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# DISPOSITION AND METABOLISM OF OLANZAPINE IN MICE, DOGS, AND RHESUS MONKEYS

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**ABSTRACT:**

**Olanzapine (OLZ) is a novel antipsychotic agent with a high affinity for serotonin (5-$HT_2$), dopamine ($D_1$/$D_2$/$D_4$), muscarinic ($m_1$–$m_5$), adrenergic ($\alpha_1$), and histamine ($H_1$) receptors. The pharmacokinetics, excretion, and metabolism of OLZ were studied in CD-1 mice, beagle dogs, and rhesus monkeys after a single oral and/or intravenous dose of [$^{14}$C]OLZ. After oral administration, OLZ was well absorbed in dogs (absolute bioavailability of 73%) and to the extent of at least 55% in monkeys and 32% in mice. The terminal elimination half-life of OLZ was relatively short in mice and monkeys (~3 hr) and long in dogs (~9 hr). In mice and dogs, radioactivity was predominantly eliminated in feces; but, in monkeys, the major route of elimination of radioactivity was urine. Dogs and monkeys excreted in urine, respectively, 38% and 55% of the dose over a 168-hr period, whereas the fraction of the dose excreted in urine of mice over the collection period (120 hr) was 32%. OLZ was subject to substantial first-pass metabolism; at the $t_{max}$, OLZ accounted for 19%, 18%, and 8% of the radioactivity, in mice, dogs, and monkeys, respectively. The ratio of AUC OLZ to AUC radioactivity was, respectively, 10%, 14%, and 4% in mice, dogs, and monkeys. The principal urinary metabolites in mice were 7-hydroxy OLZ glucuronide, 2-hydroxymethyl OLZ, and 2-carboxy OLZ accounting for ~10%, 4%, and 2% of the dose. Metabolites that were present in urine in lesser amounts were 7-hydroxy OLZ, *N*-desmethyl OLZ, and *N*-desmethyl-2-hydroxymethyl OLZ. In dogs, the major metabolite accounting for ~8% of the dose was 7-hydroxy-*N*-oxide OLZ. Other metabolites identified were 2-hydroxymethyl OLZ, 2-carboxy OLZ, *N*-oxide OLZ, 7-hydroxy OLZ, and its glucuronide and *N*-desmethyl OLZ. The major metabolite in monkey urine was *N*-desmethyl-2-carboxy OLZ, and accounted for ~17% of the dose. In addition, *N*-oxide-2-hydroxymethyl OLZ, *N*-oxide-2-carboxy OLZ, *N*-desmethyl-2-hydroxymethyl, 2-carboxy OLZ, and 2-hydroxymethyl OLZ were identified in monkey urine. Thus, in mice and dogs, OLZ was metabolized through aromatic hydroxylation, allylic oxidation, *N*-dealkylation, and *N*-oxidation reactions. In monkeys, OLZ was biotransformed mainly through double oxidation reactions involving the allylic carbon and methyl piperazine nitrogen. Whereas the oxidative metabolic profile of OLZ in animals was similar to that of humans, animals were notable for not forming appreciable amounts of the principal human metabolite (*i.e.* 10-*N*-glucuronide OLZ).**

OLZ[1] (fig. 1) is a new antipsychotic drug with a thienobenzodiazepinyl structure. OLZ displays a broad pharmacological profile with potent activity at dopamine ($D_1$/$D_2$/$D_4$), serotonin (5-$HT_{2A/2C}$), muscarinic (especially $m_1$), histamine ($H_1$) and adrenergic ($\alpha_1$) receptors (1, 2). The receptor binding profile of OLZ is very similar to clozapine, although OLZ is a more potent inhibitor of these receptors.

In clinical studies with patients suffering from schizophrenia or schizophreniform disorder, OLZ was effective in the treatment of both positive and negative symptoms of schizophrenia, with a low incidence of extrapyramidal side-effects (3–5). Antipsychotic efficacy of OLZ was demonstrated in the dose range of 5–20 mg/day.

The disposition and metabolism of OLZ after a single oral dose to healthy volunteers has recently been reported (6). OLZ was well absorbed and extensively metabolized. The primary metabolic route was *N*-glucuronidation. OLZ also underwent oxidative metabolism through *N*-oxidation, *N*-demethylation, and 2-alkyl hydroxylation. This study describes the comparative absorption, pharmacokinetics, and metabolism of OLZ in mice, dogs, and monkeys. The studies were conducted after the administration of [$^{14}$C]OLZ.

[1] Abbreviations used are: OLZ, olanzapine; LSC, liquid scintillation counting; $C_{max}$, maximum plasma concentration; $t_{max}$, time to maximum concentration; AUC, area under the plasma concentration-time curve; LC-MS/MS, liquid chromatography-tandem mass spectrometry; CID, collision-induced dissociation; IV, intravenous.

