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Pharmacokinetics of Diazepam and Nordiazepam in the Cat

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Abstract □ The cat has been used extensively as an experimental model for studying the pharmacology of compounds that exhibit CNS activity including diazepam and nordiazepam. However, since little is known about the distribution and elimination of diazepam in this species, the pharmacokinetics of diazepam and nordiazepam were studied in the cat following intravenous doses of 5, 10, and 20 mg/kg of diazepam and 5 and 10 mg/kg of nordiazepam. The disappearance of diazepam and nordiazepam from blood was fitted with classical equations. Theoretical and trapezoidal areas under the curve (AUC_{th} and AUC_{tr}) were calculated. The volumes of distribution (Vd_{β}) were calculated as model-independent parameters for diazepam and nordiazepam. Intrinsic hepatic clearance, extraction ratio, and tissue binding parameters were also calculated for diazepam. From the observed data, it is apparent that the blood concentrations and the resulting areas under the curves are proportional to the dose of diazepam administered and that the pharmacokinetics of diazepam were linear over the dose range studied. In addition, nordiazepam formed after diazepam administration appeared to be proportional to the dose of diazepam administered. The terminal elimination rate constant of nordiazepam remained constant over the dose range studied. It appears that both diazepam and nordiazepam are highly bound to tissue. The total body clearance of diazepam (4.72 ± 2.45 mL/min/kg) is approximately six times that of nordiazepam (0.85 ± 0.25 mL/min/kg). Approximately 50% of an administered dose of diazepam was biotransformed to nordiazepam in the cat.

Keyphrases □ Diazepam—pharmacokinetics in the cat, nordiazepam □ Nordiazepam—pharmacokinetics in the cat, diazepam □ Pharmacokinetics—diazepam and nordiazepam in the cat after intravenous doses

The benzodiazepines are centrally active compounds that reduce anxiety, produce sedation and sleep, have anticonvulsant effects, and may cause muscle relaxation. In addition, the benzodiazepine diazepam is also used in the treatment of alcohol abusers during potentially life-threatening withdrawal episodes (1). Diazepam has a long effective duration of action, probably due to slow elimination of parent compound and biotransformation to active metabolic products including nordiazepam (2). Many of the pharmacological properties of the benzodiazepines seen in humans are present in the cat (3–5). Diazepam has been shown to be effective in the cat in the control of seizures in experimental epilepsy (6) and with the sudden abstinence of alcohol after chronic use (5). Although the cat has been used extensively to study the effects of diazepam, little is known about the pharmacokinetics of diazepam and nordiazepam in this species. Morselli *et al.* (7) described the distribution of diazepam and its major metabolites in plasma and in several areas of the brain of the cat. A direct correlation was established between blood flow to various tissues of the brain and the diazepam found in these tissues. However, the accumulation of nordiazepam, the major blood metabolite, in the brain appeared to be inversely related to blood flow. Unfortunately, this data did not lend itself to an overall pharmacokinetic profile of diazepam or nordiazepam in this species.

The present study in the domestic short-hair cat was performed to (*a*) establish the pharmacokinetics of diazepam and nordiazepam following 5-, 10-, and 20-mg/kg iv doses of diazepam and (*b*) to compare the pharmacokinetic profile of nordiazepam after nordiazepam administration to its profile observed after the administration of diazepam in this species. These data will establish the fundamental pharmacokinetic profile of diazepam and nordiazepam in the cat.

EXPERIMENTAL

Animal Model—Domestic short-haired female cats (2.5–3.0 kg) were used for all experiments. Hematocrit values were determined before surgery and drug administration and were found to be within the normal range (8). Cats were anesthetized with 0.33–0.44 mL of a solution containing 100 mg/mL of ketamine and 7.5 mg/mL of promazine[1]. An incision was made in either the right or left leg exposing a branch of the femoral vein. A butterfly infusion set[2] with the needle removed, leaving the tubing and Luer fitting intact, was used as a cannula. The catheter was inserted into the vein, exteriorized, and the incision was closed with 000 surgical silk[3]. The catheter was cleared daily and kept patent with a sodium heparin flush. Diazepam or nordiazepam (10 mg/mL) was dissolved in dimethylacetamide[4] immediately prior to administration.

Study Design—Unanesthetized fasted cats, with a femoral vein catheter previously implanted to facilitate blood sampling, were administered 5-, 10-, and 20-mg/kg doses of diazepam or 10 mg/kg of nordiazepam intravenously as a short infusion (20 s) *via* a peripheral vein. Two weeks were allowed for recuperation between doses.

