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Flumazenil Oral Absorption in Dogs

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WALA, E., L. F. McNICHOLAS, J. W. SLOAN AND W. R. MARTIN. *Flumazenil oral absorption in dogs.* PHARMACOL BIOCHEM BEHAV **30**(4) 945–948, 1988.—Flumazenil is rapidly absorbed after oral or gastric fistula administration to the dog reaching peak plasma concentrations in about an hour. Plasma level decrease rapidly thereafter reaching barely detectable levels by four hours. The onset of signs of flumazenil precipitated abstinence in diazepam-dependent dogs is well correlated with the rise of flumazenil plasma levels, however, precipitated abstinence seizures occur when plasma levels have markedly decreased. Oral dosing is a more efficient way of administering flumazenil than gastric fistula dosing.

Flumazenil Diazepam Dependence, diazepam

EXTENSIVE studies have been conducted on the precipitation of abstinence in diazepam- and nordiazepam-dependent dogs dosed orally or through a gastric fistula [4,5]. During the course of present studies it was observed that smaller doses of diazepam were required to obtain comparable plasma levels of nordiazepam when dogs were dosed orally than when dosed through a gastric fistula. This study was conducted to investigate the time course of absorption of flumazenil, a benzodiazepine antagonist (Ro15-1788; ethyl, 8-fluoro-5,6 dihydro-5-methyl-6-oxo-4H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate) when administered orally or through a gastric fistula in both drug naive dogs and in dogs treated chronically with diazepam. Orally and gastric fistula dosed dogs are being used in our laboratory to study benzodiazepine physical dependence. Thus another purpose was to relate plasma levels of flumazenil to the emergence of the precipitated abstinence syndrome in the orally dosed diazepam-dependent dog. Of particular interest has been the observations that both clonic and clonic-tonic flumazenil precipitated convulsions occur both early and late in the time course of the precipitated abstinence syndrome.

METHOD

Subjects

Four female beagle type dogs with gastric fistulas (7.2–13.4 kg) [4] and four intact female beagle type dogs (10.4–13.6 kg) were employed in these studies. The dogs had not previously received any benzodiazepines or any other psychoactive drug to our knowledge. At least two weeks elapsed between the day of surgery and participation in the experiment. The intact dogs were subsequently used to study diazepam physical dependence and the precipitated abstinence syndrome. In these studies they were administered doses of diazepam orally (240–360 mg/day in four equally divided doses) to achieve comparable plasma levels of nordiazepam (ca. 10 to 20 μg/ml) to those achieved in gastric fistula dosed dogs. All dogs received a No. 4 gelatin capsule containing a dose of 80 mg (fistula dosed dogs), 8 mg/kg (orally dosed, naive dogs) or 6 mg/kg (orally dosed, diazepam-dependent dogs) of flumazenil. Diazepam-dependent dogs received flumazenil 1 hour after the administration of diazepam. Diazepam-dependent dogs were fasted overnight before the experiment.

Abstinence Syndrome

Dogs were observed for a 4-hour period immediately after the administration of either flumazenil or placebo for signs of abstinence. All behavior and unusual postures were recorded. A Nordiazepam Precipitated Abstinence Scale (NPAS) has been derived to estimate the overall intensity of precipitated abstinence [6]. The NPAS consists of signs of abstinence that are increased in a dose-related manner after the administration of flumazenil; these signs are then weighted such that the dose-response lines are superimposable. The NPAS and the weighting factors are: gross whole body tremor (weighting factor=1), twitches and jerks (1), hot foot walking (2), and respiratory rate (1). The NPAS score for each dog is the sum of the weighted items observed in that dog.

Blood Collection

Venous blood was drawn thirty minutes before flumazenil was administered and 0.25, 0.5, 1, 2, 3, and 4 hr after administration through a gastric fistula; 0.25, 0.5, 0.75, 1, 2, 3 and 4

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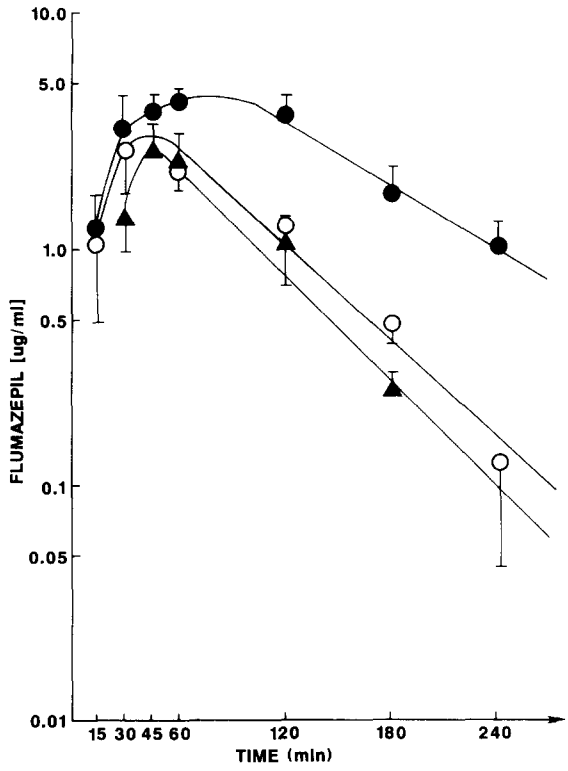