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## Materials and Methods

**Reference Compounds and Other Materials.** The following compounds were synthesized at Lilly Research Laboratories: OLZ (2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-B][1,5]benzodiazepine), [4,10a-$^{14}C_2$]OLZ ([$^{14}$C]OLZ; radiochemical purity, 98.7%; specific activity, 26.2 $\mu$Ci/mg), 4′-*N*-desmethyl OLZ (*N*-desmethyl OLZ, 2-methyl-4-(1-piperazinyl)-10*H*-thieno[2,3-B][1,5]benzodiazepine), 4′-*N*-oxide OLZ (*N*-oxide OLZ, 4-(2-methyl-10*H*-thieno[2,3-B][1,5]benzodiazepin-4-yl)-1-methylpiperazine-1-oxide), 2-hydroxymethyl OLZ (4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-B][1,5]benzodiazepine-2-methanol), 2-carboxymethyl OLZ (methyl 4-(4-methyl-1-piperazinyl)-10*H*-thieno[2,3-B][1,5]benzodiazepine-2-carboxylate), 7-ethoxy OLZ (7-ethoxy-2-methyl-4-(4-methyl-1-piperazinyl)-10*H*-thieno⟨2,3-B⟩⟨1,5⟩benzodiazepine), and 4′-*N*-desmethyl-2-hydroxymethyl OLZ (4-(1-piperazinyl)-10*H*-thieno[2,3-B][1,5]benzodiazepine-2-methanol). NEE-154 Glusulase was purchased from the DuPont Company (Wilmington, DE). $\beta$-Saccharolactone was purchased from Sigma Chemical Co. (St. Louis, MO). Scintisol was supplied by Isolab, Inc. (Akron, OH). HPLC-grade ammonium acetate, acetonitrile, triethylamine, and reagent-grade boron tribromide were purchased from Fisher Scientific (Fair Lawn, NJ). 7-Hydroxy OLZ was pre-

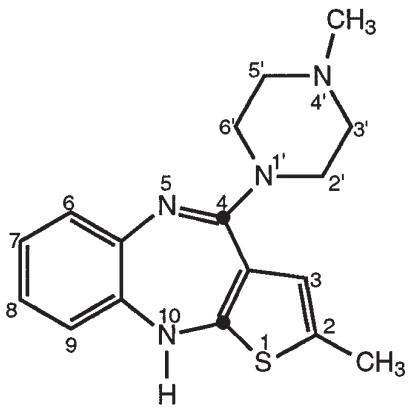

FIG. 1. *Chemical structure of OLZ; the position of $^{14}C$ atoms is indicated by bullet points.*

pared by deethylation of 7-ethoxy OLZ, and 2-carboxyl OLZ was prepared by hydrolysis of 2-carboxymethyl OLZ. Approximately 2 mg of each starting material was placed in separate siliconized tubes and dissolved in methylene chloride (2 ml). The solution was flushed with nitrogen and treated with boron tribromide solution (2 ml of 25% solution in methylene chloride). The reaction was allowed to proceed at room temperature for 2 hr. Approximately 90% of 7-ethoxy OLZ was converted to 7-hydroxy OLZ, whereas ~50% of 2-carboxymethyl was converted to the corresponding acid as determined by HPLC and electrospray LC/MS. *N*-Desmethyl-2-carboxy OLZ was prepared by oxidizing the corresponding hydroxy compound using chromium trioxide (7).

**Animal Experiments.** All animal experiments were conducted according to protocols approved by the Eli Lilly Animal Care and Use Committee. The dosing solution used for all animal studies was prepared by dissolving the required amounts of OLZ and [$^{14}$C]OLZ in 1 M HCl and titrating the solution to approximately pH 6 by the addition of 0.1 M NaOH. The appropriate volume was then obtained by the subsequent addition of water.

*Mouse.* Male CD-1 mice were obtained from Charles River Laboratories (Wilmington, MA) and acclimatized for 3 days before use. Food and water were supplied *ad libitum* at all times throughout the experiment. For radiolabeled excretion study, the mice were divided into three groups, with each group containing five mice. Each animal was administered a single oral (gavage) dose of OLZ (15 mg/kg containing 420 $\mu$Ci/kg of [$^{14C}$]OLZ). Urine and fecal samples were collected at 24-hr intervals for up to 120 hr. For pharmacokinetic study, four mice were used for each time point and dosed as described. Blood was collected and pooled from four mice at 0.5, 1, 2, 4, 7, 12, 24, 48, and 72 hr after the dose. Plasma was obtained by centrifugation and stored at −70°C until analysis. Metabolite identification was conducted in urine obtained from mice given a 20 mg/kg dose. Urine samples collected for 24-hr postdose from eight mice were combined and stored at −70°C until analyzed. Additional mice (10) were administered a single oral dose (15 mg/kg) of OLZ, and plasma was collected at ~1 hr for metabolite identification.