Sampling and Analysis—Blood specimens (0.5 mL) were obtained from the femoral vein catheter. The first specimen was obtained 2.5 min following intravenous administration and subsequent specimens were obtained for as long as 96 h. The blood specimens were collected in tubes containing dried heparin[5] and kept at −20°C until analyzed. The samples were analyzed simultaneously for diazepam and nordiazepam using the electron capture GC method of Weinfeld *et al.* (9) with the following modifications. Whole blood (0.1 mL) was extracted into 1 mL of benzene after adjusting the pH to 9.0 with a 1 M H_3BO_3–KCl–Na_2CO_3 buffer. The percent standard deviation for the intraassay variability averaged 3.6% for diazepam and 3.4% for nordiazepam over the range of 50–2000 ng/mL

[1] Ketaset Plus; Bristol Labs, Syracuse, N.Y.
[2] Model No. 44492; Abbott Laboratories, Chicago, Ill.
[3] Ethicon, New Brunswick, N.J.
[4] Model No. 4972, Eastman Kodak, Rochester, N.Y.
[5] Model No. 3206, Becton, Dickenson, Rutherford, N.J.

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Table I—Diazepam Pharmacokinetic Parameters and Percent Coefficient of Variation [a] after Intravenous Administration of Diazepam to the Cat

Parameter	Cat										
	1		2			3			4	5	6
Dose, mg/kg	5	10	5	10	20	5	10	20	10	10	10
Weight, W kg	2.7	2.5	2.7	2.4	2.7	3.0	3.0	3.0	2.5	2.8	2.8
P, μg/mL				25.4 (9.90)							
π, h^{-1}				15.4 (12.7)							
A, μg/mL	4.43 (13.7)	28.8 (20.8)	9.28 (11.4)	7.40 (6.08)	24.2 (8.84)	4.55 (10.4)	10.4 (9.70)	18.1 (3.40)	12.4 (11.6)	8.99 (13.5)	
α, h^{-1}	2.30 (32.3)	1.44 (25.8)	2.56 (21.8)	.717 (14.2)	2.63 (16.8)	1.29 (21.0)	1.26 (20.5)	1.65 (6.86)	3.58 (21.4)	2.08 (29.3)	
B, μg/mL	2.58 (23.6)	3.15 (24.2)	2.77 (13.9)	1.30 (21.3)	8.08 (8.88)	1.25 (34.7)	2.09 (34.5)	3.85 (7.40)	2.41 (21.3)	2.06 (30.5)	6.98
β, h^{-1}	0.204 (22.8)	0.123 (10.9)	0.064 (33.6)	0.0125 (95.6)	0.048 (19.8)	0.120 (56.0)	0.111 (46.9)	0.077 (11.5)	0.149 (35.2)	0.096 (46.4)	0.397 8.39
V_c, mL/kg	713	313	416	293	618	862	800	911	675	905	
Vd_β, mL/kg	1684	1783	1668	6864	2345	2997	3327	4259	3418	4047	1432
V_c/Vd_β	0.42	0.18	0.25	0.043	0.26	0.29	0.24	0.21	0.20	0.22	
AUC_{th} μg·h	14.6	45.6	46.6	116	177	14.0	27.1	60.7	19.6	25.8	18.9
AUC_{tr} μg·h/mL	15.0	59.2	49.3	116	186	13.9	31.1	66.0	22.6	26.8	19.2
CL, mL/min/kg	5.70	3.65	1.78	1.43	1.88	5.95	6.17	5.48	8.48	6.47	8.76
CL_{tr}, mL/min/kg	5.55	2.80	1.68	1.43	1.78	6.00	5.35	5.05	7.38	6.20	8.68

[a] % CV in parentheses.

of blood for diazepam and 100–4000 ng/mL of blood for nordiazepam. The interassay variability for the same range was 6.0 and 8.9% for diazepam and nordiazepam, respectively.

Blood-to-plasma ratios were determined for diazepam and nordiazepam. Diazepam and nordiazepam (1500 ng) were added to 3-mL aliquots of blood, previously collected in a tube[5] containing dried heparin. The blood and the two components were gently shaken in a reciprocal shaker[6] at ~60 excursions per minute for 1.5 h. A 0.5-mL aliquot of whole blood was removed and the remainder was centrifuged for 30 min at 2500 rpm to separate the plasma from the red blood cells. A 0.5-mL volume of plasma was taken for each analysis. Both whole blood and plasma were analyzed for diazepam and nordiazepam by a specific and sensitive HPLC method (10).