FIG. 1. Plasma levels of flumazenil. Each value is the mean±S.E.M. of 4 dogs. Comparisons are made between 4 (●) naive dogs who were dosed orally with flumazenil (8 mg/kg); the same 4 dogs (▲) dependent on diazepam (240–360 mg) who are also dosed orally with flumazenil (6 mg/kg) and 4 different (○) naive dogs who were dosed through a gastric fistula (80 mg).

TABLE 1

PHARMACOKINETICS PARAMETERS OF FLUMAZENIL IN THE GASTRIC FISTULA (NAIVE) (I) AND ORALLY (II) DOSED NAIVE DOGS AND ORALLY (III) DOSED DIAZEPAM-DEPENDENT DOGS

K_{el} (min^{-1})	$t_{1/2}$ (min)	$AUC_{0\to\infty}$ ($\mu g/ml \times min$)	Cl_T (ml/min)	V_D (l)
		I		
0.019 ±0.004	41.3 ±7.9	289.0 ±50.7	312.3 ±69.3	17.2 ±2.5
		II		
0.010 ±0.001	73.4 ±$6.2_{0.05}$	729.9 ±$69.3_{0.05}$	131.2 ±$18.1_{0.05}$	14.0 ±2.4
		III		
0.018 ±0.003	$42.2^{0.05}$ ±6.7	$226.9^{0.05}$ ±47.5	$297.2^{0.05}$ ±48.3	17.1 ±2.7

p values are indicated by super- and subscripts. Subscripts indicate a significant difference between groups I and II using an unpaired t-test. Superscripts indicate a significant difference between groups II and III using a paired comparison.

hr after its administration orally in drug naive dogs and 0.25, 0.5, 0.75, 1, 2, 3, 4, and 5 hr after its administration orally in diazepam-dependent dogs. Blood was collected into a vacutainer containing disodium EDTA. Following centrifugation plasma samples were obtained, frozen and stored at −20°C until analyzed.

Drug Analysis

A modification of the method of Rao *et al.* [7] was used to determine plasma levels. Briefly, 25 μl of internal standard: oxazepam, 30 μg/ml in methanol (drug naive dog study) or flunitrazepam, 30 μg/ml in methanol (diazepam-dependent dog study) was added to 125 μl of dog plasma. Duplicate 50 μl aliquots of each sample were absorbed on a buffered (pH 9.5) C18 1 cc Bond Elut column (Analytichem International, Harbor City, CA), rinsed twice with 100 μl of water then with 50 μl of methanol and eluted with 200 μl and 100 μl methanol rinses using the Baker 10 sp system. The methanol extract was taken to dryness under a N_2 stream. The dried extract was dissolved in 25 μl of methanol and 5 μl of the solution was injected into the HPLC. A Beckman model 332 HPLC system with a reverse-phase Supelco column (Bellefonte PA) LC-8 (5 μl particle size) was used. A flow-through cell with a variable wave length detector was used and the absorbance of flumazenil was measured at 240 nm. The mobil phase, a mixture of methanol:55% and 1 mM KH_2PO_4, pH=3.6:45% was degassed by ultrasonication prior to use. The flow rate was maintained at 1.3 ml/minute. The levels were measured by comparing peak height ratios (drug/internal standard) with the peak height ratio for known standards. Standard curves of flumazenil were obtained using both unextracted standard solutions prepared in methanol (1.25–250 ng) and standard solutions added to and extracted from plasma obtained from drug-free dogs. The two standard curves were linear over the range of 0.25 to 50 ng/μl or 0.15 to 30 μg/ml of plasma. The mean retention time for flumazenil was 3.4 minutes and the mean recovery was 82.0±5.2%. Data were analyzed using a two-way ANOVA (dogs × times).

The evaluation of pharmacokinetic parameters was obtained using a model-independent analysis [1]. Elimination rate constants (K_{el}) were estimated by linear regression analysis of the terminal part of the log plasma concentration − time curves. The elimination half-lives ($t_{1/2}$) were then obtained from the corresponding elimination rate constants. The areas under the plasma concentration time curves ($AUC_{0\infty}$) were calculated using the trapezoidal rule from time zero to the last measured concentration. The residual areas were then obtained by dividing the last concentration value by the elimination rate constant. Apparent total drug clearance (Cl_T) was calculated from the given dose and AUC (assuming 100% absorption). Apparent volume of distribution was calculated by the area method.

