Pharmacokinetics of Indapamide in Dogs

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Abstract □ Four beagle dogs received both an oral and intravenous dose (1 mg/kg) of indapamide in a crossover design. The blood levels and urinary excretion of intact indapamide were measured, and the pharmacokinetic parameters of the drug were defined. The results indicate that indapamide is completely bioavailable after an oral dose and does not undergo first-pass metabolism. Excretion of unchanged drug from the kidney accounted for only a small percentage of the drug's clearance. While the dog is very similar to the human in its handling of indapamide, the dog clears indapamide approximately twice as fast as humans.

Keyphrases □ Indapamide—pharmacokinetics in dogs □ Pharmacokinetics—indapamide, dogs □ Antihypertensive agents—indapamide, pharmacokinetics in dogs

Indapamide[1], 1-(3-sulfamyl-4-chlorobenzamido)-2-methylindoline, is a new antihypertensive agent which has been shown to be clinically effective at daily doses of 2.5 and 5.0 mg (1–5). A terminal elimination half-life of ~15 hr was observed in human volunteers who received 5.0-mg oral doses of indapamide (6). A study (7) in which humans received radiolabeled indapamide orally showed that the drug was extensively metabolized, with only ~5% of the administered drug excreted unchanged in the urine. Therefore, renal clearance was considered to account for only a small percentage of indapamide's total body clearance.

The present study in dogs was designed to define the pharmacokinetic parameters of indapamide. Dogs were given both oral and intravenous doses to determine the fraction of dose systemically available after the oral dose.

EXPERIMENTAL

Animals—Four male beagle dogs, weighing an average of 12.1 kg, were administered ~1 mg/kg of indapamide as an oral solution or as an intravenous injection in a crossover design. Two dogs initially received the oral dose, while the other two received the intravenous dose. A 2-week wash-out period was used between doses. Prior to dosing, the dogs were fasted overnight. They were maintained in metabolism cages and provided with water *ad libitum* throughout the study. Four hours after dosing, the dogs were given 480 ml (16 oz) of dog food mixed with an equal volume of water.

Treatment—The oral solution was prepared by dissolving 100 mg of indapamide[2] in 50 ml of polyethylene glycol 400[3], followed by dilution with water to a final concentration of 0.2 mg/ml. Approximately 60 ml of this solution was administered to each dog *via* a gastric tube, followed by 25 ml of water to rinse the gastric tube. The intravenous solution was prepared by dissolving indapamide in absolute ethanol to a final concentration of 5 mg/ml. After administration of the intravenous dose as a bolus, the dogs received ~75 ml of water *via* a gastric tube.

Blood samples (6 ml) for the first 12 hr after dosing were collected by an indwelling catheter in the jugular vein of the dog. Thereafter, blood samples were collected by venipuncture from the cephalic vein. The samples were collected in plastic syringes and transferred to polypropylene tubes[4] for storage. Sodium oxalate was the anticoagulant. With the intravenous dose, blood samples were collected just prior to and at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, 30, 48, and 72 hr after dosing. Blood samples were collected just prior to and at 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 30, 48, and 72 hr after the oral dose.

Urine was collected by catheterization just prior to and at 4, 8, 12, 24, 48, and 72 hr after dosing. This urine was combined with the urine collected in the metabolism cages during the appropriate time intervals, and the total urine volume was measured. All samples were divided into aliquots, and the urine and blood samples were maintained at −20° until assayed.

Assay of Indapamide in Blood and Urine—Measurement of blood and urine concentrations of indapamide was performed using a semiautomated fluorescence assay (8). The procedure was shown to be sensitive (25 ng/ml) and specific for the intact drug.

