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Pharmacokinetics of Nordiazepam in Physical Dependence and Precipitated Abstinence in Dogs

ELZBIETA P. WALA,[1] WILLIAM R. MARTIN AND JEWELL W. SLOAN

Department of Anesthesiology, Room 209, Research Facility #3, University of Kentucky, Lexington, KY 40536-0216

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WALA, E. P., W. R. MARTIN AND J. W. SLOAN. *Pharmacokinetics of nordiazepam in physical dependence and precipitated abstinence in dogs.* PHARMACOL BIOCHEM BEHAV 44(4) 857–864, 1993.—Previous studies suggested that the extensive accumulation of benzodiazepines is an important factor in the induction of physical dependence. The mechanistic basis for accumulation of nordiazepam (ND) and its metabolite, oxazepam (OX), have been examined in crossover studies in drug-naive and in ND-dependent dogs that exhibited a flumazenil-precipitated abstinence syndrome. ND and parent OX have similar pharmacokinetic profiles. Steady-state plasma levels of ND and OX cannot be predicted from single-dose pharmacokinetics. Reduced plasma clearance of ND and altered plasma protein binding were observed in dogs physically dependent upon ND. The benzodiazepine antagonist, flumazenil, significantly reduces steady-state plasma levels of total and free ND.

Nordiazepam Flumazenil Nordiazepam pharmacokinetics Nordiazepam physical dependence Flumazenil-precipitated abstinence

NORDIAZEPAM, a major metabolite of several benzodiazepines (diazepam, halazepam, clorazepate, medazepam, prazepam) possesses considerable pharmacological activity and for this reason may contribute significantly to the effect produced by the parent drugs. Although there is a body of literature covering the pharmacokinetics of nordiazepam when its disposition is altered by the continued hepatic and/or extrahepatic formation from the different prodrugs [cf. (9)], there are fewer studies of the pharmacokinetics and metabolism following administration of nordiazepam that is not formed from its precursor (7,14). The pharmacokinetic characteristics of nordiazepam in relation to physical dependence and precipitated abstinence syndrome has not been addressed.

There have been a number of observations suggesting that metabolites play an important role in the generation of physical dependence upon benzodiazepines (20). In dogs, chronic treatment with diazepam, nordiazepam, and oxazepam results in the induction of physical dependence as revealed by a flumazenil-precipitated abstinence syndrome that differs qualitatively and quantitatively (20). During multiple-dose administration of diazepam or nordiazepam to the dog, nordiazepam, and, to a lesser degree, oxazepam accumulate in plasma and brain tissue, whereas diazepam does not accumulate in diazepam-dependent dogs and oxazepam does not accumulate in oxazepam-dependent dogs (18,19,30). Because high plasma levels of nordiazepam and oxazepam may be responsible for induction of physical dependence in dogs chronically administered nordiazepam or diazepam (17,19,20), it is important to explain the mechanistic basis of accumulation of these drugs. Accordingly, the present studies were carried out to compare pharmacokinetic profiles of nordiazepam and oxazepam in drug-naive dogs and in dogs physically dependent upon nordiazepam. Another objective was to determine whether pharmacokinetic interactions occur between nordiazepam, its metabolite, oxazepam, and the benzodiazepine antagonist, flumazenil, during precipitation of abstinence in nordiazepam-dependent dogs.

The metabolic profiles of diazepam or nordiazepam in dog and man are similar. However, dogs metabolize benzodiazepines much more rapidly than humans (3), which results in a different pattern of parent drug/metabolite(s) accumulation ratio. Thus, although the dog is the most suitable animal model for testing benzodiazepines, the limitation of extrapolating pharmacokinetic data from dog to man must be recognized and appreciated.

[1] To whom requests for reprints should be addressed.

ANIMALS

Six female beagle-type dogs (bw 7.6–9.0 kg) were used in these studies. Dogs were housed in an AAALAC facility and the experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals. To our knowledge, dogs had not previously received benzodiazepines or any other CNS drugs. Dogs were not deprived of food (standard diet) and water before dosing.

SINGLE-DOSE STUDY

The acute study consisted of four crossover single-dose treatments: a) IV oxazepam; b) oral oxazepam; c) IV nordiazepam; and d) oral nordiazepam. One week elapsed between these studies. Oxazepam and nordiazepam were dissolved in propylene glycol : ethyl alcohol : saline (2 : 2 : 1) (5 mg/ml solution filtered through 0.22-μm filter) and then given IV as a bolus injection in a dose of 1.5 mg/kg. Oxazepam and nordiazepam were given orally (PO) in #4 gelatin capsules containing 120 mg of drug appropriately diluted with lactose. Venous blood samples were taken at 5, 15, 30, 45 min and 1, 2, 3, 4, and 6 h after IV and 0.5, 1, 1.5, 2, 4, 6, and 8 h after PO administration and analyzed for the plasma time courses of nordiazepam and parent and formed oxazepam.

