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Pharmacokinetics and Metabolism of Halazepam in Naive and Dependent Dogs

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WALA, E. P., J. W. SLOAN, W. R. MARTIN AND T. PRUITT. *Pharmacokinetics and metabolism of halazepam in naive and dependent dogs*. PHARMACOL BIOCHEM BEHAV **38**(3) 561–567, 1991.—The pharmacokinetic profiles of halazepam (HL) and its metabolites, desmethyldiazepam (DMDZ), oxazepam (OX), 3-hydroxyhalazepam (OH-HL), and conjugates of oxazepam (OX-CONJ) and 3-hydroxyhalazepam (OH-HL-CONJ) were studied in 4 naive dogs following single intravenous (2 mg/kg) and oral (112.5 mg/kg) administrations of HL and in 5 dependent dogs chronically dosed with HL (450 mg/kg/day q.i.d.). HL is rapidly metabolized to DMDZ as the principal metabolite but appreciable levels of HL, OX and OH-HL were measured in plasma and the brain tissue. High levels of conjugated metabolites were measured in plasma. The steady-state plasma concentrations of HL and its unconjugated metabolites can be predicted from the single dose study. Halazepam does not serve as a simple prodrug for DMDZ in producing physical dependence in dogs.

Halazepam pharmacokinetics Halazepam metabolism Halazepam dependence

HALAZEPAM (Paxipam) is a benzodiazepine used for the treatment of anxiety. The chemical structure of halazepam (Fig. 1) differs from that of diazepam only with regard to the substitution on the 1 position of nitrogen. The metabolism of halazepam resembles that of diazepam in that the major metabolic pathway for both drugs involves N-dealkylation, yielding desmethyldiazepam as the principal metabolite. Two hydroxylated metabolites of halazepam, oxazepam and 3-hydroxyhalazepam, have also been detected following halazepam administration.

Preliminary evaluation of the pharmacokinetic properties of halazepam (6) suggests that halazepam serves mainly as a precursor of desmethyldiazepam similar to several other benzodiazepines (7,8). There are only a few published data on pharmacokinetics of orally administered halazepam (3,7). Furthermore, the lack of preparations for intravenous administration has prevented the estimation of the pharmacokinetic variables of halazepam, especially systemic clearance, volume of distribution and bioavailability. One of the objectives of the present study was to evaluate the comparative pharmacokinetic profile of halazepam and its major metabolites in drug-naive dogs administered single intravenous and oral doses of halazepam and in dogs chronically dosed with halazepam. It has been reported that in dogs made physically dependent on halazepam, the benzodiazepine antagonist flumazenil precipitates an abstinence syndrome qualitatively different from that produced by nordiazepam (10). Another objective was to determine if the metabolism of halazepam could offer a hypothetical explanation of its dependence producing properties.

METHOD

Materials

Halazepam and 3OH-halazepam were supplied courtesy of Hoffmann-La Roche Co. Pure HPLC standards of desmethyldiazepam, oxazepam and diazepam were obtained from Sigma Chemical Co.

Animals

Dogs were housed in an AAALAC facility and the studies were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals*. The dogs were maintained on a standard diet with water ad lib. The dogs were not deprived of food or water before dosing.

Acute Study

Four female beagle type dogs (b.wt. 9.0–10.4 kg) were given halazepam as an intravenous (2 mg/kg) bolus injection of alcohol: saline (1:1) solution (10 mg/ml) and one week later as a single oral dose (112.5 mg/kg) in a No. 4 gelatine capsule with lactose as a filler. Blood samples were taken from a site separate from the site of the intravenous administration via a venous catheter into vacutainer tubes containing disodium EDTA at the following postdosage times: 5, 15, 30, 45 min and 1, 2, 3, 4, 5 and 6 h (IV) and 30, 45, 60, 90 min and 2, 3, 4, 5, 6, 8 and 12 h

