.60	2.18
81174	3.24	0.095	7.29	12.03	66.0	1.05	1.25
81176	2.93	0.020	34.65	22.06	63.9	1.07	1.98
81179	2.12	0.120	5.78	12.95	60.8	1.47	1.31
81184	1.29	0.083	8.35	13.84	61.4	1.42	1.77
82505	0.54	0.104	6.66	10.10	46.9	2.68	1.11
82506	2.79	0.110	6.30	9.27	44.6	1.30	1.10
Mean	1.84	0.079	8.77[a]	16.43	63.6	1.53	1.64
SD	0.93	0.038		5.94	12.5	0.50	0.42

[a] Calculated from the mean beta rate constant.

Table IV—Pharmacokinetics of Fostedil in Beagle Dogs Following Capsule Administration (12 mg/kg)

Dog No.	K_a, h^{-1}	K_{net}, h^{-1}	Half-life, h	AUC, μg·h/mL	F, %	T_{max}, h	C_{max}, μg/mL
81121	0.70	0.088	7.88	14.08	61.8	3.62	0.92
81122	1.46	0.136	5.10	6.14	39.8	2.03	0.66
81128	0.13	0.138	5.02	5.89	14.4	7.70	0.29
81147	1.10	0.095	7.29	9.56	30.6	2.68	0.72
81167	10.59	0.099	7.00	11.48	30.7	0.91	1.09
81170	0.60	0.121	5.73	9.86	41.4	3.77	0.80
81174	1.76	0.091	7.62	14.16	77.6	1.78	1.09
81176	1.36	0.076	9.12	10.76	31.2	2.48	0.69
81179	0.16	0.161	4.30	8.10	38.0	6.42	0.48
81184	0.79	0.076	9.12	8.46	37.5	3.49	0.50
82505	0.72	0.122	5.68	15.15	70.4	3.17	1.29
82506	3.08	0.138	5.02	6.98	33.6	1.29	0.84
Mean	1.87	0.112	6.19[a]	10.05	42.24	3.28	0.78
SD	2.86	0.028		3.17	18.4	2.00	0.29

[a] Calculated from the mean K_{net} rate constant.

are listed Tables II–IV, respectively. Figure 1 is a semilogarithmic concentration-time plot of the mean data for each oral and intravenous formulation with the NONLIN generated best-fit curve. As expected for a compound with limited water solubility like fostedil, the absorption kinetics became more complex in progressing from an oral solution to a capsule formulation. Figure 1 illustrates this change, as the two-compartment model disposition kinetics, so distinct after intravenous administration, became less pronounced after oral administration. Indeed, following capsule dosing the data were best described by a one-compartment model, since the distribution phase was completely masked by the complex absorption phase. In the fits for some of the animals, the calculated apparent K_a value approached that of alpha as determined after intravenous administration. Thus, it was impossible to accurately determine the true absorption rate constant. Only an estimated apparent absorption half-life of ~15 min is reported.

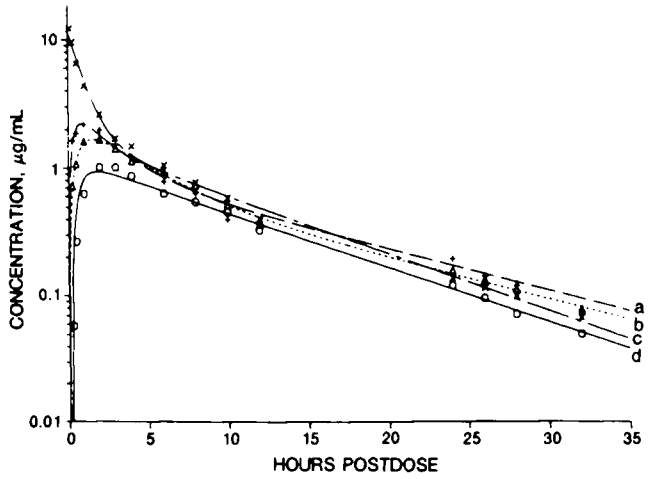

Figure 1—*Mean postdose fostedil concentration* versus *time. Key: (a) oral solution; (b) suspension; (c) intravenous administration; (d) capsule administration of fostedil (12 mg/kg) with NONLIN-generated best-fit curves.*

Studies with radiolabeled drug in rats and dogs have demonstrated the presence of extensive biliary secretion of fostedil and/or its metabolites, suggesting possible enterohepatic circulation[3]. Meal-related secondary peaks in the plasma profiles were observed for many animals in this study. This, in some cases, adversely affected the kinetic analyses. For two of the oral formulations, the mean elimination rate constants were found to be statistically significantly different ($p = 0.015$). However, these differences were considered to be of minor practical importance since the differences could be traced to data from animals displaying unusual plasma drug level profiles. In these cases, the model was inadequate to describe the possible enterohepatic circulation and the complex absorption phenomenon. Thus, the average beta half-life following oral dosing was 7.6 h.

The mean calculated C_{max} values for the oral formulations were: capsule, 0.78 μg/mL; suspension, 1.64 μg/mL, and oral solution, 2.33 μg/mL. An analysis of variance and the Duncan Multiple Range test showed all values to be significantly different from one another.

Since the same dose (12 mg/kg) was administered both orally and intravenously, the systemic availability (F) was calculated as the percent ratio of $(AUC_{0-\infty})$ oral/$(AUC_{0-\infty})$ intravenous (12). The individual F values for these animals following oral dosing are given in Tables II–IV. The mean F values were: oral solution, 71.4%; suspension, 63.6%; capsule, 42.2%. The $AUC_{0-\infty}$ values for the oral solution and suspension formulations were not statistically different ($p = 0.23$) but were greater than those for the capsule dosing. However, the results of another study in this laboratory have suggested that the low bioavailability might have been due to the type of capsule used[4].

