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Report

Hydralazine Pharmacokinetics and Interaction with Food: An Evaluation of the Dog as an Animal Model

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The intravenous and oral dose-dependent pharmacokinetics of hydralazine and the effect of concurrent administration of food with hydralazine in dogs were evaluated for comparison with published human data. Four dogs were given intravenous and oral doses of hydralazine at 0.25, 1.0, 2.5, and 4.0 mg/kg. In addition, the oral 2.5 mg/kg dose was given with a meal. Blood samples were collected at appropriate intervals and analyzed for hydralazine. Pharmacokinetic analysis showed that AUC_{oral}/dose (5552 to 13218 mg-min/ml) and F (0.36 to 0.77) increased significantly with dose, indicating saturation of first-pass metabolism, as is seen in humans. Total-body clearance (70 ml/min/kg) and steady-state volume of distribution (9 L/kg) were similar to human values. The bioavailability of hydralazine in the dog was decreased by 63% when the dose was given with a meal, which is comparable to some human data. It was concluded that the dog may be a useful model in which to study mechanisms of the hydralazine–food interaction.

KEY WORDS: hydralazine; food–drug interaction; Michaelis–Menten kinetics; bioavailability; pharmacokinetics; dog; animal model.

INTRODUCTION

Hydralazine has been used as a peripheral vasodilator in humans for many years but extreme variability in oral dose requirements has limited its usefulness (1). Its disposition is partly dependent on the acetylator phenotype (1–3), but much of the variability in oral dosage requirements may be mediated by other factors influencing the first-pass effect, such as splanchnic blood flow, protein binding, and hepatic/intestinal metabolic capacity. Contributing to this variability may be an interaction with food. Melander *et al.* (4) originally reported data which suggested that the bioavailability of hydralazine was increased when coadministered with food. Subsequently, however, Shepherd *et al.*, using a more specific assay, reported a 46% decrease in hydralazine AUC when taken 45 min after a meal (5) and, in another study, a 33% decrease when hydralazine was consumed immediately after a meal (6). They pointed out that the assay employed by Melander *et al.* (4) was susceptible to interference by hydralazine pyruvic acid hydrazone, an important metabolite which hydrolyzes to produce free hydralazine under the acidic conditions employed for derivatization. Studies in humans to elucidate the mechanisms of the food–hydralazine interaction would be difficult to perform so an animal model would be useful.

The dog has been developed successfully as a model for first-pass propranolol elimination using invasive techniques, and the effect of hydralazine on propranolol disposition has been studied (7,8). The dog possesses additional characteristics which make it an attractive species in which to study hydralazine pharmacokinetics. An early study using a nonspecific assay found the pattern of absorption and elimination in dogs to be similar to that in humans (9). In the same study, the binding of radiolabeled hydralazine to plasma was found to be slightly lower in dogs than in humans (71 vs 87%), and the partitioning into red cells was slightly higher in dogs than in humans (0.37 vs 0.25). The pharmacodynamic effect in dogs is similar to that in humans, with peripheral vasodilation causing reduced cardiac afterload (10), and hydralazine has been used to treat chronic left heart failure in dogs (10–12). Hepatic blood flow after hydralazine administration is increased in the dog in a qualitatively similar pattern to that in humans (5,7,13).

While the dog is reputed to be a poor acetylator of aromatic amines, poor acetylation ability can be an advantage in studying other mechanisms of hydralazine elimination (14). The value of this approach has been recognized by Ludden *et al.* (15), who recently published a hydralazine bioavailability study in which only slow acetylating humans were evaluated in order to decrease intersubject variability in kinetic parameters. Little is known about the kinetics of hydralazine in the dog, however, and more specific information is required to evaluate the dog's usefulness as an animal model.

The study described here was designed to provide basic intravenous and oral pharmacokinetic data on hydralazine in the dog for comparison with human data. Different doses were used to determine if dose-dependent elimination occurs after oral hydralazine administration, as has been observed in humans (16). In addition, a meal study was performed to

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determine if a pharmacokinetic interaction occurs between hydralazine and food in dogs.