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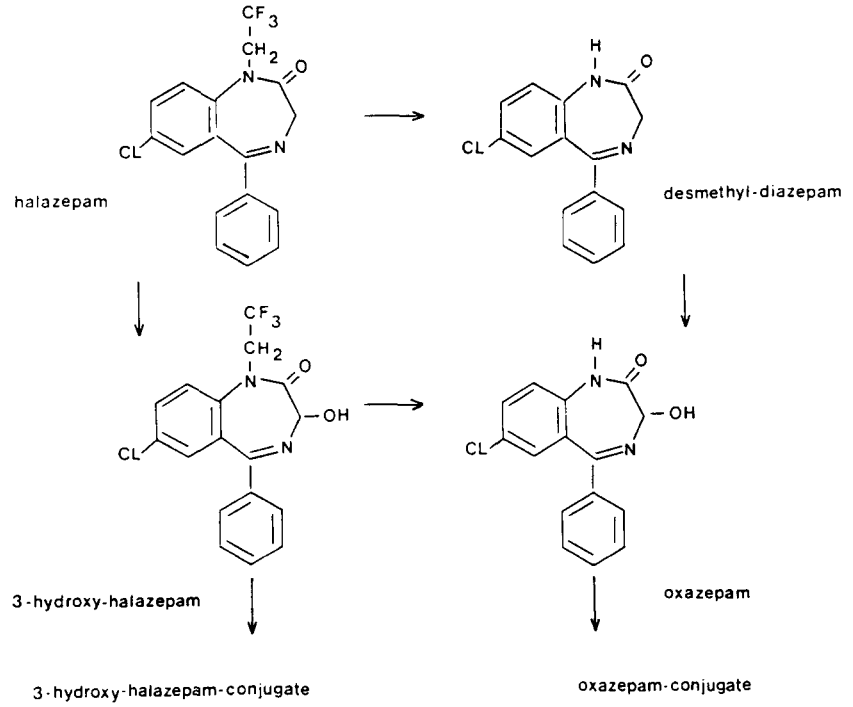

FIG. 1. Metabolic pathway of halazepam.

(PO). Plasma was analyzed for the time courses of halazepam and its metabolites.

Chronic Study

Two dogs who previously received single intravenous and oral doses of halazepam and three drug-naive dogs (b.wt. 8.4–11.2 kg) were used to study halazepam physical dependence (14). Halazepam, in No. 4 gelatine capsules, was administered orally at 7 a.m., 1 p.m., 7 p.m. and 12 a.m. in equally divided doses. The dose was escalated about 30 mg/kg/day until a dose of 450 mg/kg/day was achieved. The dogs were held at this dose for approximately 2 weeks before they participated in precipitation studies with flumazenil given orally in doses of 6, 18 and 36 mg/kg according to previously described procedures (9). "Trough" plasma levels of halazepam and its metabolites were determined several times during the dependence study. One week after the cross-over experiment was completed blood samples were drawn before and 60, 75, 90, 105 min and 2, 3, 4, 5 and 6 h after the dose of halazepam. Plasma was analyzed for the time course of halazepam and its metabolites at steady state. Finally, one hour after the morning dose of halazepam, the dogs were anesthetized with pentobarbital, a 7.5 cm spinal needle was inserted into the cisterna magna and CSF was sampled, blood was collected, and the calvarium removed. The brain was removed rapidly and dissected on ice. The brain tissues were then weighed and rapidly frozen in dry ice prior to storage at −70°C until analyzed.

Analysis of Drug

Following centrifugation, plasma samples were separated, frozen and stored at −20°C until analyzed. Duplicate determinations were performed with each plasma sample. Buffered (pH 9.5) Bond Elut C-18 1 cc columns were used for a solid phase extraction as described previously (15). Diazepam (50 ng injected) was used as the internal standard and was added to the samples prior to extraction.