Data Treatment—Pharmacokinetic parameters for the intravenous blood data were calculated by standard techniques (9, 10). A two-compartment open model (Scheme I) was used to describe the blood concentrations of indapamide following the intravenous dose. Blood concentrations (C) of indapamide for any time t after an intravenous dose are given by the relationship:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(Eq. 1)}$$

where A and B are the zero-time intercepts determined by extrapolation of the linear distribution (α) and terminal elimination (β) phases, respectively. These rate constants were calculated by the methods of residuals using a least-squares best fit to calculate the slopes. The intercompartmental transfer rate constants were then calculated as follows:

$$k_{12} = \alpha + \beta - k_{21} - k_{10} \quad \text{(Eq. 2)}$$

$$k_{21} = \frac{A\beta + B\alpha}{A + B} \quad \text{(Eq. 3)}$$

$$k_{10} = \frac{\alpha\beta}{k_{21}} \quad \text{(Eq. 4)}$$

The volume of distribution of the central compartment (V_c) was calculated as follows:

$$V_c = \frac{\text{dose}}{A + B} \quad \text{(Eq. 5)}$$

The apparent volume of distribution, V_β, for indapamide was calculated using:

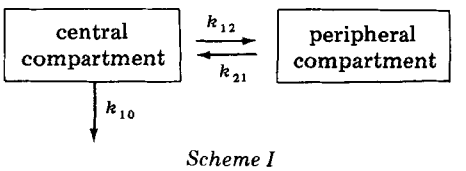
$$V_\beta = \frac{\text{dose}}{\beta AUC_{0-\infty}} \quad \text{(Eq. 6)}$$

where $AUC_{0-\infty}$ is as defined later.

The V_β of indapamide after an oral dose was calculated using Eq. 6, which is model independent. However, the oral dose was considered to be only that fraction that was available. This fraction, F, was calculated as the ratio of the area under the blood *versus* time curve after an oral dose to that following an intravenous dose.

The following additional parameters were calculated for both the oral and intravenous administrations of indapamide: area under the blood concentration *versus* time curve for the first 48 hr (AUC_{0-48}), which was calculated by the trapezoidal rule; and total area under the blood concentration *versus* time curve ($AUC_{0-\infty}$), which was calculated using:

$$AUC_{0-\infty} = AUC_{0-48} + \frac{C_{48}}{\beta} \quad \text{(Eq. 7)}$$



Scheme I

[1] RHC 2555.
[2] Revlon Health Care Group, Tuckahoe, N.Y.
[3] J. T. Baker Chemical Co., Phillipsburg, N.J.

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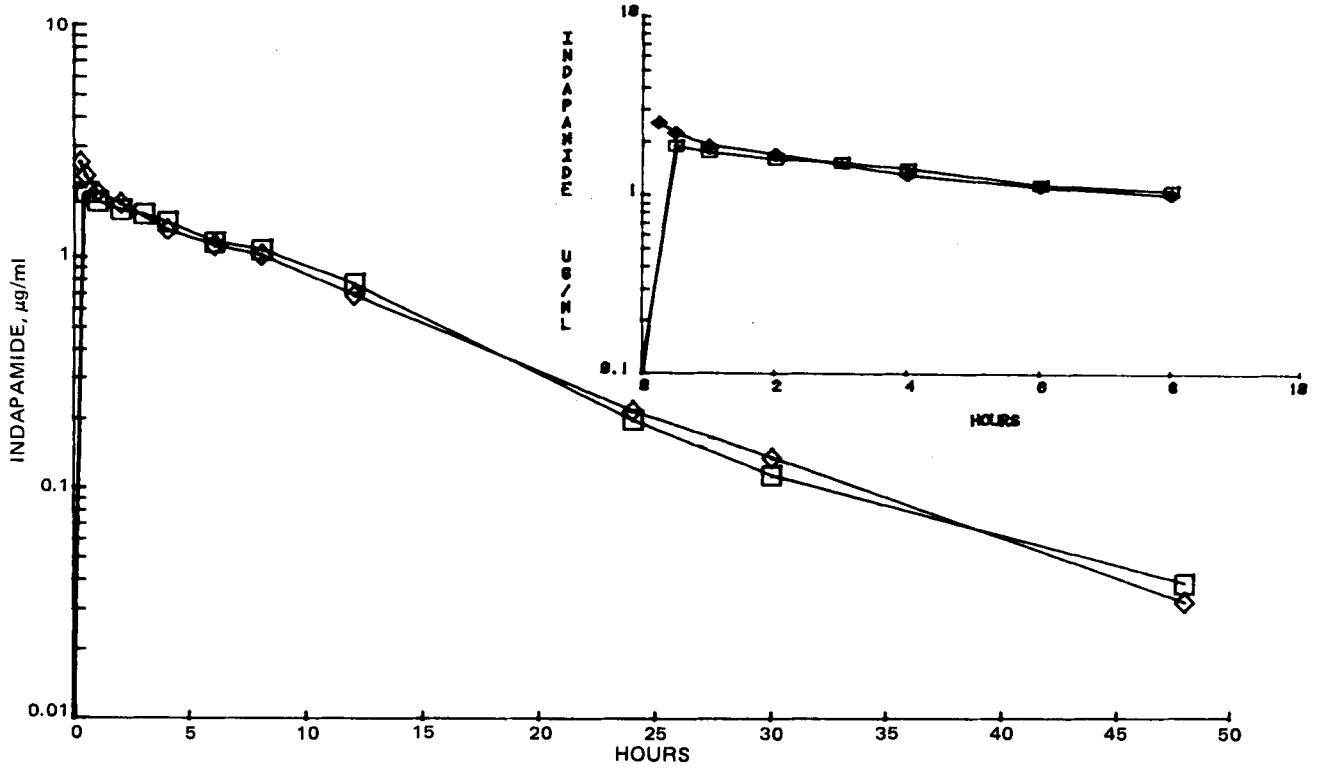