*Dog.* Four female beagle dogs (age: 2–4 years; weight: 8.7–13.1 kg) were obtained from stock animals maintained at Lilly Research Laboratories and placed in individual stainless-steel metabolism cages. Animals were fasted overnight before and 2 hr after drug administration. Animals were given a single oral (gavage) dose of OLZ (5 mg/kg containing 4 $\mu$Ci/kg of [$^{14}$C]OLZ). Urine and fecal samples were collected every 24 hr for 168 hr. Blood was drawn at 0, 0.5, 1, 3, 6, 12, 24, 48, 96, and 168 hr after dosing. Aliquots were withdrawn for determination of radioactivity, and the remainder was centrifuged to obtain plasma. Plasma was also obtained from three female dogs given a single intravenous dose of OLZ (5 mg/kg containing 5 $\mu$Ci/kg [$^{14}$C]OLZ) at 0, 0.08, 0.25, 0.5, 1, 3, 6, 12, 24, 36, 48, 72, 96, 120, 144, and 168 hr postdose. For identification of plasma metabolites, another group of three dogs were dosed orally with OLZ (5 mg/kg), and plasma was collected at 3 and 12 hr after the dose.

*Monkey.* Young adult rhesus monkeys (2 males and 2 females) weighing between 3 and 7 kg were used in the study. Each animal was given a single oral (nasogastric) dose of OLZ (5 mg/kg containing 8.9 $\mu$Ci/kg [$^{14}$C]OLZ). Blood was collected at 0, 0.5, 1, 4, 8, 12, 24, 48, 96, 120, and 168 hr postdose, whereas urine and fecal samples were collected every 24 hr up to 168 hr.

**Analysis of Radioactivity.** Total radioactivity in plasma and urine samples was determined using LSC after the addition of scintillation cocktail (Aquassure; New England Nuclear, Boston, MA). Feces was suspended in 5% aqueous sodium lauryl sulfate solution, and aliquots of the dried homogenate were combusted in a sample oxidizer. The fecal sample prepared in this manner was counted after addition of Aquassure. Mice carcasses were digested using alcoholic potassium hydroxide. The homogenate obtained in this manner was neutralized with acetic acid, and the radioactivity was determined by LSC. Quench correction was conducted by automatic external standardization.

**HPLC Assay of Plasma OLZ.** Concentrations of OLZ in mouse, dog, and monkey plasma were determined by HPLC (8). In this assay, OLZ and the internal standard (2-ethyl homolog of OLZ) are isolated from plasma using a mixed-mode, solid-phase extraction, separated with a reversed-phase method, and detected electrochemically. The upper and lower limits of quantitation of the assay were 100 and 1 ng/ml, respectively.

**Pharmacokinetics.** Noncompartmental analysis was used to determine the pharmacokinetics of OLZ and radioactivity. $C_{max}$ and $t_{max}$ were assesed by visual inspection. The terminal elimination half-life was calculated using the relationship 0.693/$k$, where $k$ is the elimination rate constant. The $AUC_{-t}$ was calculated up to the last time point ($t$) by the trapezoidal rule.

***In Vitro* Protein Binding.** [$^{14}$C]OLZ was dissolved in *n*-propyl alcohol at 0.2, 0.02, and 0.002 mg/ml, and an aliquot (15 $\mu$l) of each concentration was added into 2985 $\mu$l volume control plasma. Plasma samples were then placed in a water bath (~37°C) for 1 hr. After ultracentrifugation (360,000 *g* at 37°C for 4 hr), the amount of OLZ in the supernatant was determined by LSC. The fraction of OLZ bound to protein was calculated from the radioactivity concentrations in the spiked sample and the supernatant.

**Metabolite Isolation.** Aliquots of urine samples from each of the orally dosed dogs were combined (~80 ml total urine) and made basic by the addition of 0.1 M ammonium hydroxide (8 ml). Ethyl acetate (300 ml) was added, and the phases were mixed by shaking vigorously. The ethyl acetate layer was separated and evaporated to dryness in a water bath (40°C) under a stream of nitrogen. The aqueous fraction was lyophilized to dryness, dissolved in water, and analyzed by HPLC. Pooled mouse urine was also extracted as described. For NMR analysis, the 7-hydroxyl-*N*-oxide metabolite was isolated from urine using column chromatography. Approximately 400 g of Amberlite XAD-2 resin was packed in a 2.5 × 30 cm glass column. The remaining urine samples from each dog were combined (~1.5 liters) and passed through the column after preconditioning the column with methanol and purified water. The column was washed with water and the radioactivity eluted with methanol. Methanolic extracts were concentrated *in vacuo* at 25°C using a rotary evaporator, and the resulting residue was reconstituted in 70 ml methanol/water (1:1) for HPLC analysis. Aliquots (50 ml; 0–24 hr) of urine sample from each monkey were lyophilized to ~2 ml. The residue was reconstituted in 5 ml water:methanol (4:1, v/v) and separated by HPLC.