MATERIALS AND METHODS

Reagents and Chemicals

Acetonitrile and hexane were HPLC-grade solvents (Caledon, Edmonton, Alberta). (1,4)-Dioxane was glass distilled and *p*-nitrobenzaldehyde was recrystallized from ethanol before use. Hydralazine hydrochloride (Sigma Chemical Co., St. Louis, Mo.), 4-methylhydralazine (Ciba-Geigy Co., Basel, Switzerland), triethylamine, and phosphoric acid, 85% (Fisher Scientific Co., Fairlawn, N.J.), were used as received.

Pharmacokinetic Studies in Animals

Four random-source, cross-bred dogs weighing between 12 and 40 kg were used. After an overnight fast, a Teflon catheter was inserted into a cephalic vein for blood sampling, a rubber septum was attached, a blank blood sample was collected into a syringe containing EDTA for standard curve preparation, and a heparin lock was used to maintain catheter patency. Intravenous doses of hydralazine HCl (Apresoline injection) were administered into the opposite cephalic vein. Oral doses consisted of the appropriate volume of Apresoline solution diluted with 0.01 *N* HCl to fill a 60-ml syringe. The dilution solution was carefully administered to the dog orally over about 1.5–2 min. Doses were given in random order and route; at intervals of not less than 1 week, to allow drug washout. Four doses were used: 0.25, 1.0, 2.5, and 4.0 mg/kg. After intravenous dosing, 1-ml blood samples were collected at 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 150, 180, 210, 240, 300, 360, 420, and 480 min and, for 2.5 and 4.0 mg/kg doses, additionally at 540, 600, 660, and 720 min. After oral dosing, samples were collected at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, 120, 135, and 150 min, and so on, as for the low intravenous doses. For the meal study, the dogs were fasted overnight, given a 2.5 mg/kg oral dose of hydralazine HCl, then immediately given their normal daily maintenance ration of a commercial canned dog food (~25 g/kg of Science Diet Maintenance, Hills). This was consumed in less than 2 min in all cases. Sampling then followed the normal oral schedule.

Assay Method

Hydralazine was assayed in blood using a slight modification of a previously described HPLC method (17). The blood sample size used was 1 ml and the volume of hexane used for extraction was 5 ml instead of 3 ml. Each extraction residue was dissolved in 25 µl of acetonitrile instead of 150 µl of mobile phase. The retention time of the hydralazine derivative was 3.4 min, and that of the internal standard

