Prazepam Metabolism by Dogs

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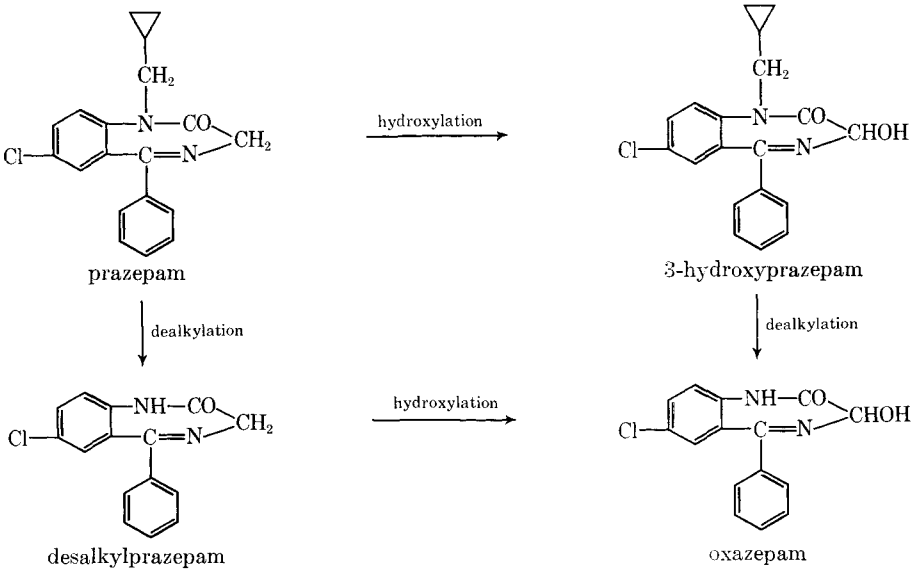
Abstract □ Prazepam-5-^{14}C [7-chloro-1-(cyclopropylmethyl)-5-phenyl-1H-1,4-benzodiazepin-2(3H)-one] was primarily eliminated with the feces in unaltered form following the oral administration of 10 mg./kg. to dogs. Prazepam resembled diazepam and oxazepam in the low blood levels and extensive fecal excretion. Further similarity of prazepam to diazepam was shown by the biotransformations involving dealkylation and hydroxylation. The major urinary metabolite from prazepam is oxazepam glucuronide. The possible presence of unchanged prazepam and/or 3-hydroxyprazepam glucuronide as well as 4′-hydroxy oxazepam glucuronide in the urinary excretion is also indicated.

Keyphrases □ Prazepam-^{14}C metabolism—dogs □ Distribution, excretion—prazepam-^{14}C □ TLC—separation, analysis □ Scintillometry, liquid—analysis

Prazepam is a new synthetic tranquilizer in the benzodiazepin series. The compound shows muscle-relaxant, calming, and anticonvulsant activities in animals. Additionally, it is well tolerated by humans and produces clinical improvement in patients with anxiety (1–3). Structurally, prazepam is 7-chloro-1-(cyclopropylmethyl) - 5 - phenyl - 1H - 1,4 - benzodiazepin-2(3H)-one. The present investigation had two objectives. The first was to determine the absorption, excretion, and tissue distribution of prazepam in dogs. The second objective was to learn whether prazepam is metabolized to oxazepam in order to ascertain whether prazepam may serve as a precursor to other tranquilizing agents by the following oxidative sequences:



MATERIALS AND METHODS

^{14}C-Labeled Prazepam—Prazepam was synthesized with ^{14}C in position 5 of the seven-membered ring (4). The preparation was >99% radiochemically pure as judged by TLC and had a specific activity of 1.31 mc./g.

Radioactivity Counting—Quantitative assays for ^{14}C were performed using a liquid scintillation spectrometer (Packard Tri-Carb model 3324). The external standardization method was used for quench corrections.

Thin-layer Chromatography—The chromatography was run on 5 × 20-Cm. (2 × 8-in.) glass plates coated with 250μ of Silica Gel G bound with calcium sulfate. The unidimensional ascending technique was used to develop the chromatograms in the following systems: Solvent 1: chloroform–acetic acid–methanol (15:1:4); Solvent 2: benzene–ethyl acetate–acetic acid (16:4:1); Solvent 3: benzene–ethyl acetate (4:1); Solvent 4: toluene–ethyl acetate–1-butanol–water (10:5:2:2); Solvent 5: heptane–chloroform–ethanol (10:10:1); Solvent 6: benzene. In Solvents 1–5 prazepam and oxazepam had the following R_f values respectively, 1: 0.93, 0.74; 2: 0.52, 0.24; 3: 0.45, 0.04; 4: 0.60, 0.21; 5: 0.37, 0.02.