**Estimation of the Amount of Metabolites.** The amount of each metabolite in urine was estimated by LSC after isolation by HPLC. An aliquot of the ethyl acetate or aqueous extract (50–200 $\mu$l) was injected into HPLC, and each metabolite was collected as it eluted the column. Scintisol (15 ml) was added, and the amount of radioactivity was determined by LSC. The total radioactivity in the sample was determined by injecting an equal aliquot into the HPLC injector and collecting the entire sample before it reached the column.

**Hydrolysis of Conjugates.** Glucuronide conjugates (~2 $\mu$g) isolated from urine were hydrolyzed to the corresponding aglycone by incubating with Glusulase (containing 2,070 units of $\beta$-glucuronidase and 150 units sulfatase) at 37°C for up to 20 hr. Incubations were also conducted in the absence of Glusulase and in the presence of $\beta$-saccharolactone (0.0325 M).

**HPLC Separation of Metabolites.** The HPLC system consisted of a Beckman pump, NEC controller, Waters Wisp autosampler, Applied Biosystem UV detector, and a Berthold radiodetector with 150 $\mu$l yittrium solid cell. Aliquots (≤200 $\mu$l) of concentrated urine or extract were analyzed on a Hypersil $C_{18}$ column (5 $\mu$m particle size, 0.46 × 25 cm) using a gradient containing A (0.1 M ammonium acetate) and B (1% triethylamine in acetonitrile). The initial solvent composition was 90% A and 10% B. After 2 min, the pump was programmed to increase solvent B by 2.5%/min until a proportion of 40% A and 60% B was achieved. The mobile phase was maintained for 8 min at that composition. The flow rate was 1 ml/min. Metabolites were isolated

TABLE 1

*Urinary and fecal elimination of radioactivity in mice, dogs, and monkeys after the administration of a single oral dose of [$^{14}$C]OLZ*

| Time | % Dose Excreted | | | | | |
|---|---|---|---|---|---|---|
| | Mouse[a,b] | | Dog[c] | | Monkey[d] | |
| | Urine | Feces | Urine | Feces | Urine | Feces |
| *hr* | | | | | | |
| 0–24 | 25.0 ± 1.8 | 46.7 ± 2.9 | 20.5 ± 3.7 | 2.7 ± 2.9 | 47.6 ± 3.6 | 6.2 ± 8.7 |
| 24–48 | 4.0 ± 1.1 | 11.6 ± 3.0 | 11.7 ± 5.1 | 22.9 ± 13.0 | 4.8 ± 0.70 | 14.0 ± 3.4 |
| 48–120 | 2.9 ± 2.2 | 6.0 ± 2.3 | 5.6 ± 1.0 | 19.0 ± 9.0 | 2.0 ± 0.70 | 8.0 ± 5.8 |
| 120–168 | NC[e] | NC | 0.60 ± 0.13 | 1.1 ± 0.27 | 0.23 ± 0.08 | 0.31 ± 0.10 |
| Total | 31.9 ± 2.8 | 64.3 ± 3.4 | 38.4 ± 2.6 | 45.6 ± 5.4 | 54.6 ± 3.7 | 28.5 ± 5.2 |
| Grand total | 97.4 ± 1.1[f] | | 84.3 ± 5.0[g] | | 83.1 ± 3.9 | |

Data represent mean ± SD.
[a] $N = 3$.
[b] % dose recovered from the carcass was 0.6%.
[c] $N = 4$.
[d] $N = 4$.
[e] NC, not collected.
[f] Includes radioactivity from cage washings and carcass.
[g] Includes cage washings.

by collecting the radioactive eluent as it eluted off the column. Several injections were made to obtain a sufficient amount of each metabolite for mass spectral identification.

**LC-MS/MS.** Isolated metabolites were analyzed by LC/MS and LC-MS/MS on a Finnigan MAT TSQ700. Metabolites were introduced into the electrospray LC interface using a Waters Model 600 pump. Metabolites were separated on an Inertsil $C_{18}$ column (5 $\mu$m particle size, 0.46 × 25 cm) using the same gradient as described with 0.05 M ammonium acetate and acetonitrile. Injection volumes ranged from 10 to 200 $\mu$l. The flow rate was 1 ml/min, and the effluent was split such that equal volumes were delivered into the ion source and a Raytest Ramona model 5LS radiodetector. MS spectra were obtained by scanning from *m/z* 200 to 600 every second. For CID experiments, the collision gas (argon) pressure was maintained at 2.0 m torr, and the collision offset voltage was −20 eV. MS and MS/MS spectra were averaged for 1 min.