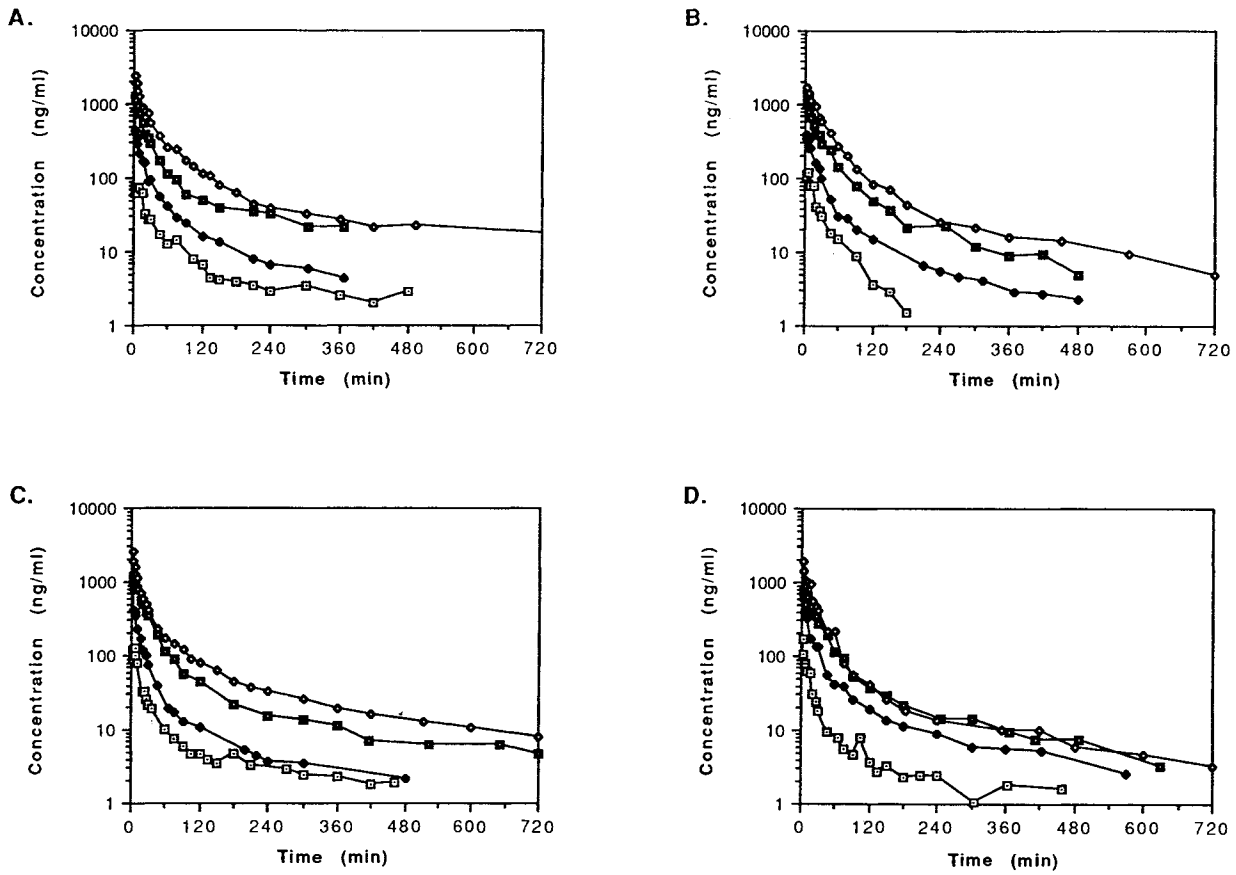

Fig. 1. Hydralazine concentration vs time profiles of the four dogs after intravenous doses of 0.25 (□), 1.0 (♦), 2.5 (■), and 4.0 (◇) mg/kg.

Table I. Hydralazine Dose-Dependent Data Analyzed Using LAGRAN, Expressed as Means (±SD) from Four Dogs

Dose (mg/kg)	Route	Cl_{tb} (ml/min/kg)	Cl_{oral} (ml/min/kg)	AUC (ng − min/ml)	V_{ss} (L/kg)	C_{max} (ng/ml)	t_{max} (min)	β (min^{-1})	$t_{1/2}$ (min)
0.25	iv	69 (10)		3,683 (566)	10 (5)			0.0079 (0.0075)	140
1	iv	78 (11)		12,771 (1,560)	8 (1)			0.0039 (0.0009)	185
2.5	iv	68 (8)		36,981 (4,192)	9 (3)			0.0033 (0.0014)	246
4	iv	67 (20)		63,697 (18,099)	9 (1)			0.0025 (0.0007)	290
0.25	po		314 (312)	1,388 (901)		27 (7)	16 (2)	0.0105 (0.0119)	124
1	po		164 (76)	7,169 (3,179)		125 (27)	11 (1)	0.0046 (0.0010)	156
2.5	po		135 (37)	19,923 (6,727)		263 (94)	27 (7)	0.0040 (0.0015)	207
4	po		102 (69)	52,872 (26,632)		1186 (424)	18 (3)	0.0033 (0.0017)	262

derivative, 4.6 min. Standard curves were prepared fresh from the blank blood samples collected from each dog at the beginning of each experiment. The standard curves were linear over the concentration range 2 to 5000 ng/ml. Quintuplicate quality control samples were independently prepared to test the accuracy and precision of the method at 2 and 5000 ng/ml. They were analyzed to contain 1.9 ± 0.3 and 4620 ± 203 ng/ml, respectively.