Approximately 0.4 g samples of the ten brain areas (frontal, parietal and piriform cortex, fornix and hippocampus, thalamus and striatum, hypothalamus, cerebellum, medulla and pons) were weighed and homogenized with 4 volumes of 9% ice-cold NaCl for 60 s with a Brinkman polytron. Bond Elut, 3 cc/500 mg C-18 columns were used for the solid phase extraction of halazepam and its metabolites from the brain tissue. Columns were conditioned by washing four times with methanol and water and buffering with 300 μl of borax buffer (pH 9.5). The brain homogenates were vortexed for 20 s and 250 μl samples were placed on the column and washed with 250 μl of water. Twenty-five μl of the internal standard solution (diazepam 100 μg/ml in methanol) was added to the column followed by two 250 μl washes with water and one 100 μl wash with methanol. The Bond Elut columns were inserted into a SP-10 Baker System which was attached to a vacuum source. A 2 ml conical centrifuge tube was placed under each column. The columns were eluted three times with 200 μl of methanol. The eluants were evaporated under N_2, reconstituted in 25 μl methanol and 5 μl samples were injected into the HPLC.

Cerebrospinal fluid was centrifuged and then a 990 μl sample was spiked with 10 μl of internal standard solution (diazepam 100 μg/ml in methanol), vortexed and 5 μl injected into the HPLC.

The levels of halazepam and its metabolites were measured by comparing the peak area ratios of drug to internal standard against similarly prepared known concentrations of chromatographically pure standards. Levels of the conjugates were determined as oxazepam and 3OH-halazepam equivalents. The conjugates were identified after the 24-h hydrolysis of the samples at pH = 5 and 37°C with 5000 U/ml of B-glucuronidase/sulfatase (type H-1, activity 365000 U/g; Sigma Co.). Each plasma sample was incubated in duplicate with and without the enzyme and analyzed

thereafter by the same procedure as described for unchanged drugs. The levels of conjugates were calculated as differences of unchanged drugs in the parallel samples. The complete hydrolysis was assumed since HPLC peaks (ausf 0.05) of conjugated metabolites disappeared after treatment with the enzyme. For each halazepam-dependent dog the extent of halazepam, 3-hydroxyhalazepam, nordiazepam and oxazepam binding to plasma protein was determined by equilibrium dialysis. Plasma samples (0.8 ml) were dialyzed in duplicate at 37°C for 20 h against isotonic phosphate buffer pH 7.4. Free fractions were calculated as the ratio of free (dialysate) and total (plasma after dialysis) drug concentrations. Plasma levels of unbound drugs were estimated for each dog as the total plasma levels multiplied by the free fraction.

HPLC

The Waters 600 HPLC system consisting of a programmable multiwavelength detector, data module, reverse-phase Supelco LC-8 column (5 μm particle size) and guard LC-8 column was used for analysis of samples. The mobile phase, 2 mM KH_2PO_4 (pH 3.6), methanol and acetonitrile (45:54:1), was filtered and degassed before use. Operating conditions involved UV absorption at 240 nm and 0.5 ausf, mobile phase flow rate was 1.8 ml/min in the isocratic condition, chart speed was 1 cm/min, and the volume of injected samples was 5 μl.

Standard Curves

Standard curves of halazepam, desmethyldiazepam, oxazepam and 3OH-halazepam were obtained using both unextracted standard solutions prepared in methanol and standard solutions added and extracted from plasma and brain tissue obtained from drug-naive dogs. The nonextracted and extracted standard curves were linear over the range of 2.5 to 250 ng (injected on the column) with a limit of sensitivity of approximately 1.25 ng at ausf = 0.5 and day to day coefficient of variation less than 3% at a level of 50 ng. The mean recovery for oxazepam, desmethyldiazepam, 3 hydroxyhalazepam and halazepam was 84 ± 4%, 79 ± 5%, 73 ± 7% and 101 ± 1% from plasma and 98 ± 5%, 94 ± 4%, 66 ± 9% and 72 ± 7% from brain tissue, respectively.

