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The Effects of Flumazenil-Precipitated Abstinence on the Pharmacokinetics of Chronic Oxazepam in Dogs

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WALA, E. P., J. W. SLOAN, W. R. MARTIN AND T. A. PRUITT. *The effects of flumazenil-precipitated abstinence on the pharmacokinetics of chronic oxazepam in dogs*. PHARMACOL BIOCHEM BEHAV 35(2) 347–350, 1990.—The pharmacokinetics of oxazepam was studied in naive dogs and in oxazepam-dependent dogs without and with administered flumazenil (6 mg/kg). Oxazepam is eliminated with a relatively short elimination half life (ca. 150 min) in both acutely and chronically treated dogs. It exhibits only a modest first pass metabolism (ca. 10%) and its bioavailability following oral administration is about 22%. The steady state concentration of oxazepam in chronically treated dogs was lower than was predicted from single dose studies. Flumazenil did not change the rate of absorption or elimination of oxazepam-dependent dogs. The total steady state plasma concentration of oxazepam was significantly reduced by flumazenil administration suggesting a displacement interaction between flumazenil and oxazepam.

Oxazepam Flumazenil Dependence, oxazepam

EXTENSIVE studies have been performed with oxazepam-dependent dogs. During these studies it was observed that high doses of oxazepam were required to obtain comparable plasma levels of oxazepam to those achieved in dogs dependent on much lower doses of diazepam or nordiazepam. After oral administration of the benzodiazepine antagonist flumazenil (RO 15-1788) to oxazepam-dependent dogs, symptoms of precipitated abstinence have been demonstrated (Sloan, Martin and Wala, unpublished data). Previous interaction studies showed that flumazenil did not alter bioavailability (5) or plasma levels (9,10) of benzodiazepines and pretreatment with benzodiazepines did not change the pharmacokinetics of acutely administered flumazenil in man (8). To our knowledge, no data on the plasma concentration time course of benzodiazepines in benzodiazepine-dependent animals following precipitation of abstinence with flumazenil have been reported.

The present study was designed to determine whether: 1) there are differences in the pharmacokinetic profiles of oxazepam after its acute and chronic administration and 2) whether pharmacokinetic interactions occur between flumazenil and oxazepam following flumazenil-precipitated abstinence.

METHOD

Acute Study

Four female beagle type dogs (8.9–10.8 kg) were employed in these studies. The dogs were given oxazepam on two occasions: 1) an intravenous bolus injection of an alcohol-saline (1:1) oxazepam solution (5 mg/ml) in a dose of 1 mg/kg was administered and 2) one week later a 90 mg dose of oxazepam was administered orally in a No. 4 gelatine capsule. Blood samples were taken at 5, 15, 30, 45 min and 1, 2, 3, 4, 5, and 6 hr after the intravenous administration and at 30, 45, 60, 90 min and 2, 3, 4, 5, 6 hr after oral administration.

Chronic Study

Six female beagle type dogs (9.9–10.2 kg) who had not previously received any benzodiazepines were used to study oxazepam physical dependence and the precipitated abstinence syndrome (Sloan, Martin and Wala, unpublished data). Oxazepam was administered orally in escalating doses until a dose of 270 mg/kg/day (administered in four equally divided doses) was achieved. Approximately 9 weeks were required to reach the stabilization dose. The dogs were held at this dose for two weeks before blood was collected at 15, 30, 45, 60, 75, 90, 105 min and 2, 3, 4, 5, and 6 hr after the morning dose of oxazepam. Plasma samples were analyzed for the time course of unchanged oxazepam. Later, the dogs participated in precipitation studies with flumazenil given orally in doses of 6, 18 and 36 mg/kg according to previously described procedures (11). After the cross-over

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experiment was completed, another 6 mg/kg dose of flumazenil was administered orally one hour after the morning dose of oxazepam. Blood samples were collected just before and 15, 30, 45 min and 1, 2, 3, 4 and 5 hr after flumazenil administration. Plasma samples were analyzed to determine the time course of flumazenil and unchanged oxazepam in oxazepam-dependent dogs following precipitation with flumazenil.