Pharmacokinetic Analysis—Following rapid intravenous infusion of diazepam and nordiazepam, blood concentration–time curves were analyzed by a weighted iterative nonlinear least-squares regression technique (11). Data points were fitted with one of the following equations:

$$C_b = Be^{-\beta t} \quad \text{(Eq. 1)}$$

$$C_b = Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(Eq. 2)}$$

$$C_b = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t} \quad \text{(Eq. 3)}$$

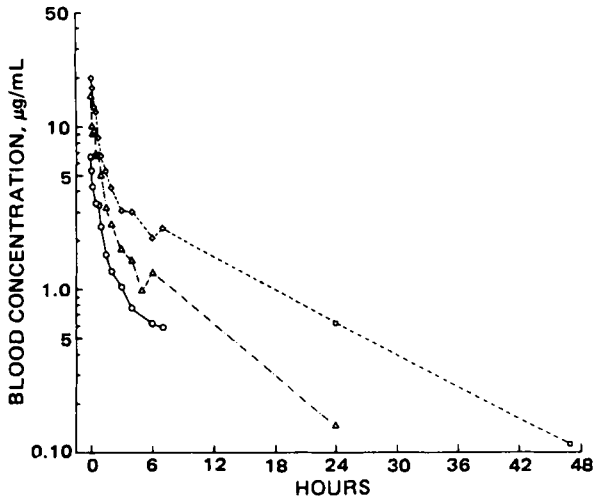

Figure 1—*Diazepam blood concentrations for Cat 3 after intravenous administration of 5- (○), 10- (△), and 20- (□) mg/kg doses of diazepam.*

[6] Eberbach Corp., Ann Arbor, Mich.

In all equations, C_b is the blood diazepam or nordiazepam concentration at time t after the dose. P, A, and B are coefficients and π, α, and β are hybrid rate constants that describe the disposition and elimination phases, respectively.

The equation selected for fitting the data was determined by comparing the sum of the squared deviations using the F-ratio test (12) and by scatter of the actual data points around the fitted function. Model-dependent pharmacokinetic parameters such as the theoretical areas under the blood concentration–time curves (AUC_{th}) were calculated from the following equations:

$$AUC_{th} = \frac{B}{\beta} \quad \text{(Eq. 4)}$$

$$AUC_{th} = \frac{A}{\alpha} + \frac{B}{\beta} \quad \text{(Eq. 5)}$$

$$AUC_{th} = \frac{P}{\pi} + \frac{A}{\alpha} + \frac{B}{\beta} \quad \text{(Eq. 6)}$$

The volume of the central compartment (V_c) was calculated by dividing the dose (mg/kg) by the sum of the coefficients, and the volume of distribution (Vd_β) was determined with the following:

$$Vd_\beta = \frac{\text{Dose}}{(AUC_{th}) \cdot \beta} \quad \text{(Eq. 7)}$$

where β is the terminal elimination rate constant. The theoretical total body clearance (TBC_{th}) was determined from the relationship of dose to the theoretical area under the curve:

$$TBC_{th} = \frac{\text{Dose}}{AUC_{th}} \quad \text{(Eq. 8)}$$

Table II—Pharmacokinetic Parameters for Nordiazepam after Intravenous Diazepam Administration to Cats

Cat	Dose, mg/kg	Wt, kg	β, h^{-1}	$AUC_{0-\infty}$, μg·h/mL	$\frac{(AUC_{nor})\ D}{(AUC_{nor})\ N}$ [a]
1	5	2.7	0.0424	47.2	0.45
	10	2.5	0.0356	108	0.79
2	5	2.7	0.0503	51.3	
	10	2.4	0.0239	105	0.69
	20	2.7	0.0329	188	
3	5	3.0	0.0135	97.0	
	10	3.0	0.0297	114	0.50
	20	3.0	0.0189	425	
4	10	2.5	0.119[b]	38.0	0.20
5	10	2.8	0.0501	87.8	0.37
6	10	2.8	0.0250	166	0.50

[a] Area under the nordiazepam blood concentration–time curve following diazepam administration divided by the area under the nordiazepam blood concentration–time curve following an equal dose of nordiazepam. [b] In Cat 4 of the series, β and AUC_{tr} of nordiazepam after diazepam administration were determined to be aberrant values and were not included in calculating the mean value of these parameters (26).