CHRONIC STUDY

The same dogs were used to study physical dependence on nordiazepam. Nordiazepam was administered PO in escalating doses until a dose of 36 mg/kg/day (administered in 120-mg doses at 7:00 a.m., 2:30 p.m., and 10:00 p.m.) was achieved. Approximately 2 weeks were required to reach the stabilization dose. One dog lost weight precipitously and for that reason was stabilized on a dose of 27 mg/kg/day. Dogs were held at the stabilization dose for 2 weeks before they participated in precipitation studies. The benzodiazepine antagonist, flumazenil, was given PO in a dose of 18 mg/kg at weekly intervals for 3 weeks and thereafter at 2-week intervals (weeks 4–14). The intensity of the precipitated abstinence syndrome was measured using a BPAS scale (20). "Trough" plasma levels of nordiazepam and oxazepam were determined weekly during 18 weeks of stabilization. After the precipitation experiment was completed (week 15), 2-ml blood samples were collected at 0.5, 1.5, 2, 3, 4, 6, and 8 h after the morning dose of nordiazepam and analyzed for the time courses of nordiazepam and oxazepam at stabilization. The next day, 1 h after the morning dose of nordiazepam, an 18-mg/kg dose of flumazenil was administered PO in a gelatin capsule. Two-milliliter blood samples were collected before and 15, 30 min and 1, 1.5, 2, 3, 4, 5, and 7 h after flumazenil administration and analyzed for the time courses of nordiazepam, oxazepam, and flumazenil. Nordiazepam-dependent dogs were subsequently employed in microdialysis experiments (28).

PLASMA PROTEIN BINDING

For each dog, the extent of plasma protein binding was determined after single PO doses of oxazepam and nordiazepam and after 18 weeks of chronic PO administration of nordiazepam with and without coadministration of flumazenil. Plasma samples were collected 2 h after a single or chronic morning dose of nordiazepam (1 h after the dose of flumazenil) and analyzed for the free and total drugs as follows. Plasma (0.8 ml) was dialyzed in duplicate against (0.8 ml) isotonic phosphate–sodium chloride buffer (pH 7.3) at 37°C for 20 h in a light-free shaking water bath. Concentrations of nordiazepam, oxazepam, and flumazenil were determined at equilibrium in buffer (free) and in plasma (free plus bound) by high-performance liquid chromatography (HPLC). The extent of plasma protein binding (free fraction) was calculated from the ratio of free to total concentrations.

ENZYMATIC HYDROLYSIS

Plasma samples were collected 8 h after single and chronic (weeks 1, 3, 8, 14, and 17 of stabilization) PO doses of nordiazepam. Each sample was incubated in duplicate for 20 h (pH = 5, 37°C) with and without 5,000 U/ml β-glucuronidase/sulfatase (type H-1, activity 365,000 U/g; Sigma Chemical Co., St. Louis, MO). The samples were thereafter analyzed by the same procedure as described for unchanged oxazepam. The levels of conjugates were calculated as differences of unchanged oxazepam in control and hydrolyzed sample. The optimal hydrolysis conditions (time, pH, enzyme concentration) were evaluated before the hydrolysis experiment.

ANALYSIS

Blood samples were collected from a site separate from the site of IV administration through a venous catheter into vacutainer tubes containing disodium EDTA. Following centrifugation, plasma samples were separated and stored at −20°C until analyzed. Duplicate determinations were performed with each plasma sample. Levels of total and free nordiazepam, oxazepam, and flumazenil were determined by HPLC as reported previously (27,30). The retention times are equal to about 3, 7, and 10 min for flumazenil, oxazepam, and nordiazepam, respectively, and to 5 min for flunitrazepam (internal standard). There was no interference between any of the drugs. Blood chemistry (veterinary profile #1, Smith Kline Bioscience Laboratories, Philadelphia, PA) was determined on several occasions.