Analysis of Drug Concentrations

Blood was taken from a site separate from the site of intravenous administration of oxazepam. Samples were collected via a venous catheter into vacutainer tubes containing disodium EDTA. Following centrifugation, plasma samples were separated, frozen and stored at −20°C until analyzed. Duplicate determinations were performed with each plasma sample. Plasma concentrations of flumazenil and oxazepam were determined by HPLC (14). Flunitrazepam (50 ng injected) was used as an internal standard and was added to the samples prior to the extraction on Bond Elut columns. The limit of sensitivity was 1.25 ng injected or 0.15 μg/ml of the original plasma sample. The mean retention times for flumazenil, flunitrazepam and oxazepam were 2.14 ± 0.02, 3.52 ± 0.03 and 4.78 ± 0.18 min, respectively. The mean recoveries were 82.0 ± 5.2% and 86.5 ± 5.3% for flumazenil and oxazepam, respectively.

Data Analysis

Noncompartmental analysis have been used for estimation of pharmacokinetic parameters (6). Areas under the plasma concentration curve (AUC) were calculated from time zero to t_n by means of the trapezoidal rule. The areas from t_n to infinity were estimated from the last measured concentration point (c_n) and the slope (β) of log drug concentration versus time line (3). Bioavailability (F) was calculated as the ratio of the AUC after oral and intravenous administration and adjusted for differences in the size of the intravenous and oral doses of oxazepam. Systemic clearance (Cl_s) after intravenous administration of oxazepam was calculated as dose/AUC. The apparent volume of distribution (V_{darea}) was determined as dose/AUC*β (13). After chronic oral administration of oxazepam at regular time intervals the average steady-state plasma levels (C_{ss}) of oxazepam were estimated by dividing the area under the plasma level time curves during dosage intervals by time interval (t). Data were analyzed using paired and unpaired *t*-tests.

RESULTS

The mean plasma concentration time curves following intravenous and oral administration of oxazepam are given in Fig. 1. Following intravenous injection of 1 mg/kg of oxazepam, the plasma concentration of unchanged drug declined biexponentially with a rapid initial distribution phase and an elimination phase with an apparent half life of 151.4 ± 18.2 min. After the oral administration of 90 mg of oxazepam the unchanged drug reached a maximal plasma concentration of 0.94 ± 0.26 μg/ml at 105.0 ± 28.7 min and then declined with an average apparent elimination half life of 149.3 ± 19.5 min. Table 1 summarizes the mean pharmacokinetic parameters calculated after intravenous and oral administration of single doses of oxazepam to 4 naive dogs. Following IV administration, the systemic clearance of oxazepam was equal to 48.2 ± 5.2 ml/min. Elimination rate constants estimated by log regression analysis of the postdistribution and the postabsorption phases were almost identical after IV and oral administration of oxazepam. Oxazepam was distributed extensively outside the blood as was indicated by the large volume of

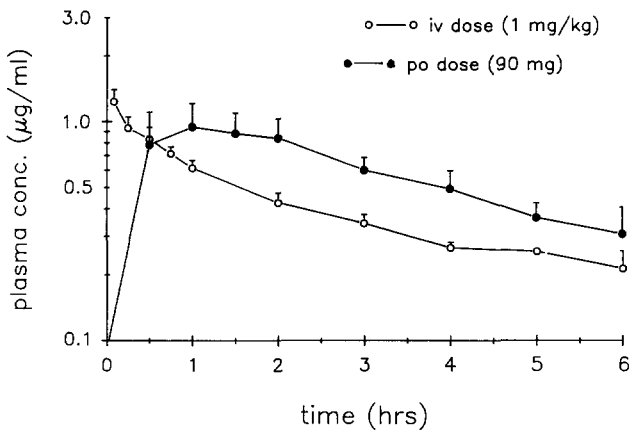

FIG. 1. Mean plasma concentrations of oxazepam (μg/ml) ± SEM (n = 4) following the acute intravenous and oral administration of oxazepam to naive dogs.

distributions. A paired *t*-test revealed no difference in the elimination and distribution parameters calculated after IV and oral administration of oxazepam to the same dogs. Based on area comparison after IV and oral administration of oxazepam the calculated availability factor was equal to 22.1 ± 5.8%. This value was considerably lower than the 88% availability estimated for the IV route using a hepatic blood flow of 41 ml/min/kg and oxazepam systemic clearance (4).