Pharmacokinetics

Plasma concentration of halazepam and its metabolites in each dog was analyzed by model-independent pharmacokinetics (5). The elimination rate constant (β) was calculated by regression analysis as a slope of the postdistribution and postabsorption phases of the log drug concentration versus time curves. Areas under the plasma concentration time curves (AUC) were calculated by means of the trapezoidal rule and the estimated final concentration time point and terminal elimination rate constant were then used to determine the area extrapolated to infinity. Systemic clearance (Cls) was calculated as dose divided by AUC. The apparent volume of distribution (V_{area}) was calculated by analogous statistical moment techniques. Bioavailability (F) was calculated as the ratio of AUC after single oral and intravenous administrations and adjusted for the differences in the doses. At steady state AUC was calculated during the dosing intervals by the trapezoidal rule. The average plasma concentration at steady state (Css) was estimated by dividing the AUC by the time interval. The maximal plasma concentration (C_{max}) and the time to reach peak concentration (T_{max}) were estimated by visual inspection of the data. Accumulation index (R) was calculated as a ratio of the mean peak plasma concentrations observed following chronic and single oral administration of halazepam.

Statistics

Statistical evaluation of the data was performed by two-way analyses of variance and paired and unpaired *t*-tests. Although two of the chronically administered dogs were previously dosed with a single oral dose of halazepam the data obtained following single and chronic oral administration were analyzed by group comparison. Therefore, the degree of freedom was slightly overestimated for chronic dogs.

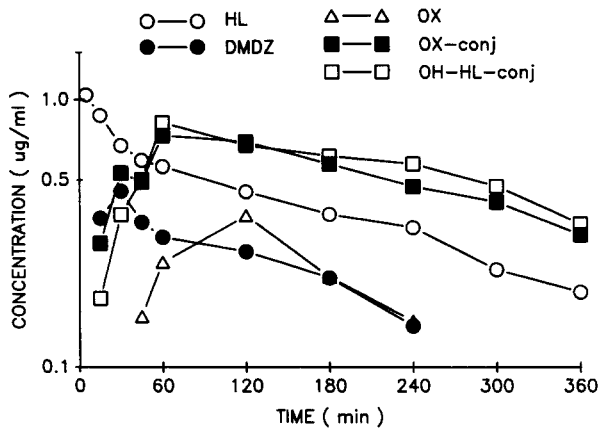

FIG. 2. Plasma levels of halazepam (HL) and its metabolites, desmethyldiazepam (DMDZ), oxazepam (OX), oxazepam conjugate (OX-conj) and 3-hydroxyhalazepam conjugate (OH-HL-conj), measured following intravenous (2 mg/kg) administration of (HL) to naive dogs. Data are mean of 4 dogs.

RESULTS

The mean plasma concentration time curves for halazepam and its metabolites following single intravenous and singe oral administrations of halazepam to four drug-naive dogs are shown in Fig. 2 and Fig. 3, respectively. Table 1 summarizes the pharmacokinetic parameters derived following acute and chronic administration of halazepam. After intravenous administration the initial rapid distribution of halazepam is followed by elimination with a $t_{1/2}$ of about 4.5 h. Halazepam is extensively distributed as indicated by the volume of distribution equal to about 4 l/kg and is moderately cleared with systemic clearance equal to