Figure 1—*Average indapamide blood concentrations after an oral (□) or intravenous (◇) dose of 1 mg/kg.*

where $\hat{C}_{48}$ was calculated from the slope of the terminal linear segment of the semilogarithmic plot of blood concentration *versus* time. The total body clearance (Cl_T) was calculated using:

$$Cl_T = V_\beta \beta \qquad \text{(Eq. 8)}$$

Average renal clearance (Cl_R) was determined by plotting the rate of excretion of unchanged indapamide in urine ($\Delta U/\Delta t$) as a function of the blood concentration of indapamide at the midpoint of the collection interval. The slope of this graph was calculated by linear regression and was equal to Cl_R. The total renal clearance was also estimated by dividing the total amount of indapamide recovered in the urine by $AUC_{0-\infty}$.

RESULTS AND DISCUSSION

A two-compartment open model was chosen to describe the blood indapamide concentrations following the intravenous dose to dogs. This selection was made after visual inspection of the data. While these data possibly might be better described by an equation containing three or four exponential terms, the data at the latter time points were not sufficient to allow such analysis. The two-compartment model, however, appeared to be adequate since the fit of the theoretical to the experimental data was acceptable for all dogs, with correlation coefficients of at least 0.98. In addition, the AUC_{0-48} values calculated from the parameters of the best fit were within 5% of the areas calculated by the trapezoidal rule.

Linear regression of the terminal elimination phase after an oral dose did not give evidence for a two-compartment model but for only a one-compartment one. This phenomenon occurs when the absorption rate constant of a drug is sufficiently close to the distribution rate constant that the distributive phase is not observed following absorption (9). The absorption rate of indapamide was not measured in this study since a sufficient number of points during the absorption phase was not obtained. Thus, only the analysis of the intravenous data was used to define the pharmacokinetic parameters of indapamide.

Blood Concentrations—Mean blood concentrations of indapamide in four dogs after an oral or intravenous dose of 1 mg/kg are shown in Fig. 1. After the oral dose, the average maximum blood concentration (C_{max}) obtained for indapamide was 1.95 μg/ml and the average time of peak blood concentrations (T_{max}) was 1.3 hr. Indapamide was rapidly absorbed after dosing with an oral solution.