PHARMACOKINETICS

Noncompartmental analysis has been employed for estimation of pharmacokinetic parameters (6). Areas under the plasma concentration time curves (AUCs) were calculated by the linear trapezoidal method and the residual area extrapolated to infinity was added. The elimination half lives ($t_{1/2}$) were calculated from the terminal slopes. The volume of distribution (V_d) and systemic clearance (Cl) were determined using the area method. Bioavailability (F) was determined as a ratio of AUCs (corrected for the doses) after PO and IV administrations. Maximum concentration (C_{max}) and maximum time (T_{max}) were estimated by the visual inspection of the data. Mean total steady-state plasma levels (C_{ss}) were calculated by dividing the AUC during dosage intervals by the time interval (t). For each dog, the single-dose oral availability (F) was employed in calculations of plasma clearance at steady state. Ratio of formation and elimination clearances was calculated according to mass balance consideration as the ratio of mean steady-state levels of metabolite ($C_{ss\,m}$) and parent drug ($C_{ss\,p}$) (16). Free clearance (Cl_u) was estimated by dividing total oral plasma clearance by free fraction (ff). Accumulation index (R) was calculated as the ratio of AUCs after single and chronic oral dose administrations of nordiazepam to the same dog. The fraction of nordiazepam converted to systemically appearing oxazepam (f_m) was generated from the AUCs of parent and formed oxazepam obtained following IV and

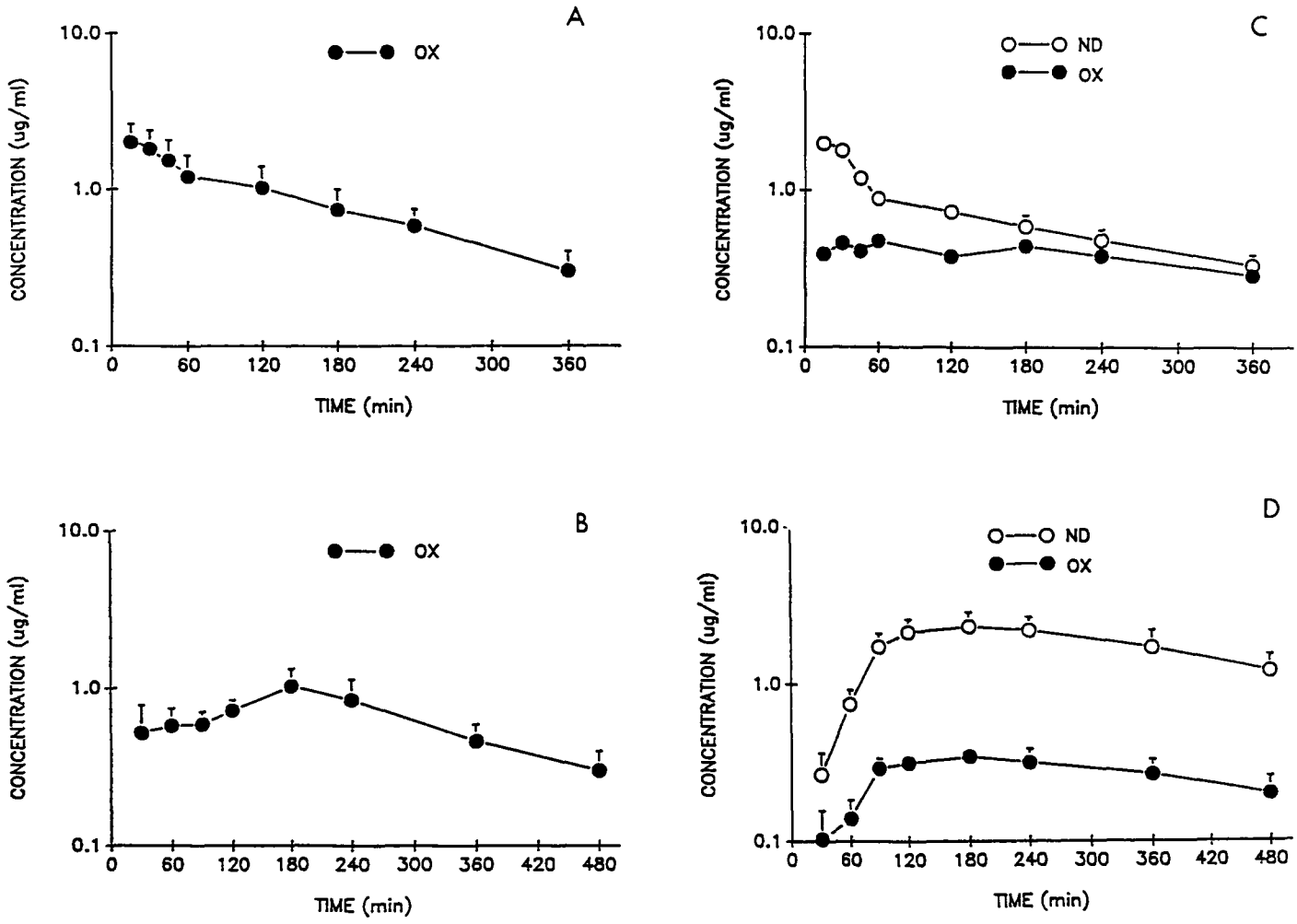

FIG. 1. Plasma concentrations of nordiazepam (ND) (○) and oxazepam (OX) (●) determined following crossover administrations of single doses of: (A) OX, IV 1.5 mg/kg; (B) OX, PO 120 mg; (C) ND, IV 1.5 mg/kg; (D) ND, PO 120 mg. Values are mean ± SEM of six dogs.