The thin-layer chromatograms were scanned for radioactivity with a radiochromatogram scanner (Packard model 7200). The area under each peak was determined with a compensating polar planimeter (Keuffel and Esser). In this manner, the R_f value and the relative quantity of each compound were obtained.

Identification of Benzophenones—The three benzophenones involved in this study are aromatic amines of sufficiently weak basicity to permit their extraction from *N*-hydrochloric acid with ethyl acetate. The intensity of the yellow pigmentation of these three compounds is sufficient to permit their location on TLC plates visually as well as by radioscanning.

Additionally, these benzophenones were tested for the presence of a primary aromatic amine by running the Bratton-Marshall (5) assay directly on the chromatograms. This was done by spraying the chromatograms successively with 4 *N* HCl, 0.5% sodium nitrite, 0.5% ammonium sulfamate, and 0.1% *N*-(1-naphthyl)ethylenediamine hydrochloride.

Fate and Metabolism of Ring-labeled Prazepam—Four beagles, two of each sex weighing 7.0–8.6 kg., were used. Quantities of ^{14}C-prazepam tagged at C-5 corresponding to 10 mg./kg. body weight were weighed into four gelatin capsules, and were fed to the dogs. Each animal was placed into a separate metabolic cage and was allowed access to food and water.

Blood samples were withdrawn from the jugular vein of all four dogs at 0.5, 1, 2, 4, 6, and 24 hr. after drug administration. Portions

(4.5 ml.) of blood were mixed with 0.5 ml. of sodium oxalate solution, and diluted to 250 ml. with water. Aliquots (1.0 ml.) of the diluted solutions were counted for total radioactivity.

Urine and feces were collected for 24 hr. from all four dogs. Each urine collection was diluted to 400 ml., and 1.0-ml. aliquots were counted for radioactivity directly. Each fecal collection was washed from the cage with water, dioxane and methanol, held at 40° overnight, and filtered. The residue was re-extracted three times with 50% dioxane, and 1.0-ml. aliquots of the combined filtrates (3.0 l.) were counted for radioactivity by scintillation spectrometry.

Two of the dogs (one of each sex) were sacrificed 24 hr. after dosing. Their livers, kidneys, and brains were excised, homogenized in a blender (Waring) with 50% dioxane, and filtered. In the same way, the residues were re-extracted twice. The combined extract volumes were 2.5 l. for the livers, and 1.5 l. for the kidneys and brains. Aliquots (1.0 ml.) of the extracts were counted for radioactivity.

Collections of blood, urine and feces were made from the remaining two dogs at 48, 72, and 96 hr., after which these animals were also sacrificed to obtain the livers, kidneys, and brains. These specimens were treated as described above.

Urine and feces specimens collected 24 hr. after ^{14}C-prazepam administration to Dog No. 3 were studied. The radioactivity of the urine was 28.4×10^3 dpm./ml., and the aqueous dioxane extract of feces showed 59×10^3 dpm./ml.

A 5.0-ml. portion of the urine was adjusted to pH 7.0 and extracted with three equal volumes of ethyl acetate. The aqueous layer was brought to pH 5.5 and incubated at 37° for 4 hr. with 80 mg. of β-glucuronidase (Mann, specific activity = 50–70 units/mg.). Following incubation, a second extract was prepared using three 5.0-ml. volumes of ethyl acetate. All of the extracts were counted for radioactivity. In addition, the urine and the ethyl acetate extracts were analyzed by TLC.

A 10.0-ml. portion of the dog urine was hydrolyzed (6) by heating with an equal volume of concentrated HCl for 1 hr. at 100°. The hydrolysate was cooled and kept at 0–5° during partial neutralization by the addition of 4.4 g. of NaOH (4.8 g. required for neutralization). The acidic solution was extracted with three 5-ml. portions of ethyl acetate in order to recover the weakly basic chloroaminobenzophenones without extraction of other amines. The extract was analyzed by scintillation spectrometry, and TLC followed by radioscanning and testing for primary aromatic amine.