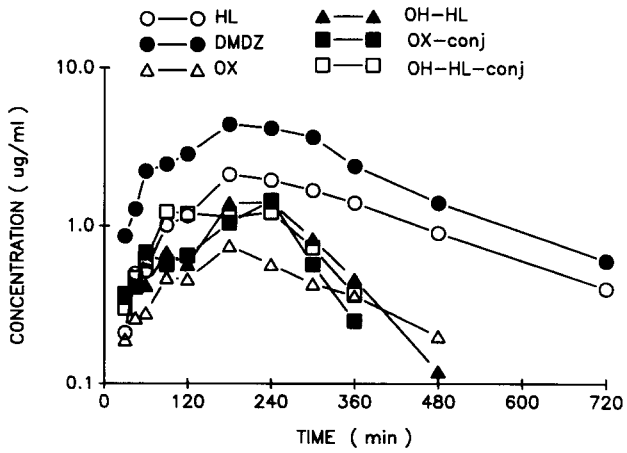

FIG. 3. Plasma levels of halazepam (HL), desmethyldiazepam (DMDZ), oxazepam (OX), 3-hydroxyhalazepam (OH-HL) and oxazepam (OX-conj) and 3-hydroxyhalazepam (OH-HL-conj) conjugates measured following single oral (112.5 mg/kg) dose of HL to naive dogs. Data are mean of 4 dogs.

TABLE 1

PHARMACOKINETIC PARAMETERS FOR HALAZEPAM AND ITS METABOLITES DETERMINED FOLLOWING SINGLE INTRAVENOUS (2 mg/kg) (I), SINGLE ORAL (1125 mg) (II) AND CHRONIC ORAL (450 mg/kg/day) (III) ADMINISTRATION OF HALAZEPAM TO DOGS

	Dose (mg)	AUC (μg*min/ml)	β (h^{-1})	$T_{1/2(\beta)}$ (h)	Cl_S (ml/min/kg)	$V_{d(area)}$ (L/kg)	T_{max} (h)	C_{max} (μg/ml)	C_{ss} (μg/ml)	F (%)	R	fu
					I (n=4)							
OX-CONJ		284.4 ± 103.3	0.19 ± 0.01	3.6 ± 0.2			1.2 ± 0.4	0.73 ± 0.3				
3OH-HLCONJ		278.5 ± 107.7	0.29 ± 0.09	2.7 ± 0.6			2.2 ± 1.0	0.81 ± 0.4				
OX		89.2 ± 34.6	0.51 ± 0.24	2.2 ± 0.7			1.4 ± 0.3	0.42 ± 0.2				
DMDZ		104.3 ± 10.3	0.25 ± 0.05	3.1 ± 0.8			0.5 ± 0.0	0.48 ± 0.1				
3OH-HL												
HL	19.35 ± 0.6	220.3 ± 48.7	0.17 ± 0.03	4.4 ± 0.9	10.3 ± 1.9	4.1 ± 1.3						
					II (n=4)							
OX-CONJ		304.7 ± 54.0	0.74 ± 0.26	1.4 ± 0.5			3.7 ± 0.5	1.9 ± 0.7				
3OH-HLCONJ		368.0 ± 56.8	0.74 ± 0.19*	1.1 ± 0.2			2.7 ± 0.7	2.6 ± 0.6				
OX		277.0 ± 83.9	0.28 ± 0.03	2.6 ± 0.3			2.7 ± 0.5	1.2 ± 0.3				
DMDZ		2108.5 ± 1035.1	0.32 ± 0.13	3.3 ± 1.1			3.2 ± 0.2	4.4 ± 1.5				
3OH-HL		399.2 ± 130.9	0.45 ± 0.12	2.3 ± 1.0			2.4 ± 0.7	1.9 ± 0.8				
HL	1125.0	1199.9 ± 472.8	0.31 ± 0.12	3.4 ± 1.1			2.5 ± 0.6	2.4 ± 1.1		10.4 ± 5.6		
					III (n=5)							
OX-CONJ		4565.7 ± 901.4‡					2.7 ± 0.4	19.9 ± 3.4‡	12.7 ± 0.2		10.5	
3OH-HLCONJ		979.4 ± 83.5‡					1.9 ± 0.3	5.4 ± 1.0	2.7 ± 0.2		2.1	
OX		220.9 ± 100.2					1.7 ± 0.1	1.1 ± 0.5	0.6 ± 0.3		0.9	0.149 ± 0.02
DMDZ		1056.1 ± 305.6					1.6 ± 0.3†	3.4 ± 0.9	2.9 ± 0.8		0.8	0.036 ± 0.00
3OH-HL	1125.0 (q.i.d.)	264.1 ± 121.6					2.4 ± 0.8	1.4 ± 0.7	0.7 ± 0.3		0.7	0.135 ± 0.03
HL		270.1 ± 65.2					1.7 ± 0.3	0.8 ± 0.2	0.7 ± 0.2		0.3	0.037 ± 0.01