Pharmacokinetic Analysis

The concentration vs time data were analyzed using LAGRAN (18). The pharmacokinetic parameters, Cl_{tb}, Cl_{oral}, AUC, V_{ss}, and F were calculated according to published methods (19). C_{max} and t_{max} were taken directly from the concentration vs time data.

Statistical Analysis

A crossover design was used. Comparison of dose groups was accomplished using a repeated-measures analysis of variance, with $\alpha = 0.05$. The Scheffé test ($\alpha = 0.05$) was used to compare means. The significance of trends in the data was established by testing the slopes using simple linear regression ($\alpha = 0.05$). The AUC_{oral} of hydralazine under fasted and fed conditions was compared using the Wilcoxon signed rank test ($\alpha = 0.05$).

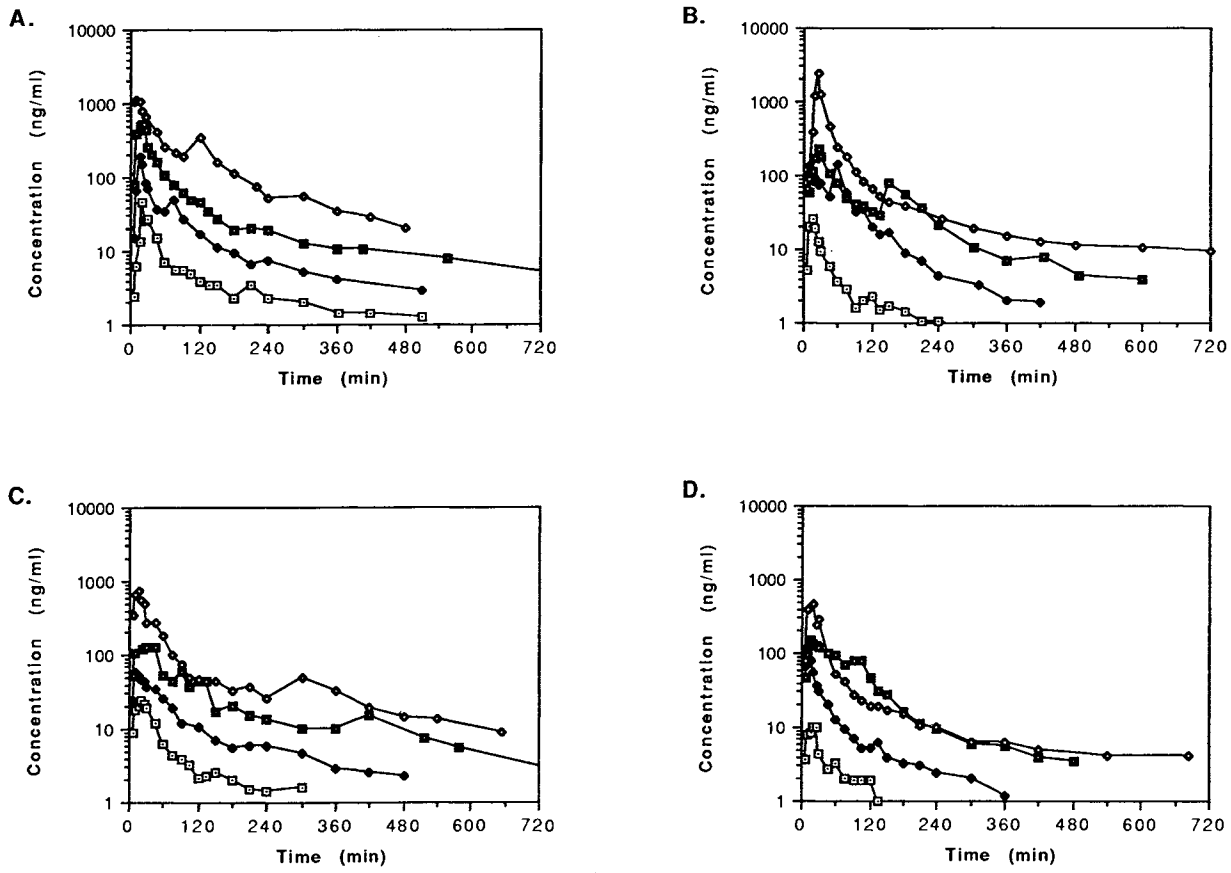

Fig. 2. Hydralazine concentration vs time profiles of the four dogs after oral doses of 0.25 (□), 1.0 (♦), 2.5 (■), and 4.0 (◇) mg/kg.

RESULTS

Intravenous Data

Log hydralazine concentration vs time curves are shown in Fig. 1 for the four intravenous doses in each dog, and the resulting pharmacokinetic parameters are summarized in Table I. There were no significant differences among dose groups for total-body clearance, AUC/dose, V_{ss}, or β. Mean Cl_{tb} was 70 ml/min/kg, mean V_{ss} was 9 L/kg, and mean β was 0.0044 min^{-1}.

Oral Data