A 100-ml. portion of the fecal extract was diluted with 200 ml. of water and extracted twice with 50-ml. portions of ethyl acetate. The ethyl acetate extract was concentrated to 2 ml., and hydrolyzed with HCl as described above, diluted with eight volumes of water, and extracted with ethyl acetate to collect the radioactive reaction products. These extracts were assayed for total radioactivity and were resolved by chromatography.

RESULTS

The ^{14}C levels of the blood of dogs treated with ^{14}C-prazepam are shown in Table I. The values, expressed as a percentage of the administered dose, were calculated on the basis that the blood volume of the dogs was 90 ml./kg. (7). All of the levels were low; the maximum concentrations observed at 2–4 hr. after treatment, represented about 0.2% of the dose. The male dogs seemed to absorb this drug faster than the females. Plots of the data showed the mean half-life of radioactivity to be approximately 14 hr.

Table I—Radioactivity in Blood of Dogs After Oral Administration of Ring-labeled Prazepam

Time, hr.	Dose Radioactivity in Blood %[a] Dog 1 (m., 7.2 kg.)	Dog 2 (m., 8.6 kg.)	Dog 3 (f., 8.0 kg.)	Dog 4 (f., 8.3 kg.)
0.5	0.12	0.07	0	0
1	0.21	0.16	0	0
2	0.24	0.25	0.27	0.14
4	0.24	0.21	0.33	0.15
6	0.17	0.19	0.23	0.08
24	0.06	0.26	0.08	0.06
48	—	0.03	—	0
72	—	0.03	—	0
96	—	0	—	0

[a] Based upon blood volume of 90 ml./kg. (7).

Table II—Radioactivity in Urine, Feces, and Tissues of Dogs After Administration of Ring-labeled Prazepam

Specimen	Time, hr.	Dose Radioactivity, % Dog 1	Dog 2	Dog 3	Dog 4
Liver	24	0.266	—	2.62	—
	96	—	0.024	—	0.009
Brain	24	0.003	—	0.005	—
	96	—	0.001	—	0.001
Kidney	24	0.008	—	0.005	—
	96	—	0.002	—	0.001
Urine	24	1.74	4.61	4.59	1.86
	48	—	4.16	—	0.65
	72	—	0.41	—	0.07
	96	—	0.08	—	0.01
Feces	24	88.69	80.12	79.25	58.91
	48	—	5.47	—	34.36
	72	—	0.84	—	1.95
	96	—	0.26	—	0.12
Total recovery		96.7	96.0	86.5	97.9

Table II presents the ^{14}C content of the urine, feces, and tissues. The recovery of radioactivity was almost quantitative from the dogs sacrificed 96 hr. after drug administration, due mainly to very extensive excretion with the feces. The urinary excretion of radioactivity varied widely, from 1.7–4.6% of the dose in 24 hr. and from 2.6–9.3% in 96 hr.

The dog urine contained one radioactive component which overshadowed the others. This compound (R_f 0.14 in Solvent 1, 0.07 in Solvent 3, and 0.05 in Solvents 2, 4, and 5) was obviously neither prazepam nor oxazepam and was readily extracted into ethyl acetate only after incubating the urine with β-glucuronidase. Chromatograms of the ethyl acetate extract showed the single major tagged component to migrate as oxazepam in five solvent systems.

The acid-hydrolyzed dog urine showed five radioactive bands upon TLC in Solvent 3 (Table III). The slowest moving bands (at R_f 0.01 and 0.36) were colorless and represented 9 and 6%, respectively, of the total sample radioactivity. The identities of these two components are unknown; they may represent products obtained by incomplete hydrolysis. The three remaining labeled bands at R_f 0.45 (8% of the sample ^{14}C), 0.83 (72%), and 0.95 (4%) displayed the characteristic yellow color of the 2-amino-5-chlorobenzophenones. The major band at R_f 0.83 gave a positive Bratton-Marshall test and migrated as did 2-amino-5-chlorobenzophenone. This ketone is the hydrolysis product expected from oxazepam glucuronide and the quantity formed agrees with the amount of oxazepam collected by extracting the β-glucuronidase-treated urine. Although the band at R_f 0.45 was also a primary aromatic amine and corresponded chromatographically to 4′-hydroxy-2-amino-5-chlorobenzophenone, additional evidence is needed before this ketone can be conclusively considered to be the hydrolysis product of 4′-hydroxyoxazepam glucuronide (8). The fastest moving radioactive band (R_f 0.95) was not a primary aromatic amine and corresponded to 2-cyclopropylmethylamino-5-chlorobenzophenone. This ketone suggests that the urine contained some unchanged prazepam and/or 3-hydroxyprazepam glucuronide. The results obtained with Solvent 6 were confirmatory. The major band (R_f 0.34) accounted for 73% of the radioactivity, had a yellow color, reacted with Bratton-Marshall reagent, and showed an R_f corresponding to 2-amino-5-chlorobenzophenone. Likewise, the minor peaks at R_f 0.06 and 0.62 showed the same properties as 4′-hydroxy-2-amino-5-chlorobenzophenone and 2-cyclopropylmethylamino-5-chlorobenzophenone, respectively.