**NMR Spectroscopy.** Proton and carbon-13 NMR spectra were recorded in $d_6$-DMSO or $CDCl_3$ on a Bruker AMX spectrometer operating at 500 MHz. Chemical shifts are reported in ppm relative to tetramethylsilane.

## Results

**Excretion of Radioactivity.** Mice administered a single oral dose (15 mg/kg) of OLZ eliminated 64.3 ± 3.4% (mean ± SD) and 31.9 ± 2.8% of the radioactivity, respectively, in feces and urine over a 120-hr period (table 1). The majority of the dose (>87%) was excreted during the first 48 hr of dosing. Less than 1% of the administered dose was recovered in the carcasses.

In dogs, ~84% of the radioactivity was recovered after 168 hr, with slightly more radioactivity eliminated in the feces (45.6 ± 5.4%) than in the urine (38.4 ± 2.6%). Greater than 50% of the dose was recovered within 48 of dosing (table 1).

In monkeys, renal excretion was the primary mode of radiocarbon elimination accounting for 54.6 ± 3.7% of the dose. Another 28.5 ± 5.2% of the dose was eliminated *via* the feces over the same period. Greater than 50% of the dose was eliminated in the urine and feces 24 hr after the dose (table 1). There was no difference between males and females with respect to the amount of radioactivity in either the urine or feces.

**Pharmacokinetics.** *Mice.* Pharmacokinetic parameters of OLZ and radioactivity in mice are shown in table 2. OLZ was quantitated in plasma using an HPLC assay with a lower limit of quantitation of 1 ng/ml. The $C_{max}$ of OLZ was 421 ng/ml and occurred at 0.5 hr after the dose. The corresponding value for radioactivity was 2,260 ng-eq/ml and was reached at a much later time (4 hr). At 0.5 hr, OLZ accounted for ~19% of plasma radioactivity. This is indicative of the extensive metabolism of OLZ in the mouse. Similarly, OLZ accounted for 10% of the total $^{14}$C AUC. The plasma terminal half-life of OLZ was 3.2 hr. Radioactivity in plasma declined slowly with a half-life of 10.6 hr. The plasma radioactivity *vs.* time curve (fig. 2) showed elevated concentrations at both 0.5 and 4 hr, suggesting enterohepatic recycling.

*Dogs.* The mean $C_{max}$ of OLZ was 172 ± 69 ng/ml and occurred between 1 and 3 hr in 3 of the 4 animals tested. The fourth animal had a $t_{max}$ of 6 hr. The elimination of OLZ from plasma seemed to be biphasic (fig. 3), with the terminal phase displaying a half-life of 9.2 ± 1.4 hr.

The mean $t_{max}$ for radioactivity in plasma was 1 ± 0.0 hr, and the $C_{max}$ was 949 ± 296 ng-eq/ml. Plasma radioactivity declined with a mean half-life of 27.6 ± 12.0 hr. The ratio of AUC OLZ to AUC radioactivity was 0.14.

After a single IV dose of OLZ to three dogs, the mean $C_{max}$ and AUC for OLZ were, respectively, 871 ± 241 ng/ml and 2,633 ± 1,041 ng * hr/ml. The corresponding values for plasma radioactivity were 1,145 ± 195 ng-eq/ml and 18,813 ± 2,598 ng-eq * hr/ml. Thus, after an IV administration, at the $t_{max}$ OLZ accounted for 76% of the radioactivity, compared with a value of 18% after an oral dose. Because the amount of radioactivity excreted in urine after the oral and IV doses was almost the same (38.4% and 39.7% of the dose), the decreased bioavailability after oral administration is likely due to first-pass metabolism. The ratio of AUC OLZ to $^{14}$C AUC was the same as that obtained after oral dosing. The absolute oral bioavailability of OLZ was calculated to be 73%.

*Monkeys.* The mean $C_{max}$ of OLZ and radioactivity were 60 ± 18 and 757 ± 169 ng eq/ml, and were reached on average within 1.5 hr postdose. Therefore, at the $C_{max}$ OLZ accounted for ~8% of the plasma radioactivity. On the basis of AUC, the fraction of plasma radioactivity represented by OLZ was ~4%.