PO administration of nordiazepam and oxazepam (10). The oral availabilities of nordiazepam and oxazepam and the correction factor for the molecular weight differences were employed in the above calculations. Data were analyzed using paired *t*-test and a two-way analysis of variance (ANOVA) (dogs × weeks) with the weeks variance partitioned into regression and deviation from regression variance.

RESULTS

Figure 1 shows the mean plasma concentration time curves generated following administration of single IV (1.5 mg/kg) and single oral (120 mg) doses of nordiazepam and oxazepam to dogs. IV administered nordiazepam and oxazepam have almost identical biexponential plasma time courses, described by a rapid initial distribution followed by elimination with an apparent half-life time equal to 3.6 h. Oral administration of equal doses of nordiazepam and oxazepam results in significantly higher plasma levels of nordiazepam than oxazepam; however, the time peaks and the terminal half-lives are similar for both drugs. In dogs, as in man, nordiazepam is metabolized to oxazepam. The formed oxazepam appears in plasma immediately after IV and oral administration of nordiazepam and declines approximately in parallel with the parent drug.

Figure 2 shows that in nordiazepam-dependent dogs untreated or precipitated with a single oral dose of the benzodiazepine antagonist, flumazenil, stable plasma levels of nordiazepam and oxazepam are sustained during 8-h dosing intervals. However, 30 min after administration of flumazenil at each time point plasma levels of nordiazepam are significantly lower than in untreated animals. Flumazenil reaches a maximum concentration in plasma at about 1 h and then rapidly declines with a half-life time equal to 1.7 h.

Table 1 summarizes the mean pharmacokinetic parameters of nordiazepam and oxazepam derived in acute studies. The volume of distribution, terminal half-life time, total plasma clearance, and free plasma clearance of nordiazepam and parent oxazepam are not significantly different. Terminal half-life of formed oxazepam is independent of the route of nordiazepam administration (5.3 h for IV and 6.5 h for PO) but is longer than the $t_{1/2}$ for parent oxazepam (3.6 h). Absolute systemic availabilities of nordiazepam and oxazepam from the identical oral preparations are equal to 33 and 14%, respectively. The amount of IV and orally administered nordi-

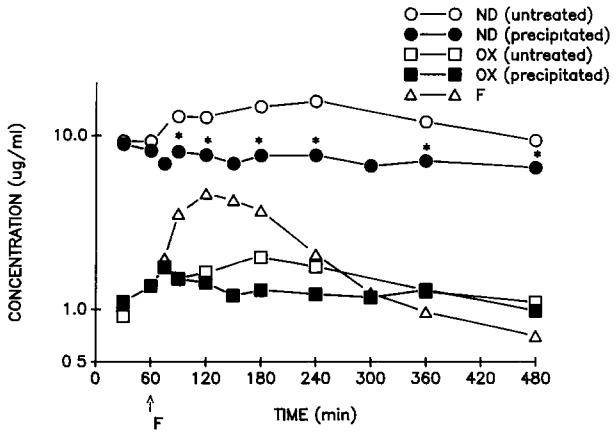

FIG. 2. Mean plasma concentrations of nordiazepam (ND), oxazepam (OX), and flumazenil (F) in ND-dependent dogs (n = 6). A stabilization dose of ND (36 mg/kg/day) was administered every 8 h. One hour after the morning dose, dogs were either untreated or received a single oral dose (18 mg/kg) of flumazenil. Levels of ND before (○) and after (●) administration of F, levels of OX before (□) and after (■) administration of F, and levels of F (△). *Significantly different from untreated (no flumazenil) dogs ($p < 0.05$), paired t-test.

azepam that is converted to oxazepam is about 85 and 58%, respectively. The major proportion of formed oxazepam is conjugated (ca. 85%).

Table 2 summarizes the pharmacokinetics of nordiazepam and oxazepam in nordiazepam-dependent dogs untreated and precipitated with flumazenil. Based upon the elimination rate constant obtained in the single-dose study, the accumulation factor of nordiazepam administered every 8 h is equal to about 1.3. However, in nordiazepam-dependent dogs accumulation indexes for total and free nordiazepam are equal to about 10 and 6, respectively, which indicates that after chronic treatment plasma levels are higher than predicted from single-dose pharmacokinetics. Total plasma clearance of nordiazepam is significantly reduced and there is a trend for the free plasma clearance and total and free oxazepam/nordiazepam concentration ratios to decrease at steady state. Following administration of flumazenil, the mean stabilization plasma levels of total (bound + free) and free nordiazepam are significantly reduced. Further, total and free plasma clearances and total and free oxazepam/nordiazepam steady-state concentration ratios are enhanced.