Urinary excretion of unchanged drug for both the oral and intravenous dosings was less than 1% of the administered dose over the 32-h collection period.

Protein Binding Studies—The *in vitro* protein binding of [^{14}C]fostedil at

[3] B. A. Bopp, unpublished results.

[4] E. W. Thomas, unpublished results.

concentrations of 0.1–100 μg/mL ranged from 95–97% in dog plasma, and the binding appeared to be similar in the plasma from the male and the female dogs. The binding was not concentration dependent, and saturation of the binding sites was not apparent at concentrations up to 100 μg/mL.

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Inhibition of Human Polymorphonuclear Leukocyte Cell Responses by Ibuprofen

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Received September 12, 1983, from the *Medical Research Laboratory, Department of Medicine, Hartford Hospital, Hartford, CT 06115.* Accepted for publication November 19, 1983.

Abstract □ The stimulation of cell swelling, cell aggregation, polymorphonuclear leukocyte locomotion, and lysosomal enzyme release in response to chemoattractant were all inhibited by ibuprofen, a nonsteroidal anti-inflammatory agent. The dosages needed to induce 50% inhibition (ID_{50}) were 5.9, 7.6, 60, and 95 μg/mL, respectively. Aside from the differences in ID_{50}, there was also a difference in the degree of maximum inhibition (I_{max}) of the complement C5a-stimulated responses observed, so that at achievable serum drug concentrations of 20–50 μg/mL, inhibition of 67–78% for cell swelling, 69–82% for cell aggregation, 20–35% for migration response, and 17–38% for lysosomal enzyme release were demonstrated. Also observed were a minor stimulatory effect on nitroblue tetrazolium reduction and an inhibitory effect on the ability to kill *Staphylococcus aureus*, but only at very high concentrations (~2 mg/mL).

Keyphrases □ Ibuprofen—inhibition of polymorphonuclear leukocyte cell responses □ Polymorphonuclear leukocytes—inhibition of response, ibuprofen □ Anti-inflammatory agents—ibuprofen, inhibition of polymorphonuclear leukocytes

Although the acute inflammatory reaction is usually the combined result of vascular, humoral, and cellular responses, there is considerable evidence that the contribution of the polymorphonuclear leukocyte[1] is indispensable (1). This is derived from studies showing inhibition of experimental inflammatory response in PMN-depleted animals, the presence of lysosomal enzymes in inflammatory exudates in humans, and the production of tissue damage in experimental animals by PMN lysosomal lysates. Moreover, one mechanism of action of the effects of a variety of anti-inflammatory drugs, which includes corticosteroids and colchicine, is their ability to inhibit the release of lysosomal enzymes from PMN. Consequently, it is important to determine whether nonsteroidal anti-inflammatory agents, in addition to their inhibitory activity on prostaglandin synthesis (2), also have direct inhibitory effects on PMN function. Although the inhibitory effect of these drugs on PMN migration has been shown previously (3–5), a systematic study to quantitate the effects on this and other PMN responses is lacking. Therefore, we studied and quantitated the effects of the anti-inflammatory drug ibuprofen on PMN responses.

EXPERIMENTAL SECTION

Preparation of Leukocytes and Serum—Blood for these studies was obtained from healthy male and female laboratory and hospital personnel. Polymorphonuclear cells were prepared from heparinized (50 U of preservative-free sodium heparin per mL of blood) whole blood by sedimenting erythrocytes with 6% hetastarch–whole blood at a ratio of 1:5. The leukocyte-rich plasma was washed once with a commercially obtained medium[2] at pH 7.4 and centrifuged at 500×*g*. The cell pellet was resuspended in the same medium, and the PMN concentration was adjusted to 5×10^6 PMNs/mL. This preparation was used for PMN locomotion and bactericidal assays. For leukocyte aggregation, swelling, and lysosomal enzyme release assays, the leukocyte pellet was prepared with Hank's balanced salt solution[1]. To lyse contaminating erythrocytes, the cells were resuspended in ice-cold 0.15 M NH_4Cl (pH 7.5) for 10 min, with five 2-min interval inversions, followed by another wash with HBSS. The final cell pellet was resuspended in HBSS to a cell concentration of 10^7 PMNs/mL. Serum was prepared from clotted whole blood.

Chemotactic Factor—The chemotactic factor used in PMN locomotion studies was derived from zymosan-activated serum prepared by incubation of 5 mg of zymosan with 1 mL of serum at 37°C for 30 min. A 3% solution of this material in the medium[2] was used in the lower compartment.

Partially purified complement C5a fragments were used as the chemotactic factor in leukocyte aggregation, swelling, and lysosomal enzyme release studies. C5a was derived by the activation of normal serum with zymosan in the presence of 1 M ε-aminocaproic acid[3] and purified by gel filtration[4] (6). Active fractions were identified by the lysosomal enzyme release assay, lyophilized, and then dialyzed against 0.1 M phosphate buffer (pH 7.4), and

[1] Polymorphonuclear leukocyte will be abbreviated throughout the text as PMN; Hank's balanced salt solution will be abbreviated as HBSS.

[2] Medium 199; Difco Laboratories, Detroit, Mich.

[3] Sigma Chemical Co., St. Louis, Mo.

[4] Sephadex G-100; Pharmacia Fine Chemicals, Piscataway, N.J.

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