The mean elimination half-life of OLZ was 3.4 ± 1.2 hr. The

TABLE 2

*Mean pharmacokinetic parameters of OLZ and radioactivity in mice, dogs and monkeys after the administration of a single oral dose of [$^{14}$C]OLZ*

| Parameter | Mouse[a] | Dog | Monkey |
|---|---|---|---|
| Dose (mg/kg) | 15 | 5 | 5 |
| $C_{max}$ (ng or ng-eq/ml) | | | |
| OLZ | 421 | 172 ± 69 | 60 ± 18 |
| $^{14}$C | 2,260 | 949 ± 296 | 757 ± 169 |
| OLZ as % of $^{14}$C | 19% | 18% | 8% |
| $t_{max}$ (hr) | | | |
| OLZ | 0.5 | 3.3 ± 2.1 | 1 ± 0.0 |
| $^{14}$C | 4 | 1 ± 0.0 | 1.5 ± 1.7 |
| $t_{1/2}$ (hr) | | | |
| OLZ | 3.2 (7–12 hr)[b] | 9.2 ± 1.4 (3–48 hr)[b] | 3.4 ± 1.2 (1–12 hr) |
| $^{14}$C | 10.6 (7–48 hr)[b] | 27.6 ± 12.0 (24–96 hr)[b] | 5.3 ± 0.7 (4–12 hr) |
| AUC (ng or ng-eq · hr/ml) | | | |
| OLZ | 1,522 | 1,923 ± 325 | 536.6 ± 208.3 |
| $^{14}$C | 15,201 | 13,405 ± 2,123 | 14,429 ± 1,572 |
| OLZ as % of total $^{14}$C | 10% | 14% | 4% |

[a] Mouse data obtained from pooled plasma, for dogs and monkeys values represent mean ± SD ($N$ = 4).
[b] Points used in the determination of the respective half-lives.

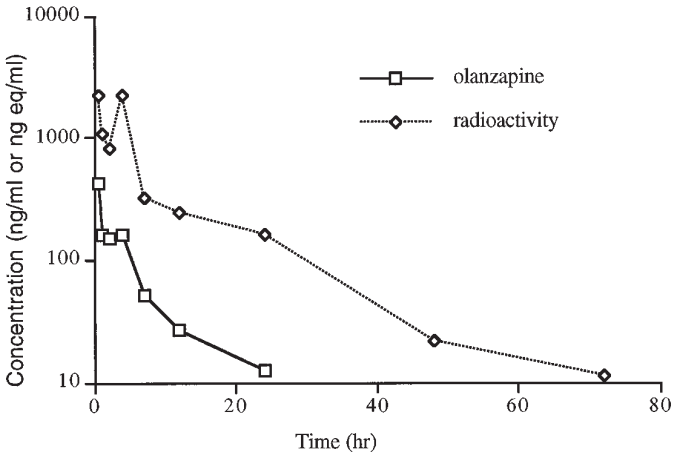


FIG. 2. *Plasma concentration vs. time profiles for OLZ and total radioactivity in mice given an oral dose of 15 mg/kg of [$^{14}$C]OLZ.*

Radioactivity is expressed as mean ± SD ($N$ = 4), whereas OLZ concentrations were obtained from pooled plasma.

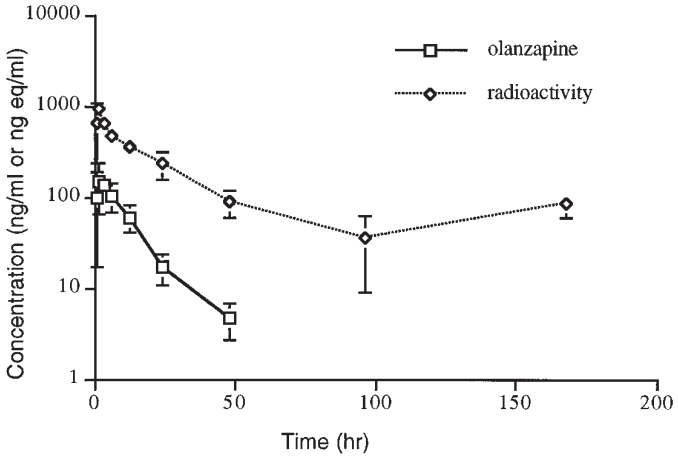


FIG. 3. *Plasma concentration vs. time profiles for OLZ and total radioactivity in dogs given an oral dose of 5 mg/kg of [$^{14}$C]OLZ.*

Data are expressed as mean ± SD ($N$ = 4).

elimination of radioactivity from plasma was biphasic (fig. 4), with the initial and terminal phases having half-lives of, respectively, 5.3 ± 0.7 and 98.7 ± 26.5 hr.

***In Vitro* Plasma Protein Binding.** The plasma protein binding of OLZ was similar in the three species studied, with mean binding being 77%, 75%, and 83% in mice, dogs, and monkeys, respectively. The binding was concentration-independent (10–1,000 ng/ml). The extent of protein binding was lower in these species than that reported for humans at 93% (6).