The results of plasma protein binding (free fractions) of nordiazepam and oxazepam (Tables 1 and 2) revealed that the free fraction of nordiazepam is significantly higher following an acute dose of nordiazepam than at steady state and that flumazenil significantly elevates free fractions of oxazepam but not nordiazepam.

Figure 3 compares the total and free plasma clearances and the free fractions of nordiazepam in the individual dogs that were administered a single dose of nordiazepam in naive state, stabilized on chronic doses of nordiazepam, and precipitated with a single dose of flumazenil. In each dog, total and free plasma clearances and free fraction of nordiazepam are lower following chronic then single-dose treatments with nordiazepam. A single dose of flumazenil tends to enhance total and free plasma clearances and free fraction of nordiazepam.

Figure 4 shows that across the time of chronic treatment with nordiazepam the percent of conjugated oxazepam in plasma tends to be lower than after a single oral dose. A two-way ANOVA (dogs × weeks) revealed no significant between-dogs variance for percent of conjugated oxazepam and a significant between-weeks variance, $F(6, 4) = 7.2$, $p <$

TABLE 1

PHARMACOKINETIC CHARACTERISTICS OF NORDIAZEPAM (ND) AND OXAZEPAM (OX) FOLLOWING A SINGLE IV AND PO ADMINISTRATION OF ND AND OX

	Drug Administered					
	Oxazepam		Nordiazepam			
Dose and Route of Administration	1.5 mg/kg IV	120 mg PO	1.5 mg/kg IV		120 mg PO	
Drug Determined	OX	OX	OX	ND	OX	ND
AUC ($\mu g\ ml^{-1}\ min^{-1}$)	419.5 ± 115.1	418.6 ± 80.7	266.2 ± 22.2	380.8 ± 54.5	272.3 ± 83.3	1291.7 ± 374.8
$t_{1/2}$(h)	3.6 ± 0.8	3.8 ± 0.6	5.3 ± 0.8	3.6 ± 0.6	6.5 ± 1.9	4.1 ± 0.8
$V_{d\ area}$(l/kg)	1.6 ± 0.4			1.2 ± 0.7		
Cl($ml\ min^{-1}\ kg^{-1}$)	4.9 ± 0.9			4.5 ± 0.9		
Cl_u($ml\ min^{-1}\ kg^{-1}$)	48.5 ± 9.0			51.0 ± 10.4		
C_{max} ($\mu g\ ml^{-1}$)		1.3 ± 0.3	0.58 ± 0.04		0.39 ± 0.04	2.8 ± 0.5
T_{max}(h)		2.3 ± 0.5	1.6 ± 0.5		3.0 ± 0.4	2.7 ± 0.4
C_m/C_p			0.83 ± 0.21		0.26 ± 0.06	
$(C_m/C_p)_u$			1.61 ± 0.73		0.51 ± 0.19	
F(%)		14.1 ± 4.5				33.6 ± 8.9
f_m(%)			85.5 ± 19.9		58.2 ± 14.5	
ff		0.100 ± 0.007			0.168 ± 0.042	0.097 ± 0.013

Values are mean ± SEM of six dogs used in this study. AUC, area under the curve; $t_{1/2}$,half-life time; $V_{d\ area}$, volume of distribution; Cl, total plasma clearance; Cl_u, free plasma clearance; C_{max}, peak concentration; T_{max}, time peak; C_m/C_p, OX/ND AUC's ratio for total drugs; $(C_m/C_p)_u$, OX/ND AUC's ratio for free drugs; F, systemic availability; f_m, fraction of ND converted to OX; ff, free fraction.