Figure 2 shows the mean plasma concentrations of oxazepam at specified times in oxazepam-dependent dogs, untreated and after the administration of flumazenil (6 mg/kg). After chronic administration of oxazepam at a dose of 675 mg every 6 hr, the maximal plasma concentration of unchanged drug was 4.7 ± 0.9 μg/ml which was achieved at 130.0 ± 23.1 min after administration. Plasma levels then declined with the apparent half life of 126 ± 22.2 min. The half life of oxazepam calculated after chronic administration did not differ significantly from that calculated after the oral administration of a single 90 mg dose (unpaired *t*-test). When oxazepam-dependent dogs received flumazenil, the maximal plasma concentration of oxazepam was 1.9 ± 0.4 μg/ml. This peak concentration was observed at 101.5 ± 19.1 min after the last dose of oxazepam and 45 min after flumazenil administration. The plasma concentration of oxazepam declined with the apparent half

TABLE 1

PHARMACOKINETIC PROFILES OF OXAZEPAM IN 4 NAIVE DOGS (MEAN ± SEM) FOLLOWING A SINGLE INTRAVENOUS (I) AND A SINGLE ORAL (II) DOSE OF OXAZEPAM

	I	II
Dose (mg)	9.1 ± 1.12	90.0
AUC o → ∞ (μg*min/ml)	176.3 ± 15.8	272.9 ± 33.6
Cl_s (ml/min)	48.2 ± 5.2	
β (min^{-1})	0.0048 ± 0.0005	0.0049 ± 0.0007
$t_{1/2}$ (β) (min)	151.4 ± 18.2	149.3 ± 19.5
V_{area} (L)	10.9 ± 0.78	
C_{max} (μg/ml)		0.94 ± 0.26
t_{max} (min)		105.0 ± 28.7
F (%)		22.1 ± 5.8

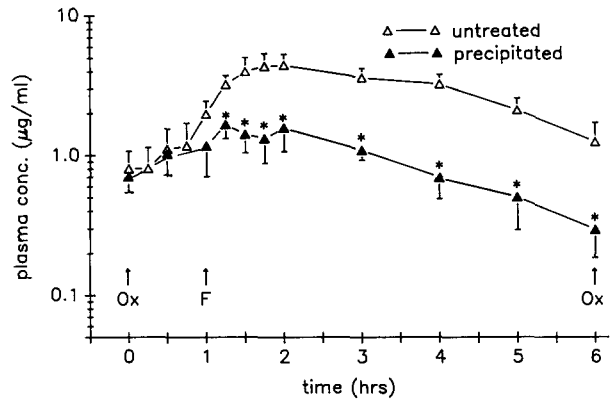

FIG. 2. Plasma concentrations of oxazepam in oxazepam-dependent dogs (n = 6). A stabilization dose of oxazepam (OX) was administered every 6 hr (270 mg/kg/day). One hour after the morning dose dogs were either untreated or received flumazenil (F) which precipitated an abstinence syndrome. *Significantly different from untreated ($p<0.025$), paired t-test.

life time of 106.3 ± 11.7 min. The plasma levels of oxazepam were statistically significantly lower ($p<0.025$) in the oxazepam-dependent dogs after flumazenil administration than in the untreated oxazepam-dependent dogs at each time point (Fig. 2). The plasma concentration of oxazepam before flumazenil administration was not different in these two experiments. The apparent half life times of oxazepam, however, did not differ significantly between the two treatment conditions. Table 2 summarizes the pharmacokinetic parameters of oxazepam derived in each oxazepam-dependent dog in the absence and presence of flumazenil treatment.

The mean steady state concentration of oxazepam (about 5.8 μg/ml) can be predicted from the single dose study. The measured mean steady-state concentration was 2.7 ± 0.6 μg/ml. Based on the elimination rate constant obtained in the single dose study the accumulation factor for oxazepam administered every 6 hr was about 1. The average steady state concentration of oxazepam in oxazepam-dependent dogs following precipitation of abstinence with flumazenil was 1.0 ± 0.3 μg/ml and was significantly lower ($p<0.001$) than the mean steady state concentration observed in nonprecipitated oxazepam-dependent dogs. Plasma levels of flumazenil and its pharmacokinetic parameters in oxazepam-dependent dogs are reported separately (16).

DISCUSSION

Oxazepam has a low solubility both in water and organic vehicles and for this reason is not available in parenteral preparations. Since the bioavailability of orally administered oxazepam has not been well established, most of the pharmacokinetic calculations were based on its complete absorption and therefore the reported values of clearance were subject to some limitation. The availability of oxazepam from the micronized water suspension administered orally to dogs was reported to be about 70% (2). Availability of oxazepam from tablets was reported to be equal to 96% in man (15). In our studies a low hepatic extraction ratio of oxazepam in the dog was calculated following the intravenous administration of oxazepam since its systemic clearance was only about one-tenth of the assumed hepatic blood flow. This estimate suggests that oxazepam is probably subject to a negligible first past effect and should have a high systemic availability after oral administration. The actual availability (ca. 22%) calculated after oxazepam's oral administration to nondependent dogs was much lower than the value predicted (88%). The most likely explanation of the low availability of oxazepam is its incomplete absorption from the dosage form used in our studies. The poor water solubility of oxazepam or its chemical or metabolic degradation in the gut can contribute to this phenomena. The low availability factor can partly explain low plasma levels of oxazepam observed in dogs chronically administered high doses of oxazepam. As can be inferred from the half lives of oxazepam in dogs, the drug will not accumulate when it is administered at 6-hr intervals. No differences in oxazepam distribution and elimination were observed following single intravenous and oral administrations. In dogs, oxazepam can be characterized as a slowly cleared drug with a short elimination half life time and a large volume of distribution.