RESULTS

Figure 1 shows the plasma concentrations of flumazenil at the specified times after its administration. In drug naive dogs, a mean peak plasma concentration of 4.4±0.4 μg/ml was reached within about 90 minutes following oral administration while a peak concentration of 2.6±0.8 μg/ml was observed 45 minutes after giving the same dose of flumazenil through a gastric fistula. After the peak levels were reached, the plasma concentrations declined less rapidly when

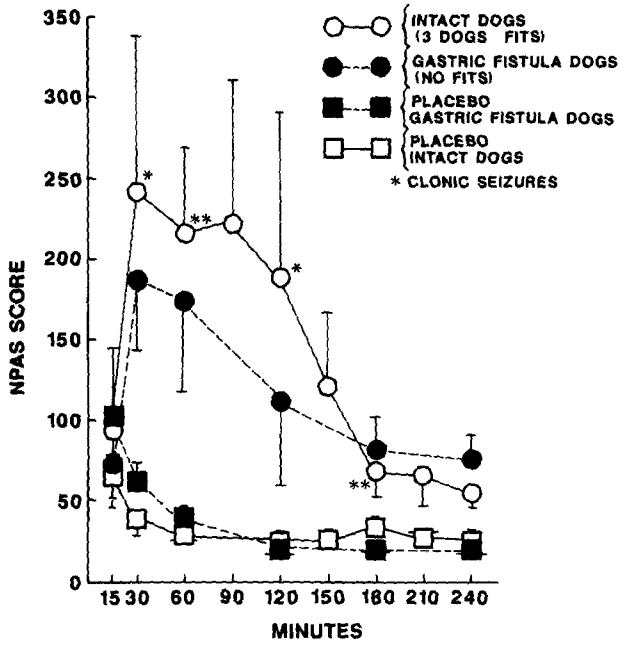

FIG. 2. The time course of flumazenil precipitated abstinence intensity (NPAS) in diazepam-dependent dogs. The mean scores (±S.E.M.) are shown for observations made in four orally dosed dogs dependent on 240–360 mg/day of diazepam and six gastric fistula dogs who were dependent on approximately 420 mg/day. The gastric fistula dogs received 80 mg of flumazenil, the orally dosed dogs received 6 mg/kg. Three of the four orally dosed dogs had clonic seizures. The time period and number of clonic seizures following the administration of flumazenil is indicated by asterisks.

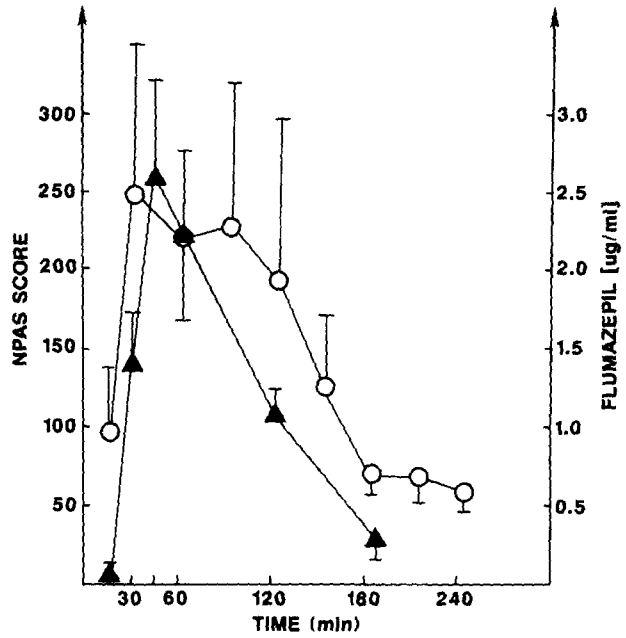

FIG. 3. Comparison of the time course of precipitated abstinence (NPAS score) (●) with plasma levels of flumazenil (▲) in four diazepam-dependent dogs (240–360 mg/day) who received flumazenil (6 mg/kg orally). Each value is the mean±S.E.M.

flumazenil was administered orally than when given through a gastric fistula. The half-life was in the range of 56.2–85.1 minutes after oral administration and 24.7–61.0 minutes after administration by gastric fistula. The AUC in orally dosed dogs was nearly twice that observed in dogs dosed through a fistula. Total body clearance in orally dosed dogs ranged from 98.5 to 178.6 ml/min and was much lower than clearance after dosing through the fistula which ranged from 204.5 to 512.3 ml/min. Flumazenil was distributed extensively outside the blood. Values for the apparent volume of distribution ranged between 8.7 to 18.9 l and 9.8 to 20.9 l, respectively, in dogs dosed orally or through the gastric fistula. The mean±SEM values of the individually derived pharmacokinetic parameters are summarized in Table 1. An unpaired t-test revealed a statistically significant difference ($p<0.05$) in the elimination parameters but no difference in the distribution parameters between the two groups studied.