TABLE 2

PHARMACOKINETIC CHARACTERISTICS OF NORDIAZEPAM (ND) AND OXAZEPAM (OX) IN ND-DEPENDENT DOGS (36 mg/kg/DAY) (120 mg t.i.d.) UNTREATED AND PRECIPITATED WITH FLUMAZENIL (18 mg/kg)

	Drug of Administration			
	Nordiazepam		Nordiazepam and Flumazenil	
Drug of Determination	OX	ND	OX	ND
AUC_{ss} ($\mu g\ ml^{-1}\ min^{-1}$)	$692.5^{0\,01} \pm 111.2$	$6015.8^{0\,01} \pm 926.3$	$526.8 \pm 91.2_{0\,005}$	$3030.6 \pm 444.0_{0\,001}$
Cl_{ss} ($ml\ min^{-1}kg^{-1}$)		$1.3^{0\,25} \pm 0.4$		2.5 ± 0.9
$Cl_{ss\,u}$ ($ml\ min^{-1}kg^{-1}$)		30.8 ± 11.5		$47.2 \pm 16.5_{0\,05}$
C_{ss} ($\mu g\ ml^{-1}$)	1.4 ± 0.2	12.5 ± 1.9	1.1 ± 0.2	$6.3 \pm 0.9_{0\,05}$
$C_{ss\,u}$ ($\mu g\ ml^{-1}$)	0.13 ± 0.03	0.56 ± 0.11	0.14 ± 0.02	$0.34 \pm 0.07_{0\,05}$
$(C_m/C_p)_{ss}$	0.12 ± 0.01		$0.17 \pm 0.01_{0\,005}$	
$(C_m/C_p)_{ss\,u}$	0.24 ± 0.01		$0.45 \pm 0.05_{0\,005}$	
ff	0.090 ± 0.007	$0.043^{0\,01} \pm 0.003$	$0.133 \pm 0.009_{0\,025}$	0.053 ± 0.004
R	4.4 ± 1.7	10.6 ± 5.6		
R_u	2.8 ± 0.9	5.9 ± 3.5		

Values are mean ± SEM of 6 dogs. Superscripts indicate the significant p values for the paired t comparison between single (Table 1) and chronic nordiazepam administration. Subscripts indicate the significant p values for the paired t comparison between untreated and precipitated dogs. $R = AUC_{ss}/AUC$; $R_u = AUC_{ss\,u}/AUC_u$.

0.001, with significant regression, $F(1, 4) = 17.3$, $p < 0.001$, and significant deviation from regression, $F(5, 41) = 5.2$, $p < 0.001$).

DISCUSSION

Results of this crossover pharmacokinetic study confirm observations (18) that nordiazepam and oxazepam accumulate in dogs physically dependent upon nordiazepam. In dogs, the accumulation of nordiazepam and oxazepam cannot be predicted from single-dose pharmacokinetics. This observation is not in agreement with the general assumption that in man steady-state plasma levels of benzodiazepines are predictable based upon single-dose studies (11). It must be emphasized, however, that in dogs and humans the rate of metabolism of benzodiazepines is different (3,12). Thus, to achieve similar plasma levels in man and dogs much higher doses of benzodiazepines must be administered to dogs. Most of the multiple-dose human pharmacokinetic data are generated following relatively low doses and short-term administration of benzodiazepines and for this reason may not be applicable to the chronic benzodiazepine use and abuse at higher doses for long periods of time. It has been reported that during long-term therapy with benzodiazepines high interpatient variability in plasma levels is observed but the factors contributing to this variance are not well established (8). There is a discrepancy in the literature on the metabolism and nonlinear pharmacokinetics of benzodiazepines [cf. (9)]. Some investigators have suggested that inhibition (15) or induction of *N*-demethylation of diazepam (22) and inhibition of 3-hydroxylation of nordiazepam (13,25) can cause unpredictable steady-state plasma levels. In man, nordiazepam does not alter the kinetics of diazepam (1). The possibility of alteration of the conjugation pathway and plasma protein binding during chronic treatment with benzodiazepines have received relatively little attention.

Our study shows that the pharmacokinetics and metabolism of nordiazepam is significantly different in naive and physically dependent dogs. Single-dose data indicate almost identical pharmacokinetic characteristics of nordiazepam and parent oxazepam. The close relationship between plasma clearances of nordiazepam (4.5 ml/min/kg) and oxazepam (4.9 ml/min/kg) suggests that, in the dog, 3-hydroxylation of nordiazepam and conjugation of oxazepam occur with similar rates. The parallel slopes for nordiazepam and formed oxazepam indicate formation rate-limited kinetics, and thus no accumulation of oxazepam should be expected upon chronic administration of nordiazepam. Total plasma levels of nordiazepam are higher than levels of formed oxazepam, is in agreement with the observed differences in plasma protein binding (about 96% for nordiazepam and about 90% for oxazepam) and with the lower than 100% conversion of nordiazepam to oxazepam. Most of oxazepam in plasma is conjugated, which confirms previous observations (24,26).