Blood levels were significantly higher for the intravenous dose than the oral dose for only the 1st hr after dosing. At 4 hr after dosing, the average blood concentration of indapamide was higher after the oral dose than after the intravenous dose. The *AUC* values for the blood concentration *versus* time curves are shown in Table I. The average values for $AUC_{0-\infty}$ were 23.3 and 23.9 μg hr/ml for the oral and intravenous doses, respectively. It can be concluded from these data that the *AUC* values after the oral and intravenous doses were essentially the same and that the fraction of drug available was 1.0. Therefore, these results indicate that indapamide, although extensively metabolized (7), is completely bioavailable after an oral dose and does not undergo first-pass metabolism by the gut or liver.

A similar conclusion could be arrived at by using only the data obtained after the oral dose and calculating F by the following (9):

$$F = \frac{Q}{Q + \dfrac{\text{dose}}{(AUC_{0-\infty})_{po}}} \qquad \text{(Eq. 9)}$$

Table I—Pharmacokinetic Parameters for Intravenous and Oral Indapamide in Dogs

Parameter	Intravenous[a]	Oral
AUC_{0-48}, μg hr/ml	23.5 ± 3.2	23.0 ± 1.4
$AUC_{0-\infty}$, μg hr/ml	23.9 ± 3.4	23.3 ± 1.4
C_{max}, μg/ml	—	1.95 ± 0.16
T_{max}, hr	—	1.3 ± 1.2
A, μg/ml	1.06 ± 0.54	—
α, hr^{-1}	2.48 ± 0.92	—
$t_{1/2}\alpha$, hr	0.32 ± 0.16	—
B, μg/ml	1.94 ± 0.28	1.95 ± 0.12
β, hr^{-1}	0.087 ± 0.006	0.086 ± 0.002
$t_{1/2}\beta$, hr	8.04 ± 0.56	8.09 ± 0.20
k_{12}, hr^{-1}	0.71 ± 0.36	—
k_{21}, hr^{-1}	1.73 ± 0.82	—
k_{10}, hr^{-1}	0.13 ± 0.02	—
V_c, liters/kg	0.34 ± 0.07	—
V_β, liters/kg	0.49 ± 0.06	0.50 ± 0.04
Cl_T, ml/min/kg	0.71 ± 0.09	0.72 ± 0.04
Cl_R[b], ml/min/kg	0.060 ± 0.008	0.059 ± 0.012

[a] Mean ± *SD*. [b] Amount of indapamide excreted in the urine (0–48 hr)/$AUC_{0-\infty}$.

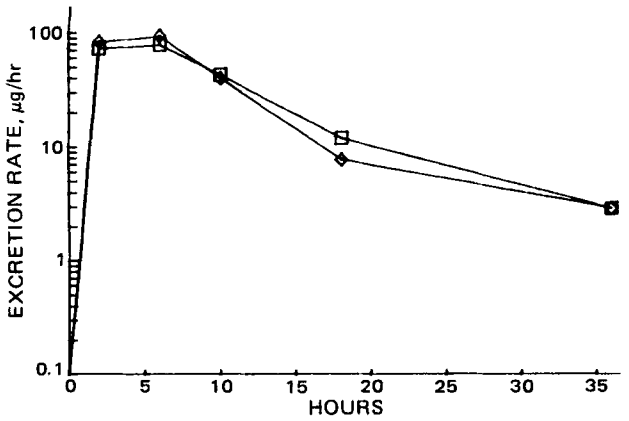

Figure 2—*Average indapamide urinary excretion rates after an oral (□) or intravenous (◇) dose of 1 mg/kg.*

where Q is the liver blood flow in dogs [43 ml/min/hr (11)], the dose is 1 mg/kg, and the average $AUC_{0-\infty}$ is 1.95 μg hr/ml kg. A value of 0.98 was calculated for F using these parameters.

The pharmacokinetic parameters were calculated for each dog after the intravenous and oral doses and are shown in Table I. The average terminal half-life for elimination of unchanged indapamide was 8 hr after both the oral and intravenous dose. The average V_β was 0.50 for the oral dose and 0.49 liter/kg for the intravenous dose. Thus, there was no apparent difference in the way indapamide was handled after either an oral or intravenous dose. The average V_c after the intravenous dose was calculated to be 0.34 liter/kg.