Figure 2 shows the log concentration vs time curves for the four oral doses of Apresoline liquid in the four dogs. There are secondary peaks in most of the individual oral curves, occurring from about 75 to 420 min after dosing. Most curves contained at least one secondary peak, and some contained two or three.

Significant differences in oral AUC/dose and F were observed between the 0.25 and the 4 mg/kg doses. Linear regression indicated that there were significant trends toward higher AUC/dose and F with increasing dose (Figs. 3 and 4). Cl_{oral}, β (Table I), and C_{max}/dose were not significantly dose related. The mean slopes of the terminal elimination phases were not significantly different from those after iv doses (Table I). The time to peak concentration (t_{max}) was quite variable (range, 5–45 min; Table I).

Meal Effect Data

Mean (±SD) concentration vs time curves are presented in Fig. 5. Fasted and postprandial pharmacokinetic parameters of hydralazine are compared in Table II. A significant (mean 63%) reduction in hydralazine AUC was observed when an oral 2.5 mg/kg dose was accompanied by a meal (Table II). Apparent oral clearance was tripled (Table II). The mean C_{max} was apparently doubled, but this increase was not statistically significant due to the high variability in this parameter. The t_{max} and β values were not significantly different between the two conditions.

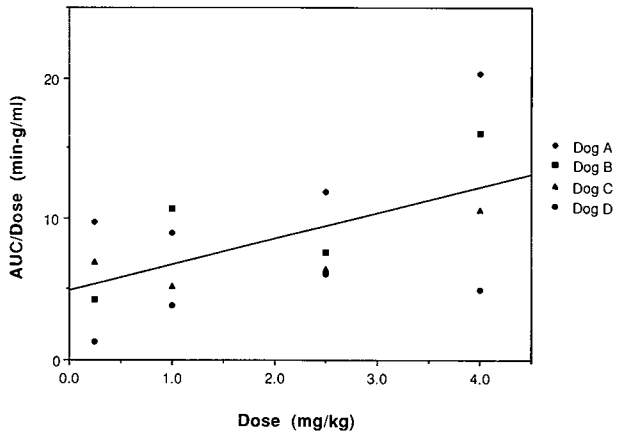

Fig. 3. Dose-corrected AUC vs dose with best-fit simple regression line for the four subjects.

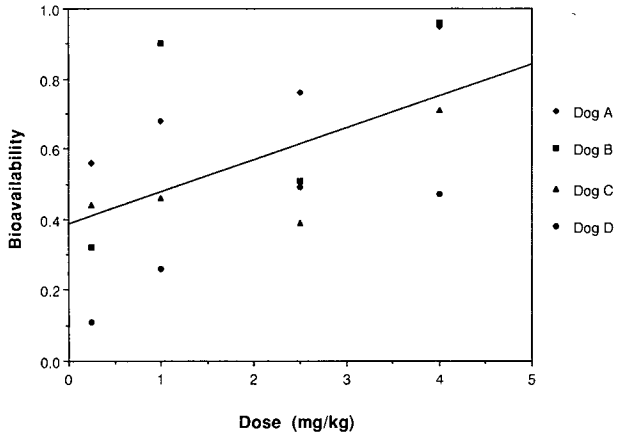

Fig. 4. F vs dose with best-fit simple regression line for the four subjects.