Data on flumazenil plasma levels from orally dosed diazepam-dependent dogs are also presented in Fig. 1. As can be seen, flumazenil was rapidly absorbed reaching a peak plasma level of 2.5±0.6 μg/ml in about 45 minutes. The plasma level declined rapidly with a half-life time in the range of 27.0 to 59.2 minutes. Values for the total body clearance and apparent volume of distribution ranged between 169.4 to 390.4 ml/min and 13.6 to 25.2 l, respectively (Table 1). A paired t-test showed significant differences ($p<0.05$) in the elimination parameters of orally administered flumazenil in the non-dependent and diazepam-dependent dog. An analysis of variance of the plasma level data showed no significant between dogs variance and highly significant across time variance ($p<0.005$).

Plots of the time course of the intensity of the abstinence syndrome as well as the time of occurrence of the first clonic seizure in gastric fistula and orally dosed dogs are presented in Fig. 2. As can be seen, the abstinence syndrome is well developed within the first hour following administration of flumazenil either through a gastric fistula or orally. Further, precipitated abstinence seizures were seen within thirty minutes when plasma levels of flumazenil were nearly at peak levels and as late as 180 minutes when plasma levels were about $^1/_{10}$ of peak levels following the administration of flumazenil. Plots of the time course of plasma concentrations of flumazenil and the time course of the intensity of the precipitated abstinence syndrome in diazepam-dependent dogs as measured by a composite score of several signs of abstinence are compared in Fig. 3. A good concordance across time between plasma levels of flumazenil and the intensity of precipitated abstinence is seen.

DISCUSSION

These observations show that flumazenil is rapidly absorbed following gastric fistula or oral administration in naive and diazepam-dependent dogs and that it has a rapid onset of action in diazepam-dependent dogs. Significant plasma levels are presented 30 min after administration when signs of precipitated abstinence can be clearly demonstrated. Further, the intensity of abstinence decreases as plasma levels fall. As can be seen from Fig. 2, clonic convulsions occur both early and late in the orally dosed dogs when plasma levels of flumazenil ranged from 2.7 to 0.25 μg/ml.

One dog had a grand mal seizure shortly after the experiment ended at 4 hours. These observations suggest that factors other than plasma levels of flumazenil also determine the susceptibility of diazepam-dependent dogs to seizures.

Flumazenil has a large apparent volume of distribution that indicates its extensive distribution outside the plasma space and binding to tissue. Although flumazenil has a lower lipophilicity than most of the benzodiazepines it has relatively low binding to the plasma proteins [2] which may contribute to its high tissue uptake and rapid onset of action. Flumazenil was easily detectable in the brain at times when the plasma levels were to low to be detected [3]. Plasma levels fall more slowly in the dog after oral and fistula dosing than in the rat following intraperitoneal administration [3].

Plasma levels of flumazenil after its administration in average dose of 80 mg to the naive dogs through a gastric fistula were comparable to these observed after its administration in an average oral dose of 60.7 mg to diazepam-dependent dogs. Since flumazenil (pKa=1.7) is expected to be absorbed more easily from the small intestine than from the stomach this greater efficiency of absorption seen in the orally dosed dogs when compared to the fistula dogs may be related to the geometry of the fistula or the changes in the physiology of the absorption site in the fistula dogs.

The elimination half-life is practically identical in the naive fistula and orally dosed diazepam-dependent dogs. However, after oral administration of an average dose of 92.4 mg to the naive dogs, the half-life time for flumazenil was almost twice as long as that observed in the diazepam-dependent dogs. Results from the present study are different from data reported by Klotz *et al.* [2] which showed that the acute coadministration of benzodiazepines do not change elimination of flumazenil. To our knowledge no data exist in the literature concerning the pharmacokinetic characteristics of flumanzenil in benzodiazepine-dependent dogs. Several design features may account for these differences and include (1) acute and chronic administration of diazepam (2) species and (3) the dependent variable. The data indicate that chronic administration of diazepam in some way accelerates the metabolism or elimination of flumazenil in the dog. The drug naive dogs received a dose of 8 mg/kg while the drug-dependent dogs a dose of 6 mg/kg after the overnight fasting. Although no differences were found in the disposition parameters after the two different flumazenil oral doses in man [8], dose-related changes in the disposition of flumazenil have not been studied in the dog.

There is a good concordance between the intensity of signs of abstinence (except seizures) and plasma levels of flumazenil which is consistent with observations that the intensity of precipitated abstinence is related to the dose of flumazenil [6] again excepting seizures.

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