Plasma indapamide concentrations at 2 and 8 hr after dosing were measured, and the average ratio of whole blood to plasma concentrations for all samples was 5.4 ± 1.4.

Urinary Excretion and Renal Clearance—The average urinary excretion rate profiles after the intravenous and oral doses are shown in Fig. 2. Comparison of data after the oral and intravenous dose revealed no significant differences in the amount or rate of urinary excretion of unchanged indapamide. Within 48 hr after oral or intravenous dosing, averages of 8.2 and 8.1% of the dose were eliminated in the urine as unchanged indapamide, respectively. It was estimated that only ~0.2% of the dose was excreted in the urine as unchanged indapamide between 48 and 72 hr.

Renal clearance and average incremental renal clearance data for all dogs are presented in Table II. The renal clearance during the 4–8-hr interval was significantly higher than the values obtained for the other time intervals. This point caused considerable error in the calculation of renal clearance as evidenced by the increase in the correlation value when it was omitted. This temporal dependence of renal clearance was not related to the blood indapamide concentrations but instead may have been related to the administration of food and water to the dogs at 4 hr after dosing. This water intake may have increased the urine flow during the 4–8-hr period and thus decreased indapamide reabsorption.

However, while the renal clearance of indapamide appears to have been dramatically altered during this period, there was little change in total drug clearance because of the small involvement of the kidneys.

Renal clearance was also estimated by dividing the amount of unchanged indapamide excreted in the urine by 48 hr (U_{0-48}) after dosing by the $AUC_{0-\infty}$. This value should closely approximate the true value since a negligible amount of unchanged indapamide is excreted in the urine after 48 hr. The average values were 0.72 ml/min after the oral dose and 0.74 ml/min after the intravenous dose (Table I). These values compare favorably with those obtained graphically (0.79 and 0.87 ml/min, respectively).

Table II—Average Renal Clearance of Indapamide in Four Dogs following Oral or Intravenous Dosing

Time Interval, hr	$C_B(\bar{T})$[a], μg/ml	$\Delta U/\Delta t$, μg/hr	$\Delta U/\Delta t/C_B(\bar{T})$, ml/min
	Oral Dosing		
0–4	1.633	73.1	0.746
4–8	1.168	79.8	1.139
8–12	0.826	43.6	0.880
12–24	0.416	12.2	0.489
24–48	0.088	3.0	0.568
	Cl_R[b] = 0.89 ml/min; r^2 = 0.88		
	Cl_R[c] = 0.787 ml/min; r^2 = 0.97		
	Intravenous Dosing		
0–4	1.715	84.0	0.816
4–8	1.141	95.2	1.391
8–12	0.817	40.6	0.828
12–24	0.410	7.9	0.321
24–48	0.087	3.0	0.575
	Cl_R[b] = 1.005 ml/min; r^2 = 0.81		
	Cl_R[c] = 0.873 ml/min; r^2 = 0.98		

[a] Blood indapamide concentration at midpoint of time interval. Extrapolated values were used for those times when no blood sample was collected. [b] Renal clearance calculated by the graphical method using all points. [c] Renal clearance calculated by the graphical method excluding the 4–8-hr point.

Values of C_{max} for blood concentrations of indapamide in humans (6) were ~0.3 μg/ml after a total dose of 5 mg (~0.07 mg/kg). These values, when corrected for dose, are approximately double the C_{max} values obtained in the present study. This difference could be explained by the differences in the V_β values of the two species. The values for dogs and humans are 0.5 and 0.3 liter/kg, respectively.

For humans, the elimination half-life has been reported as 15 hr (6); in the present study in dogs, a half-life of only 8 hr was observed. This difference may reflect a difference in the hepatic clearance rate in the two species since the renal clearance is ~10% or less of the total clearance for both species. Indeed, in both dogs and humans, indapamide clearance is considerably less than the liver blood flow, which may be a result of the drug's binding to the blood constituents and/or its intrinsic clearance.

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