Pharmacokinetic parameters in dogs are in good agreement with those reported in man (7). In dogs, as in man, oxazepam is relatively slowly absorbed from the gastrointestinal tract with peak plasma levels occurring after 1–3 hr. The mean steady-state concentration of chronically administered oxazepam was lower than the steady-state concentrations estimated following adminis-

TABLE 2

PHARMACOKINETIC PROFILE OF OXAZEPAM IN OXAZEPAM-DEPENDENT DOGS DOSED ORALLY (270 mg/kg/day), (n = 6), UNTREATED (I) AND FOLLOWING PRECIPITATION WITH FLUMAZENIL (6 mg/kg) (II)

	AUC t→t+1 (μg*min/ml)		C_{ss} (μg/ml)		β (min^{-1})		C_{max} (μg/ml)		t_{max} (min)	
Dog No.	I	II	I	II	I	II	I	II	I	II
1	1844.6	797.7	5.1	2.2	0.0035	0.0064	8.7	3.8	105.0	120.0
2	566.6	286.1	1.6	0.8	0.0044	0.0072	3.1	2.3	75.0	60.0
3	501.0	295.5	1.4	0.8	0.0075	0.0059	2.8	1.3	120.0	60.0
4	901.3	251.2	2.5	0.7	0.0043	0.0049	3.5	1.2	120.0	75.0
5	818.7	104.6	2.3	0.3	0.0134	0.0123	3.5	0.6	240.0	180.0
6	1286.1	440.0	3.6	1.2	0.0070	0.0058	6.9	2.2	120.0	120.0
Mean	986.4	$362.5^{0.005}$	2.7	$1.0^{0.001}$	0.0067	0.0070	4.7	$1.9^{0.01}$	130.0	101.5
± SEM	±206.0	±97.4	±0.6	±0.3	±0.0015	±0.0015	±0.9	±0.4	±23.1	±19.1

Superscript indicates the significant p values for the paired t comparison between untreated and precipitated dogs.

tration of the single dose of oxazepam. This discrepancy is consistent with observations reported after single and multiple dose administration of oxazepam in man (1). The same absorption and elimination characteristics were observed in dogs treated acutely and chronically.

In dogs administered flumazenil t_{max} and β for oxazepam were not statistically different from these observed in untreated dogs suggesting that flumazenil did not change the rate of absorption or elimination of oxazepam. However, the maximal and steady state plasma concentrations of oxazepam following flumazenil were significantly lower than those obtained in the same dogs untreated with flumazenil.

The above observation suggests an increase in oxazepam total plasma clearance following flumazenil administration. Since we find that oxazepam has a negligible first pass effect a variation in its intrinsic clearance would not significantly affect its availability in dogs administered oxazepam chronically and precipitated with flumazenil. Our interpretation of the oxazepam kinetic study may be somewhat distorted since it is based on the measurement of the total rather than the free fraction of oxazepam. The oxazepam free fraction is independent of its total concentration (12), but we cannot rule out the possibility of a flumazenil-oxazepam protein binding interaction. In this case both the clearance and volume of distribution will increase with an increase of oxazepam-free fraction. Consequently, at steady state the concentration of total oxazepam will be dependent on any change in the free fraction. The possible protein binding interaction of oxazepam with flumazenil will result in a transitory elevation of the oxazepam free fraction, such that only a short-term increase in pharmacological effect is likely.

In conclusion, multiple dosing with oxazepam would not be expected to lead to excessive accumulation of unchanged oxazepam, but the formation and accumulation of the glucuronide conjugate of oxazepam was not determined in oxazepam-dependent dogs. Flumazenil precipitated abstinence results in a significant decrease of plasma levels of total oxazepam. Altered protein binding or tissue distribution of oxazepam could contribute to this phenomena. These problems will be further investigated in future studies.

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