During multiple-dose administration, AUC_{ss} of nordiazepam is much higher than $AUC_{0\to\infty}$ after a single oral dose. Thus, plasma clearance at steady state is significantly reduced in comparison to clearance calculated in acute studies. This phenomenon can be caused by several different mechanisms. The possibility of enhanced absorption in nordiazepam-dependent dogs is unlikely; however, it cannot be ruled out without additional data from chronic parenteral studies. In the present study, dogs were neither withdrawn nor administered tracer doses of radioactive drug; thus, the exact contribution of the elimination and distribution parameters to the altered clearance cannot be defined. However, as previously reported from our laboratory (18) the terminal half-life time of nordiazepam determined during withdrawal abstinence in nordiazepam-dependent dogs (32 mg/kg/day q.i.d.) was equal to about 17 h and is significantly longer than the single-dose half-life (about 4 h) reported herein. Thus, a correlation between total clearance of nordiazepam and its half-life time can be anticipated. In each dependent dog, free plasma clearance of nordiazepam tends to decrease; however, the differences are of borderline significance due to the high between-subjects variability. Further, there is a trend for the total and free oxazepam/nordiazepam concentrations ratios to decrease at steady state. Because the metabolite to parent drug plasma concentration ratio depends solely upon formation (Cl_f) and elimination (Cl_m) clearances of metabolite (16), the observed alteration of oxazepam/nordiazepam ratio can be explained

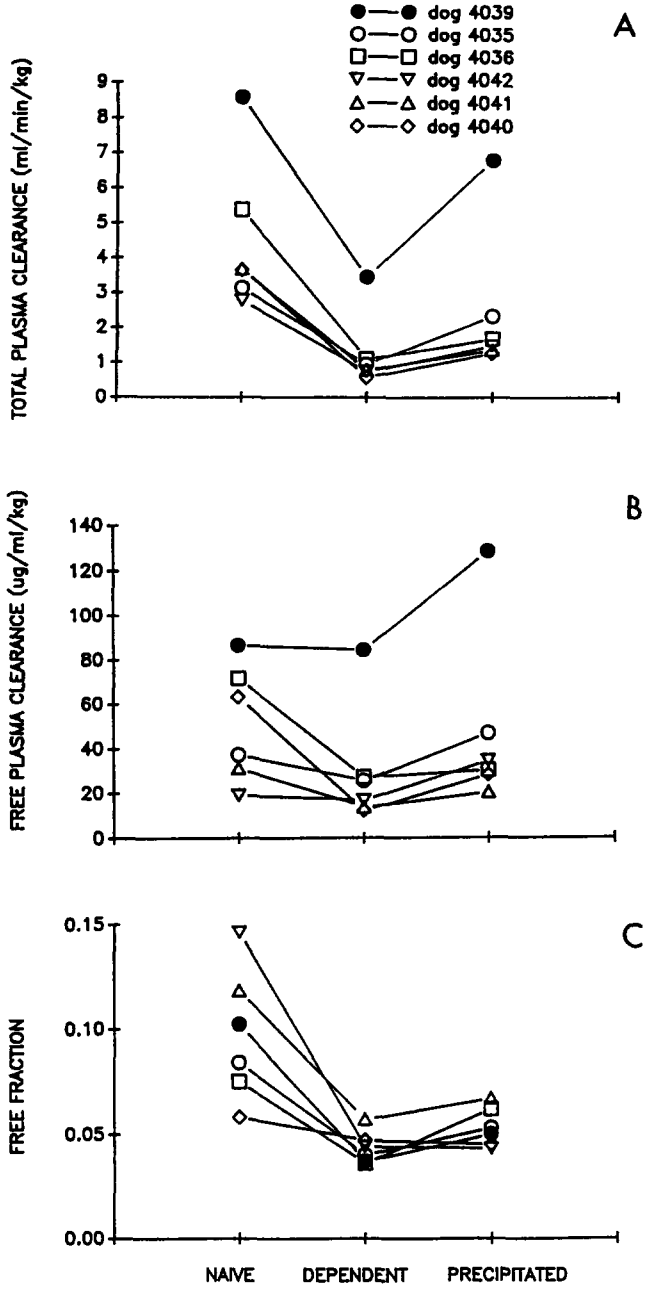

FIG. 3. Total (A) and free (B) plasma clearances and free fractions (C) of nordiazepam determined following single (naive) and chronic (dependent) doses of nordiazepam and chronic doses of nordiazepam with coadministered single dose of flumazenil (precipitated). The different symbols and connected points represent the individual dogs in the study.