DISCUSSION

Previously reported human pharmacokinetic data are summarized in Table III for comparison with the canine data from this study in Tables I and II. The total body clearance of hydralazine in the dog (Table I) is similar to that in humans (Table III) and does not change over the intravenous dose range examined. This parameter is about three to four times resting hepatic blood flow, indicating the existence of extrahepatic pathways. The rate of hydralazine pyruvic acid hydrazone production in canine blood has not yet been reported, but the high clearance value is indirect evidence that this metabolic pathway could be as important in the dog as it is in the human.

V_{ss} of hydralazine in the dog (Table I) may be somewhat larger than in the human (Table III), although the ranges of individual estimates overlap [3.0–14.1 L/kg for dog vs 1.3–7.9 L/kg for human (3,20)]. This could, in part, account for the smaller β values observed in the dog.

The occurrence of the secondary peaks in the oral concentration vs time profiles may indicate that hydralazine undergoes some enterohepatic recirculation. This phenomenon would be less important after an intravenous dose, because

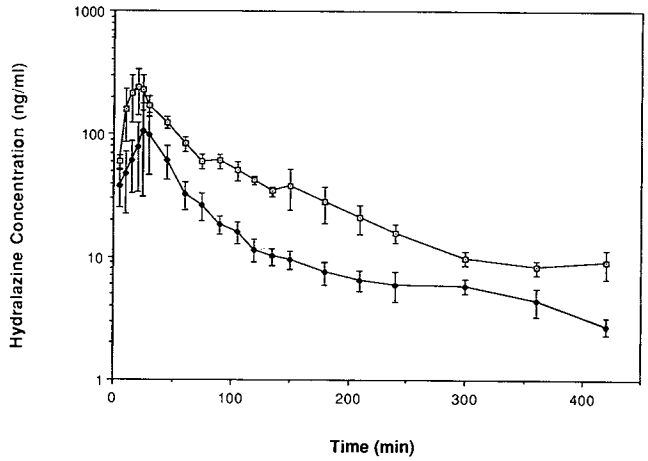

Fig. 5. Log mean (±SE) hydralazine concentration vs time after 2.5 mg/kg oral doses of hydralazine · HCl liquid in fasted (□) or fed (♦) dogs.

Table II. Mean (±SD) Pharmacokinetic Parameters from Four Dogs Given 2.5 mg/kg of Hydralazine HCl Orally Without (Fasted) or with (Fed) Food

Condition	Cl_{oral} (ml/min/kg)	AUC (ng − min/ml)	C_{max} (ng/ml)	t_{max} (min)	β (min^{-1})	$t_{1/2}$ (min)
Fasted	135 (37)	19,923 (6,727)	263 (187)	27 (13)	0.0040 (0.0015)	207
Fed	436 (241)	7,348 (3,985)	126 (136)	26 (17)	0.0069 (0.0035)	114

with the large volume of distribution of hydralazine in the dog, the concentration of drug reaching the liver and hence the rate of biliary excretion would be much lower. The intravenous curves would therefore not be visibly perturbed by this process. Another explanation for the secondary peaks could be that the rate of hydralazine absorption varies along the length of the gastrointestinal tract. While this phenomenon has not been mentioned in the literature on humans, some smaller fluctuations in concentration can be seen in the individual oral profiles reported by Shepherd *et al.* (21). The presence of these peaks warrants further investigation.