Table III—Pharmacokinetic Parameters and Percent Coefficient of Variation [a] for Nordiazepam after Intravenous Nordiazepam Administration to Cats

Cat	Wt, kg	Dose, mg/kg	A, μg/mL	α, h^{-1}	B, μg/mL	β, h^{-1}	$Area_{th}$, μg·h/mL	AUC_{tr}, μg·h/mL	CL_{tr}, mL/min/kg	V_c, mL/kg	Vd_β, mL/kg	V_c/Vd_β
1	2.7	5			4.31	0.0474	91	105	0.80		1004	
1	2.5	10	6.21 (23.5)	1.82 (43.6)	4.91 (10.7)	0.0389 (8.8)	130	136	1.23	899	1890	0.45
2	2.4	10	5.61 (27.0)	1.52 (45.0)	3.11 (11)	0.0236 (12)	136	152	1.08	1146	2754	0.37
3	3.0	10	4.81 (15)	0.957 (35)	6.24 (7.2)	0.0285 (10)	224	229	0.73	904	1543	0.58
4	2.5	10	15.6 (41)	8.26 (39)	5.51 (5.7)	0.0329 (11)	170	187	0.88	473	2217	0.26
5	2.8	10	27.9 (65)	7.95 (51)	4.75 (11)	0.0255 (17)	214	237	0.70	306	1647	0.16
6	2.8	10	4.19 (31.5)	0.776 (76)	6.09 (15)	0.0188 (28)	327	332	0.50	972	1602	0.60

[a] % *CV* in parentheses.

The model-independent areas under the blood concentration time curves were also calculated from time zero to infinity (AUC_{tr}) by adding the area from time t, of the final measurable concentration estimated by trapezoidal summation (AUC_{0-t}), to the area from time t to infinity ($AUC_{t-\infty}$) calculated with the following (13):

$$AUC_{t-\infty} = C_b{}^t/\beta \qquad \text{(Eq. 9)}$$

where $C_b{}^t$ is the observed blood concentration at time t and β is the terminal elimination rate constant estimated by nonlinear regression. The calculated total clearance (TBC_{tr}) was calculated as the dose divided by AUC_{tr}. In addition, the intrinsic clearance CL_I was calculated as:

$$CL_I = \frac{HBF \cdot CL_{tr}}{HBF - CL_{tr}} \qquad \text{(Eq. 10)}$$

where HBF is the hepatic blood flow. The extraction ratio (ER) was calculated as:

$$ER = \frac{CL_I}{HBF + CL_I} \qquad \text{(Eq. 11)}$$

as described by Wilkinson and Shand (14). The blood-to-plasma distribution ratio was calculated from the concentration of drug in whole blood divided by the concentration of drug in plasma.

RESULTS AND DISCUSSION

The mean ±*SD* blood-to-plasma distribution ratios for diazepam and nordiazepam in the cat were 0.56 ± 0.13 and 0.65 ± 0.19, respectively, which are similar to the 0.64 and 0.60 values observed for diazepam and nordiazepam in humans (15). From the blood-to-plasma distribution ratios, it is possible to estimate from blood concentration data the amount that would be in a corresponding plasma sample by simply dividing the blood concentration by the distribution ratio (16). In addition, it is possible to correct the pharmacokinetic parameters V and CL calculated from the blood concentration data for comparison with similar parameters calculated from plasma concentration data in other studies (16). Similarly, it was possible to calculate blood free fraction from known plasma free fraction values.

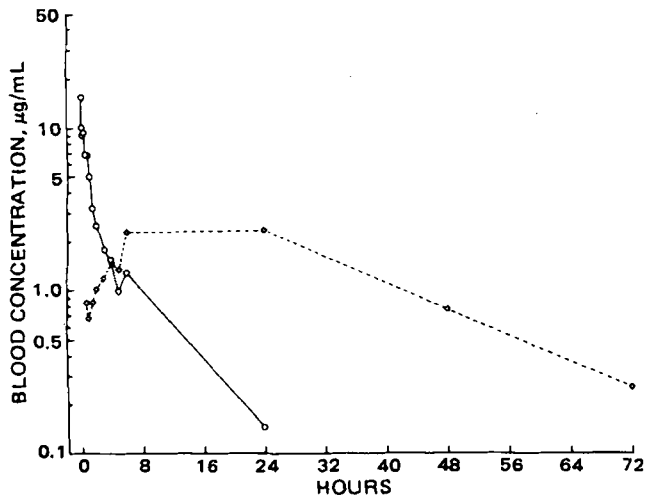

Figure 2—*Diazepam and nordiazepam blood concentrations after 10-mg/kg diazepam intravenous administration to Cat 5. Key: (○) diazepam; (◇) nordiazepam.*