TLC of the feces extract showed one predominant component which migrated as prazepam in all five solvents. Hydrolyzed feces, however, yielded chromatograms indicating the presence of three radioactive components (Table III). One of these compounds accounted for 91% of the radioactivity on chromatograms developed in both solvents, and this band was identified as 2-cyclopropylmethylamino-5-chlorobenzophenone by its R_f values, yellow color,

Table III—TLC of Hydrolyzed Dog Urine and Feces

Sample	Solvent 3				Solvent 6			
	R_f	%	Color[a]	B–M[b]	R_f	%	Color[a]	B–M[b]
2-Amino-5-chlorobenzophenone (ACB)	0.81	—	Y	+	0.34	—	Y	+
N-Cyclopropylmethyl ACB	0.96	—	Y	−	0.57	—	Y	−
4′-Hydroxy ACB	0.46	—	Y	+	0.01	—	Y	+
Hydrolyzed urine	0.01	9.4	−	−	0.01	12.6	−	−
	0.36	6.3	−	−	0.06	10.2	−	+
	0.45	8.2	Y	+	0.34	72.7	Y	+
	0.83	71.7	Y	+	0.62	3.6	−	−
	0.95	4.3	Y	−				
Hydrolyzed feces	0.02	3.0	−	−	0.03	8.7	−	+
	0.60	5.6	−	+	0.57	91.3	Y	−
	0.98	91.4	Y	−				

[a] Y, yellow band observed; —, no visible color observed. [b] Primary aromatic amine by Bratton-Marshall assay on TLC plate: +, positive test; −, negative test.

and lack of a primary aromatic amine function. Therefore, the feces was concluded to contain unchanged prazepam. The other labeled components cannot be identified at this time although one is a primary aromatic amine.

DISCUSSION

In general, prazepam resembled diazepam (9, 10) and oxazepam (6) more than chlordiazepoxide (11) in its absorption and excretion by the dog. Characteristic of these three benzodiazepins were the low blood levels and the extensive excretion into the feces. It seems that this group of drugs readily enters the enterohepatic circulation, resulting in high fecal elimination even when low doses are injected intravenously (9).

Like diazepam, prazepam is dealkylated and hydroxylated by the dog, and subsequent conjugation yields oxazepam glucuronide, the principal end product. It is interesting to note that small quantities of oxazepam glucuronide were also detected in the urine of dogs treated with chlordiazepoxide (12). The evidence from experiments conducted *in vivo* suggested that the main pathway of diazepam metabolism involves dealkylation followed by hydroxylation (9, 10, 13). This view is supported by recent experiments showing that diazepam is dealkylated faster than it is hydroxylated in isolated perfused rat and mouse livers (14). However, it is still not clear whether *N*-alkyl oxazepams are converted more rapidly into oxazepam than is desalkylprazepam. This question holds pharmacological interest since the *N*-alkyloxazepams are more active than desalkylprazepam in muscle relaxant, antifighting, and anticonvulsant evaluations (15).

SUMMARY

Four dogs were administered a single oral dose (10 mg./kg.) of ring-labeled prazepam. Urine, feces, and blood were assayed for ^{14}C at intervals up to 96 hr. after treatment. The radioactivity in selected tissues was determined at 24 and 96 hr. following dosing. Most of the radioactivity (79–95%) was recovered in the feces and 1.7–9.3% was found in the urine. Although this was not a materials balance study (since the animals were not assayed in their entirety), the ^{14}C recoveries averaged 89% after 24 hr. and 97% after 96 hr. Unaltered prazepam accounted for 91% of the ^{14}C in the feces. Oxazepam glucuronide was responsible for about 72% of the urinary radioactivity. Tentatively identified in the urine were unchanged prazepam and/or 3-hydroxyprazepam glucuronide, and 4′-hydroxyoxazepam glucuronide.

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