The trend toward rising oral dose-normalized AUC with increasing dose may indicate saturation of first-pass metabolism (Fig. 3). This is consistent with observations in hypertensive humans (16). The dose dependency of hydralazine F in the dog (Fig. 4) supported the AUC/dose data in suggesting saturation of first-pass metabolism at therapeutic doses. Although Michaelis–Menten kinetics may operate at the doses used, the conventional AUC method of determining F may be reasonably accurate for hydralazine because this drug is rapidly absorbed and has a large V_{ss} (22–24). C_{max} values are more variable in dogs (Table I) than those in humans (Table III), although the ranges overlapped between dogs and slow acetylating humans at both the 0.25 and the 1.0 mg/kg dose levels. The high variability in C_{max} could partially explain why C_{max}/dose vs dose were not significantly related. However, a similar trend to that in humans was observed. Values for t_{max} after administration of the liquid formulation were similar in the dog and human (Tables I and III), averaging around 20 min in both species.

The meal study (Table II, Fig. 5) revealed that, in agreement with the human data of Shepherd *et al.* (5,6), coadministration of hydralazine with food caused a reduction in bioavailability, as measured by AUC changes. These findings are not consistent with the study by Melander *et al.* (4), which Shepherd *et al.* (5) have pointed out used a nonspecific assay method for the measurement of hydralazine. Cl_{oral} was increased significantly in the dog, and C_{max} was reduced in three of the four subjects. Remaining unchanged were t_{max} and β. It is interesting to note that none of the individual concentration vs time curves showed secondary peaks after a meal. If their origin in the fasted animals was enterohepatic recycling, no peaks would be expected to appear after a meal, because the gall bladder contracts frequently and the small and more or less continuous release of drug into the duodenum is not sufficient to cause a noticeable rise in drug concentration.

It is not possible to use the peripheral blood data to differentiate among the possible mechanisms which may contribute to the food effect on hydralazine bioavailability. These include changes to hydralazine absorption, first-pass metabolism, hepatic blood flow, and protein binding. Further investigations using invasive methods are being conducted to gain a greater understanding of this interesting, controversial, and clinically significant phenomenon.

The data presented here indicate that the parent drug kinetics of hydralazine are quantitatively similar between dogs and slow-acetylating humans. Both species show high systemic clearance, indicating significant extrahepatic metabolism, a large volume of distribution, saturation of presystemic metabolism at therapeutic doses, and reduced bio-

Table III. Previously Reported Human Pharmacokinetic Data on Hydralazine

Dose (mg/kg)	Acetylation status	Route	Plasma (P) or blood (B)	Cl_{tb} (ml/min/kg)	AUC (ng − min/ml)	F (%)	V_{ss} (L/kg)	C_{max} (ng/ml)	t_{max} (min)	$t_{1/2}$ (min)	β (min^{-1})	Reference No.
0.30		iv	P	78			1.9			54	0.013	20
0.38		iv	P	138	2832		6			40	0.018	3
0.25	Slow	po	B					23				16
0.33	Slow	po	B					76	15			15
0.33 (tab)	Slow	po	B					49	65			15
0.5	Slow	po	P		1416	35				28	0.024	3
0.5	Slow	po	B					62				16
1.0	Slow	po	B					209				16
1.0	Slow	po	P		3680	31		165	18	41	0.017	21
0.5	Fast	po	B					15				16
1.0	Fast	po	P		1142	16				26	0.026	3
1.0	Fast	po	B					65				16
1.0	Fast	po	P		1072	10		51	25	13	0.053	21
2.0	Fast	po	B					302				16

availability of hydralazine when coadministered with food. The dog may therefore be a useful animal model for studying the pharmacokinetics and mechanisms of pharmacokinetic interaction of hydralazine with food.

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NOMENCLATURE

Cl_{tb}	Total-body clearance
Cl_{oral}	Apparent average oral clearance
AUC	Area under the hydralazine concentration-time curve
V_{ss}	Steady-state volume of distribution
C_{max}	Maximum concentration of hydralazine reached after an oral dose
t_{max}	Time at which C_{max} is reached
β	Slope of the log-linear terminal elimination phase of the concentration–time curve
F	estimated oral bioavailability

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