Typical diazepam blood concentration–time curves following the administration of 5, 10, and 20 mg/kg of diazepam to a representative cat are presented in Fig. 1. The decline of diazepam blood concentrations were described by exponential functions. Visual inspection of the observed points about the fitted curve indicated a satisfactory randomness of scatter. Percent coefficients of variation of the parameters, P, π, A, α, B, and β were <50% (Table I), indicating that the good fit was dependent on each of these parameters. The disposition (α) phase was rapid with a harmonic mean half-life[7] of 0.35 h, and the harmonic mean terminal elimination half-life was 5.46 h.

The theoretical areas are virtually identical with the areas calculated from the trapezoidal rule. There is a proportional increase in diazepam blood concentrations between the 5-, 10-, and 20-mg/kg dose (Fig. 1). This relationship was supported by a doubling of the AUC_{tr} of diazepam with a doubling of the administered dose of diazepam (Table I). The mean ±*SD* total body clearance of diazepam is 4.72 ± 2.45 mL/min/kg, which represents an extraction ratio of 0.12 based on a total hepatic blood flow of 40 mL/min/kg (17) and assuming that diazepam is eliminated totally by hepatic clearance. The average intrinsic clearance (CL_I) is 5.35 mL/min/kg, which indicates that total body clearance is only slightly dependent on the hepatic blood flow. The total body clearance varied among cats, but for a single cat the clearance was constant over the dose range studied. It was concluded from the linear relationship of the blood concentrations and the areas under the blood concentration–time curves to the dose of diazepam administered, and the constant clearance within animals, that the pharmacokinetics of diazepam in the cat are linear over the dose range studied.

The mean ±*SD* of V_c and Vd_β were 651 ± 235 and 3075 ± 1603 mL/kg, respectively. The ratio of V_c/Vd_β (0.23 ± 0.094) suggests that ~23% of the dose of diazepam in the animal is in the central compartment available for elimination at any time, whereas ~77% of the dose is in a peripheral compartment unavailable for elimination. Distribution to a peripheral compartment tends to reduce the overall elimination of diazepam in humans, as previously observed by Kaplan *et al.* (18). The value for Vd_β was greater than total body water, which is generally indicative of extensive binding and/or localization in the tissue space. Previous studies with the cat also indicated that diazepam is highly bound to plasma protein (94%)[8].

From the following equation (19), one can estimate the average fraction (F_T) of diazepam in tissue space that is not bound to tissue:

$$F_T = \frac{V_T \cdot F_B}{Vd_\beta - V_B} \qquad \text{(Eq. 12)}$$

where the volume of distribution (Vd_β) was 3075 mL/kg, the volume of the blood (V_B) was 66.7 mL/kg (20), the average volume of the tissue (V_T) (21, 22) was 563 mL/kg, and the calculated fraction of the drug that is free in blood (F_B) was 0.11. Solving Eq. 12 for F_T yields a value of 0.019. Therefore, an estimate of the fraction of diazepam in the tissue that is bound to tissue protein is 0.981.

Figure 2 shows typical blood concentration–time curves for diazepam and its major blood metabolite, nordiazepam, observed after a 10-mg/kg iv dose of diazepam. The concentrations and the resulting AUC values of nordiazepam, the major blood metabolite seen after diazepam administration, appeared to increase in proportion to the dose of diazepam administered (Table II). Initially, the formation rate of nordiazepam after diazepam administration appeared to be rapid for 6–8 h and slower thereafter. Similar rate phenomena were observed in humans (18). The

[7] Since β rather than half-life appears to have a normal distribution, harmonic rather than arithmatic mean half-lives are reported. (P. R. Gwilt and S. P. Sytsma. Paper presented at 29th national meeting of the American Pharmaceutical Association, San Antonio, Texas, Nov. 1980.)

[8] Data on file, Hoffmann-La Roche, Nutley, NJ 07110.