**Metabolism.** *Mice.* Upon partitioning pooled urine (0–24 hr) between ethyl acetate and water, 19% of the radioactivity was extracted into the ethyl acetate, whereas 76% remained in the aqueous fraction. An aliquot of the aqueous fraction was separated by HPLC with radiochemical detection and yielded the chromatogram in fig. 5. The corresponding HPLC chromatogram from the ethyl acetate extract is shown in fig. 6. The individual peaks were collected and analyzed by direct infusion electrospray MS and MS/MS. The following metabolites were identified in urine of mice by comparing their LC and LC-MS/MS properties to those obtained from synthetic standards.

The metabolite that eluted as peak 1 in fig. 5 was identified as 2-carboxy OLZ on the basis of the similarity of its HPLC retention time and product ion spectrum to those obtained from a sample of synthetic 2-carboxy OLZ. The positive ion electrospray mass spectrum of the major urinary metabolite (*peak 2*, fig. 5) exhibited an $MH^+$ ion at *m/z* 505, which suggested that the metabolite was the glucuronide of a hydroxylated OLZ derivative ($M_r$ OLZ = 312). The product ion spectrum of *m/z* 505 was dominated by the fragment at *m/z* 329, which is likely due to loss of dehydroglucuronic acid from the conjugate. β-Glucuronidase hydrolysis of the conjugate resulted in 7-hydroxy OLZ, confirming the major metabolite in urine as 7-hydroxy OLZ glucuronide. Peaks 3 and 4 were characterized as *N*-desmethyl-2-hydroxymethyl OLZ and 2-hydroxymethyl OLZ, respectively, by comparison with authentic standards. Six metabolites (fig. 6) were isolated from the ethyl acetate extract for MS identification. 2-Hydroxymethyl OLZ, which was also present in the aqueous fraction, was identified as the component eluting as peak 1 in fig. 6. The metabolite that eluted as peak 2 had the same HPLC retention volume and MS/MS fragmentation as authentic 7-hydroxy OLZ. The metabolite shown as peak 3 (fig. 6) was identified as *N*-desmethyl OLZ. Unchanged OLZ was also excreted in urine (*peak 4*, fig. 6). The identities of the other radiolabeled components in fig. 6 were not

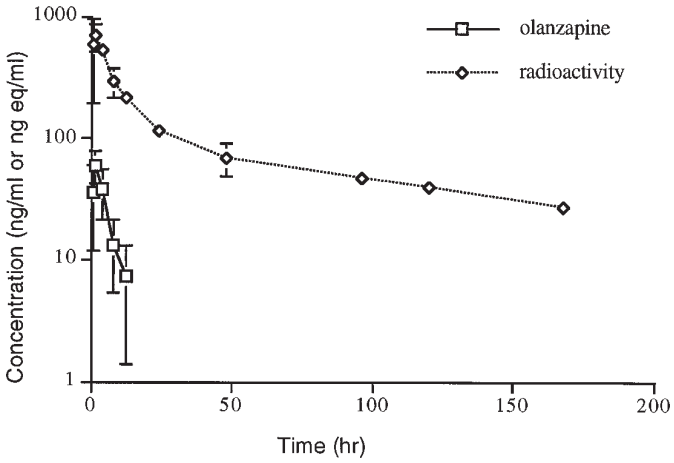


FIG. 4. *Plasma concentration vs. time profiles for OLZ and total radioactivity in monkeys given an oral dose of 5 mg/kg of [$^{14}$C]OLZ.*

Data are expressed as mean ± SD ($N$ = 4).

confirmed, although MS/MS fragmentation indicated that they were OLZ metabolites. The peak eluting between peaks 2 and 3 had an apparent $MH^+$ ion of *m/z* 327, 2 Da less than that of 2-hydroxymethyl OLZ. This metabolite could possibly be the precursor of 2-carboxy OLZ, the 2-formyl derivative of OLZ.

In plasma, in addition to the parent compound, 2-hydroxymethyl OLZ, *N*-desmethyl OLZ and the glucuronide of a hydroxy OLZ metabolite were detected. Also, mass spectral data was obtained that indicated the presence of two isomeric glutathione conjugates of OLZ. The conjugates exhibited an $MH^+$ ion at *m/z* 618, which upon CID fragmentation, gave MH-129$^+$—a characteristic loss of glutathione conjugates (9), in addition to other fragments consistent with the glutathione conjugate of OLZ. The MS/MS data also suggested that the glutathione moiety was attached to one of the carbons of the benzene ring of OLZ.

Six metabolites of OLZ were identified in the urine of mice in addition to the parent compound. Based on the percentage of the radioactivity that was extracted into the ethyl acetate (19%) and the percentage remaining in the aqueous fraction (76%), the amount of urinary radioactivity accounted for by each metabolite was estimated as shown in table 3.