*Significantly different from single IV administration ($p<0.025$); paired t-test.
†Significantly different from single PO administration ($p<0.01$); unpaired t-test.
‡Significantly different from single PO administration ($p<0.005$); unpaired t-test.
Each value is a mean ± SEM of n dogs.

TABLE 2

BRAIN (μg/g), CEREBROSPINAL FLUID (μg/ml) AND TOTAL PLASMA (μg/ml) LEVELS OF HALAZEPAM AND ITS METABOLITES IN DOGS DEPENDENT ON HALAZEPAM (450 mg/kg/day)

	Total Brain	Total Plasma	Free Plasma	Brain/ Plasma	CSF
Halazepam	0.59 ±0.1	$0.24^{0.025}$ ±0.0	0.008 ±0.002	2.8 $\pm 0.6_{0.05}$	0
Desmethyl-diazepam	2.03 ±0.5	2.9 ±0.6	0.113 ±0.034	0.95 ±0.1	1.8 ±1.0
Oxazepam	0.90 ±0.1	0.82 ±0.1	0.125 ±0.024	1.11 ±0.1	0.65 ±0.2
3-Hydroxy-halazepam	1.3 ±0.3	0.88 ±0.1	0.160 ±0.078	1.7 ±0.5	0
Oxazepam conjugate	1.8 ±0.3	$6.93^{0.005}$ ±1.0		0.26 $\pm 0.0_{0.001}$	0.52 ±0.1
3-Hydroxy-halazepam conjugate	1.0 ±0.2	2.1 ±0.4		0.50 $\pm 0.2_{0.05}$	0.64 ±0.2

Superscript indicates the significant p values for the paired t comparison between plasma and brain levels

Subscripts indicates the p value of differences from "1."

Each value is a mean ± SEM of 5 dogs. Levels in CSF are mean ± SEM of 3 dogs.

10 ml/min/kg. The principal metabolite, desmethyldiazepam, reaches peak plasma concentration (0.5 μg/ml) in about 30 min after halazepam injection and then declines with a terminal $t_{1/2}$ similar to $t_{1/2}$ of the parent drug. The mean peak plasma concentration for oxazepam (0.4 μg/ml) is observed in about 1.4 h. Plasma levels of desmethyldiazepam and oxazepam are lower than plasma levels of intravenously administered halazepam at all time points. Levels of 3-hydroxyhalazepam are below the limit of detection. The levels of conjugates are higher in comparison to postdistribution levels of halazepam. Following oral administration, halazepam is moderately absorbed and reaches the maximal plasma levels (2.4 μg/ml) in about 2.5 h. The availability of halazepam from the given oral preparation used is low (ca. 10%). There is no statistically significant difference in elimination profile of halazepam following its intravenous and oral administrations. The plasma time course of desmethyldiazepam parallels the time course of orally administered halazepam. The plasma levels of desmethyldiazepam are somewhat higher than the levels of halazepam from the first measured time point but levels of oxazepam, 3-hydroxyhalazepam and their conjugates are lower. Desmethyldiazepam and oxazepam declined with $t_{1/2}$ similar to apparent half-life of the parent drug but the conjugates decline in plasma with shorter $t_{1/2}$.