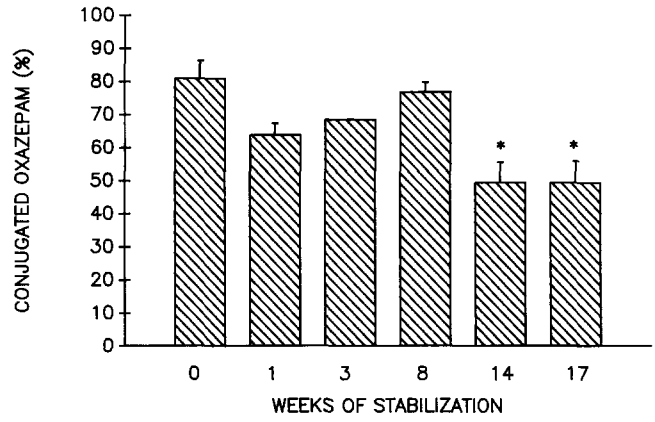

FIG. 4. Percent of conjugated oxazepam in plasma across time of chronic administration of nordiazepam. Values are mean ± SEM of six dogs. *Significantly different from single-dose study (week 0) ($p < 0.05$), paired t-test.

either by decrease in Cl_f of oxazepam or by unequal decreases in both ($Cl_f > Cl_m$). The possibility of increase in Cl_m or unequal increases in both clearances ($Cl_m > Cl_f$) can be ruled out because the percent of conjugated oxazepam in plasma at steady state is lower than after a single dose of nordiazepam. The above observations together suggest that the reduced formation of oxazepam due to inhibition of the oxidative pathway may be responsible for the extensive accumulation of nordiazepam in physically dependent dogs. Impaired metabolism of nordiazepam has been previously suggested in relation to age and liver disease (2,23).

Surprisingly, plasma protein binding of nordiazepam and oxazepam tend to be lower following a single oral dose of nordiazepam than during multiple-dose treatment. Although it is a reproducible trend also observed in dogs acutely and chronically dosed with diazepam (29), the following observations make this finding difficult to explain: a) In vitro, plasma protein binding of benzodiazepines tend to decrease with concentration and in the presence of interacting drugs (4,21,28); b) in nordiazepam-dependent dogs, clinical blood chemistries showed that total proteins, albumins, and globulins were lower than normal canine values and did not change significantly throughout 18 weeks of chronic treatment (Sloan, unpublished observations); c) the extent of plasma protein binding determined at steady state is in good agreement with the results generated in vitro (28), whereas protein binding after an acute dose is lower. Alteration of plasma protein binding during subchronic treatment with diazepam has been ruled out on a basis of constant blood to plasma distribution ratio (13). However, to our knowledge there is a lack of in vivo data that directly compare binding following acute and chronic treatments. Thus, it cannot be ruled out that the extent of binding of nordiazepam to plasma proteins in the phase of its extensive distribution following an acute dose and at steady state is different. Although an alteration of plasma protein binding and enzymatic activity have different pharmacokinetic consequences (33), the present data suggest that both can cause an accumulation of nordiazepam and oxazepam in plasma at steady state.

As was previously reported from our laboratory, in nordiazepam-dependent dogs the free steady-state levels of nordiazepam and oxazepam in plasma correlate well with the levels in the extraneuronal brain space (28). Thus, it is possible that during chronic treatment between-subjects differences in metabolism and plasma protein binding result in different levels of nordiazepam and oxazepam in the vicinity of membrane receptors. This might predispose some individuals to a greater vulnerability for the induction of physical dependence.

It is noteworthy that in nordiazepam-dependent dogs the benzodiazepine antagonist, flumazenil, significantly reduces

steady-state plasma levels of nordiazepam. This is a reproducible observation reported earlier for dogs chronically dosed with nordiazepam (18 mg/kg/day q.i.d.) and precipitated with 6 mg/kg flumazenil (32). Further, a similar pharmacokinetic interaction has been observed after flumazenil administration in dogs chronically dosed with oxazepam (270 mg/kg/day) (30). The mechanism by which flumazenil changes steady-state plasma levels of benzodiazepines is uncertain; however, some comments can be made regarding this observation: a) There is no competition between flumazenil and nordiazepam on plasma protein binding because the free fraction of nordiazepam does not change significantly in the presence of flumazenil in vivo and in vitro (31); b) the free plasma clearance of nordiazepam and free oxazepam/nordiazepam steady-state concentration ratio are significantly enhanced by flumazenil, which may suggest an alteration of the oxidative pathway; c) alternatively, flumazenil may enhance the compartmental redistribution of nordiazepam due to increase of cerebral blood flow (5). Further, oxazepam and flumazenil may bind to the same site(s) on plasma protein because flumazenil significantly elevates the free fraction of oxazepam.

In summary, this study demonstrates a variable accumulation of nordiazepam and oxazepam in dogs physically dependent upon nordiazepam and a significant reduction of steady-state plasma levels of nordiazepam during precipitated abstinence with flumazenil.

ACKNOWLEDGEMENTS

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