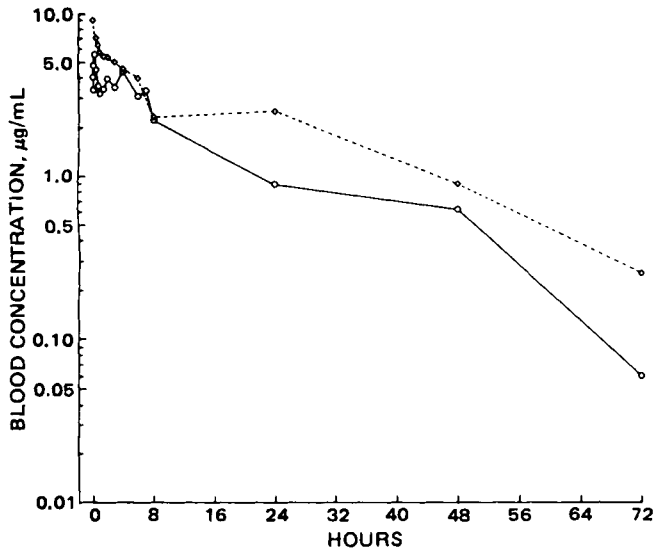

Figure 3—*Nordiazepam blood concentrations for Cat 1 after intravenous administration of 5- (○) and 10- (◇) mg/kg doses of nordiazepam.*

maximum blood concentrations of nordiazepam were generally observed at 24 h after diazepam administration. The value is only an approximation since no samples were obtained between 7 and 24 h after drug administration. The mean $\pm SD$ terminal elimination rate constant for nordiazepam was $0.032 \pm 0.012\ h^{-1}$, with a harmonic mean half-life of 21.3 h, which is 4.3 times slower than diazepam. From this data for nordiazepam after diazepam administration, it can be inferred that the amount of nordiazepam present is also directly proportional to the dose of diazepam administered and that the elimination of nordiazepam is not affected by the presence of diazepam over the dose range studied.

Corresponding nordiazepam data (Table III) were obtained following 10-mg/kg nordiazepam administration as a short (20-s) infusion to the same cats that received diazepam. In addition, one cat received 5 mg/kg of nordiazepam. Figure 3 is a typical nordiazepam blood concentration–time curve; the observed nordiazepam data could be satisfactorily described by a biexponential equation except for the cat that received 5 mg/kg, whose data appeared to be monoexponential. The disposition phase (α) for nordiazepam was rapid and variable (Table III). The terminal elimination phase (β) was much slower and less variable, with a harmonic mean elimination half-life of 21 h. The t test ($p < 0.05$) showed no significant difference between the terminal elimination half-lives of nordiazepam formed from diazepam and those for nordiazepam itself. This substantiates the earlier observation that the terminal elimination of nordiazepam after diazepam administration is not affected by the presence of diazepam nor by the rate of nordiazepam formation following diazepam administration.

The clearance of nordiazepam (mean $\pm SD = 0.85 \pm 0.24$ mL/min/kg) was approximately one-sixth the clearance of diazepam. This clearance value suggests a negligible potential for first-pass metabolism for nordiazepam. The model-independent value for the volume of distribution (Vd_β) for nordiazepam is also greater than total body water, which is generally indicative of extensive binding and/or localization in the tissue space as previously indicated for diazepam. As seen with diazepam (18), the distribution of nordiazepam into peripheral compartments reduces the overall elimination of nordiazepam from the cat.

Comparing the area of nordiazepam after nordiazepam administration to area of nordiazepam after the administration of an equivalent dose of diazepam (Table II), it was estimated that an average of 54% of the diazepam dose was biotransformed to nordiazepam in the cat. This observation of 54% conversion of diazepam to nordiazepam in the cat is in good agreement with the 50% fractional conversion of diazepam to nordiazepam observed in humans (23).

The observation that the pharmacokinetics in the cat were linear with respect to dose has been observed in humans. Greenblatt *et al.* (24) observed that in cases of diazepam overdose in humans, the terminal elimination half-lives for diazepam fell within the range observed in individuals after a therapeutic dose of diazepam. When the pharmacokinetic data in the cat are compared with those known for humans, a much more rapid elimination for diazepam and nordiazepam is apparent in the cat. The elimination half-lives of diazepam in humans are reported to be in the range of 24–48 h for adults (23) and 18 h for children, and the terminal elimination half-lives for nordiazepam are reported to be in the range of 51–120 h (25). It is apparent that nordiazepam has a longer elimination half-life than diazepam in humans; this relationship in the half-lives was also observed for the cats during this study. The first-pass effect of diazepam and nordiazepam in the cat and human is negligible compared with other animal species.

In conclusion, the cat has been previously used as an experimental model for studying the CNS pharmacology of diazepam and nordiazepam. The similarities between cat and human with respect to the pharmacokinetics of these compounds suggest that the cat may not only be a good model species for pharmacological studies but for pharmacokinetic studies as well.

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