Figure 4 illustrates the mean steady-state plasma levels of halazepam and its metabolites determined in five dogs administered oral doses of halazepam q.i.d. for about 13 weeks. Peak plasma concentrations for halazepam and its metabolites (except the oxazepam-conjugate) are not statistically significantly different in dogs administered acute or chronic oral doses of halazepam. The peak concentration of desmethyldiazepam is observed significantly earlier ($p<0.01$) in halazepam-dependent dogs than in drug-naive dogs. The steady state AUCs for oxazepam, desmethyldiazepam, 3-hydroxyhalazepam and halazepam are not statistically different from AUCs calculated after single oral dose. The AUCs for conjugates are significantly higher ($p<0.005$, unpaired t comparison) at steady state. The differences between the maximum and minimum concentrations of halazepam and its metabolites are small at steady state following oral halazepam administration in 6-h dosing intervals.

The mean concentrations of halazepam and its metabolites in brain, CSF and plasma at steady state are presented in Table 2. The brain levels were analyzed using two-way analysis of variance (dogs × brain areas). There is significant between dogs variance for desmethyldiazepam, oxazepam and the conjugates and significant between brain areas variance for oxazepam ($p<0.05$). The oxazepam levels are lower in frontal cortex than in caudate ($p<0.01$) and thalamus and striatum ($p<0.05$) and levels in caudate are higher than levels in cerebellum and piriform ($p<0.05$). The average brain concentration for each drug was calculated from the area concentrations as the weighted mean ac-

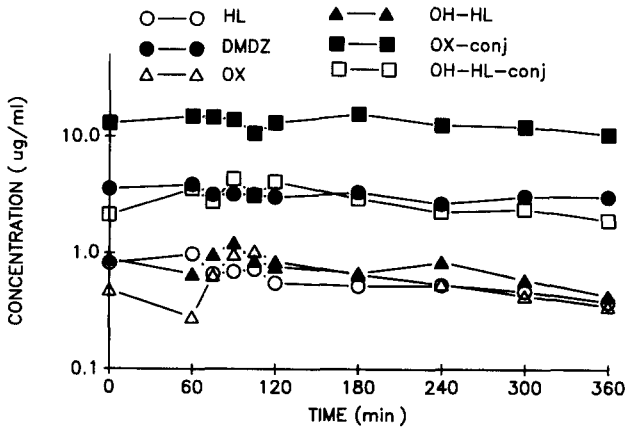

FIG. 4. Steady-state plasma levels of halazepam (HL), desmethyldiazepam (DMDZ), oxazepam (OX), 3-hydroxyhalazepam (OH-HL) and oxazepam (OX-conj) and 3-hydroxyhalazepam (OH-HL) conjugates in HL-dependent dogs administered HL chronically (112.5 mg/kg/dose, q.i.d.). Data are mean of 5 dogs.

counting for differences in the mass of different areas for each dog. The average brain levels are comparable to the total plasma levels except for the higher plasma levels of oxazepam-conjugate ($p<0.005$) and higher brain levels of halazepam ($p<0.025$). The CSF levels of desmethyldiazepam and oxazepam are unexpectedly high, while the levels of 3-hydroxyhalazepam and halazepam are below the detection limit. Since CSF was successfully collected only in 3 dogs and high between subjects variance was observed, CSF levels are not significantly different from brain or plasma levels.