*Dogs.* Pooled urine sample from the first 48 hr after dosing was used for metabolite identification. The partitioning of radioactivity between ethyl acetate and water was similar to that obtained for mouse urine with 15% extracted into ethyl acetate and 79% of the radioactivity remaining in the aqueous fraction. The HPLC separation of the radioactive components in the ethyl acetate and aqueous fractions is shown, respectively, in figs. 7 and 8. In the ethyl acetate extract, 2-hydroxymethyl OLZ, *N*-oxide OLZ, 7-hydroxy OLZ, *N*-desmethyl OLZ (*peaks* 1–4; fig. 7) were identified in addition to the parent compound.

The major component in the aqueous fraction (*peak 3*, fig. 8) had a retention time that was different from the available standards. The electrospray MS of this metabolite gave an apparent protonated molecular ion of *m/z* 345. MS/MS experiments indicated that the metabolite was an *N*-oxygenated species with a hydroxyl group on the benzodiazepine moiety. Approximately 30 mg of the metabolite was isolated from urine using XAD-2 chromatography and further purified by HPLC fractionation. The $^1$H- and $^{13}$C-NMR data obtained for the metabolite are shown in table 4. The $^1$H-NMR of the metabolite showed a downfield shift of the 4′-$CH_3$ to δ 3.08 (δ 2.21 for OLZ) and was identical to the value obtained for *N*-oxide OLZ. Similarly, the $^{13}$C-NMR exhibited a downfield shift of the 4′-$CH_3$ resonance to δ 58.09 (δ 45.73 for OLZ). Two-dimensional nuclear Overhauser enhancement was used to confirm the exact position of the hydroxyl group on the benzene ring of OLZ. The absence of a C-7 proton (δ 6.83 for OLZ) and the fact that the C-9 proton showed ortho coupling only ($J$ = 8 Hz) indicated the hydroxyl group was at the C-7 position. Thus, on the basis of combined MS and NMR data, the major urinary metabolite in dogs was identified as 7-hydroxy-*N*-oxide OLZ.

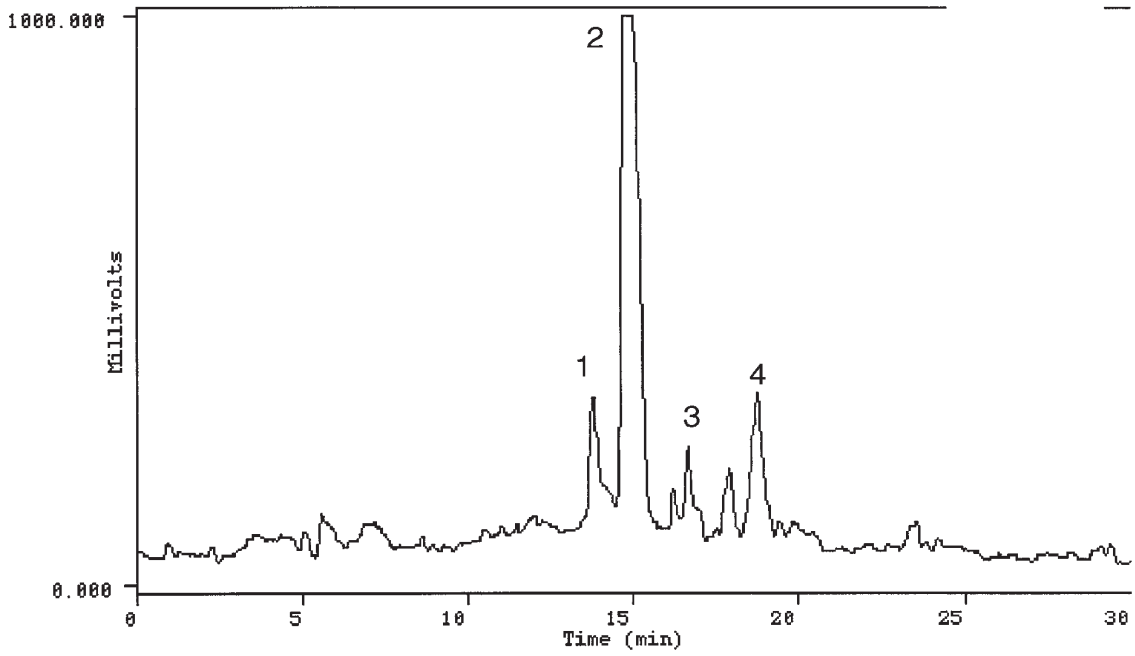


FIG. 5. *HPLC radiochromatogram obtained from the aqueous fraction of mouse urine.*

1, 2-carboxy OLZ; 3, 7-hydroxy OLZ glucuronide; 3, *N*-desmethyl-2-hydroxymethyl OLZ; 4, 2-hydroxymethyl OLZ.

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