DISCUSSION

The generated data show that intravenously administered halazepam is rapidly and extensively distributed and its apparent volume of distribution (4.1 l/kg) is in excess of the dog's total body water (0.6 l/kg) (1). The terminal half-life of halazepam equal to 4.4 h is longer than the $t_{1/2} = 30$ min previously reported for the dog (4) but considerably shorter than 35 h in man (3). Assuming hepatic blood flow equal to 41 ml/min/kg and blood/plasma ratio equal to 0.56 (2), in dogs, halazepam has intermediate hepatic extraction ratio equal to about 0.4. The absolute availability of halazepam (ca. 10%) is significantly less than the value predicted (60%) suggesting incomplete absorption and/or chemical or metabolic breakdown of halazepam in the gut (12). The following data are consistent with the possibility that halazepam is partially metabolized in the intestine: 1) The AUCs ratios (corrected for the differences in the doses) resulting from desmethyldiazepam concentration-time profiles following oral and intravenous administration of halazepam are significantly different from 1. 2) Following intravenous administration of halazepam the T_{max} for oxazepam is longer than the T_{max} for desmethyldiazepam while after oral administration T_{max} for both metabolites are almost identical. 3) An oxazepam to desmethyldiazepam plasma AUC's ratio is approximately 1:1 following intravenous but 1:10 after oral administration of halazepam. 4) After intravenous administration of halazepam, 3-hydroxyhalazepam is not detectable in plasma but its levels exceed levels of oxazepam in dogs administered a single oral dose of halazepam. 5) The AUCs of conjugates (corrected for the available dose of halazepam) are dependent on the route of halazepam administration. Our data show that following intravenous administration of halazepam, desmethyldiazepam reaches peak plasma concentration rapidly (T_{max} equal to 30 min) and then declines in parallel with the parent drug suggesting slow hepatic formation and rapid elimination of this metabolite (13). The plasma levels of oxazepam and 3-hydroxyhalazepam also appeared to follow that of halazepam pointing to a formation rate-limited elimination.

In dogs chronically dosed with halazepam stable plasma levels of halazepam, desmethyldiazepam, oxazepam, 3-hydroxyhalazepam and the conjugates are observed during 6-h dosing intervals. Total plasma levels of desmethyldiazepam exceed those of halazepam, oxazepam and 3-hydroxyhalazepam. The steady state levels of the 3-hydroxyhalazepam conjugate are in comparable range to the levels of desmethyldiazepam while the levels of oxazepam conjugate are much higher. As indicated by the accumulation index (Table 1), there is no progressive accumulation of halazepam and its unconjugated metabolites in plasma. Thus, when the dogs are chronically dosed with a daily 450 mg/kg dose of halazepam, the mean steady state plasma levels of halazepam and its metabolites, desmethyldiazepam, oxazepam and 3-hydroxyhalazepam, can be predicted from the single dose study. The significant plasma accumulation of conjugated metabolites in halazepam-dependent dogs cannot be explained by the single dose pharmacokinetics.

To our knowledge, no data on dependence-producing properties of halazepam in man have been reported. Chronic administration of halazepam produces a significant level of physical dependence in dogs as evidenced by the flumazenil precipitated abstinence syndrome (14). Despite the very large dose of halazepam, the stabilization plasma levels of desmethyldiazepam in halazepam-dependent dogs are relatively low in comparison to desmethyldiazepam plasma levels in diazepam- and nordiazepam-dependent dogs (9). The total (bound + free) plasma level of desmethyldiazepam (2.9 μg/ml) is considerably higher than the total levels of oxazepam (0.65 μg/ml), hydroxyhalazepam (0.88 μg/ml) or halazepam (0.24 μg/ml). However, the plasma levels of free desmethyldiazepam (0.11 μg/ml), oxazepam (0.12 μg/ml) and 3-hydroxyhalazepam (0.16 μg/ml) are within similar concentration ranges while halazepam levels are lower (0.008 μg/ml) (Table 2). It is also noteworthy that in diazepam- and nordiazepam-dependent dogs the levels of unbound benzodiazepines in plasma reflect their quantities in the extraneuronal brain space (Wala et al., in preparation). In steady state the brain levels of halazepam exceed its total levels in plasma by three-fold which probably can be explained by the high lipophilicity of halazepam leading to its extensive uptake into tissues. Furthermore, appreciable levels of metabolites of halazepam are measured in the brain tissue. These observations taken together suggest that in halazepam-dependent dogs the total steady state plasma levels of halazepam and its active metabolites may not reflect their levels at the receptor site. In conclusion, our data indicate that halazepam does not serve as simply another precursor of desmethyldiazepam as in the case of prazepam, chlorazepate or oxazolam (11) and that halazepam dependence as measured by precipitated abstinence is not related solely to its metabolite desmethyldiazepam.

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