Pharmacokinetics of the insulin-sensitizing agent troglitazone in cats

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Objective—To determine pharmacokinetics of troglitazone in healthy cats after IV and oral administration of a single dose of the drug.

Animals—5 healthy ovariohysterectomized adult cats.

Procedure—Using a randomized crossover design, cats were given 5 mg of troglitazone/kg of body weight IV and 40 mg of troglitazone/kg orally. Blood and urine samples were collected after drug administration, and concentrations of troglitazone in plasma and urine were determined by use of high-performance liquid chromatography.

Results—Area-moment analysis was used to calculate pharmacokinetic variables. Terminal phase half-life was 1.1 ± 0.1 hours. Steady-state volume of distribution was 0.23 ± 0.15 L/kg. After IV administration, clearance was 0.33 ± 0.04 L/h/kg. Drug was not detected in urine samples. Mean bioavailability of orally administered troglitazone was 6.9%.

Conclusions and Clinical Relevance—The overall disposition of troglitazone in cats was similar to that reported in other species, including humans. Troglitazone has low and variable oral bioavailability. Clearance of the compound is moderate. Little if any unchanged troglitazone is excreted in urine; thus, metabolism and biliary excretion play predominant roles in elimination of the drug. On the basis of troglitazone pharmacokinetics in healthy cats, as well as on the basis of pharmacodynamics of the drug in humans and other animals, a regimen that uses a dosage of 20 to 40 mg/kg administered orally once or twice per day to cats will produce plasma concentrations of the insulin-sensitizing agent that have been documented to be effective in humans. (*Am J Vet Res* 2000;61:775–778)

Troglitazone ([±]-5-[4-[6-hydroxy-2,5,7,8-tetramethylchroman-2-ylmethoxy]benzyl]-2,4-thiazolidinedione) is an orally administered insulin-sensitizing agent that was recently approved in the United States for use as a sole therapeutic agent as well as in combination with sulfonylureas or insulin to improve glycemic control in people with type-2 **diabetes mellitus (DM)**.[1] Troglitazone is a thiazolidinedione **(Fig 1)**. The precise mechanism of action is not known but may be associated with its activity as a potent and selective activator of **peroxisome proliferator-activated receptor-γ (PPAR-γ)**,[2] a nuclear receptor predominantly found in adipose tissue with little expression in skeletal muscle.[3-4] Activation of PPAR-γ induces the expression of several genes involved in uptake of fatty acids.[5-7] This leads to increased uptake of fatty acids in adipose tissue and, as a result of the difference in tissue distribution, a relative depletion of fatty acids in muscle.[8] Fatty acids in muscle interfere with glucose metabolism.[9] Thus, troglitazone's mechanism of action may result from activating PPAR-γ, thereby reversing accumulation of fatty acids in muscle and improving glucose homeostasis.[8]

Adverse effects in placebo-controlled clinical trials in humans are uncommon. Although orally administered hypoglycemic agents such as sulfonylureas may cause hypoglycemia, use of troglitazone as the sole therapeutic agent does not.[10-12] Evidence of hepatocellular injury following long-term treatment has been reported in approximately 2% of treated patients and resulted in death in a few cases.[13-14] In most people, withdrawal of the drug results in resolution of increased alanine transaminase serum activity and clinical symptoms of hepatic dysfunction. Hepatocellular injury is believed to be idiosyncratic, as determined on the basis of clinicopathologic features. Thus, individual susceptibility cannot be predicted prior to initiation of treatment. Currently, periodic monitoring of serum activity of alanine transaminase for the first year of treatment is recommended, with withdrawal of the drug if increased values or clinical signs of hepatic dysfunction develop.[13-14]

The form of DM that occurs in cats shares some of the characteristics associated with type-2 DM in humans. Therefore, troglitazone may be efficacious in the treatment of DM in cats. Although adverse effects in humans with type-2 DM that have been treated with troglitazone as the sole therapeutic agent are uncommon, it is important to conduct studies to determine pharmacokinetics of the drug as well as safety and efficacy in cats with DM before it can be recommended for clinical use in that species. The purpose of the study reported here was to obtain an assessment of the disposition of troglitazone in healthy cats as a prelude to clinical trials involving diabetic cats. Pharmacokinetics of troglitazone was investigated after IV and oral administration of the drug to healthy cats.

Materials and Methods

Cats—Five healthy ovariohysterectomized adult domestic shorthair cats that weighed 4.1 to 4.8 kg (mean ± SD, 4.5 ± 0.2 kg) were used in the study. Cats were housed at a

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university veterinary medical animal care facility. Procedures involving animals were approved by a university animal care and use committee and conducted in accordance with guidelines established by the Animal Welfare Act and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. Cats used in the study had adapted well to their environment and were accustomed to being handled. Before the study, samples were obtained from all cats for a CBC, serum biochemical analyses, and urinalysis.

Study design—Cats were assigned to 2 groups (group 1, 2 cats; group 2, 3 cats). The study was conducted in accordance with a randomized paired crossover design, with 2 dosing periods and a 4-week washout period between doses. Twelve to 18 hours before each experiment, catheters[a] were placed in the jugular vein of cats that had been sedated by administration of tiletamine-zolazepam.[b] Patency of the catheters was maintained by injection of 1.5 ml of heparinized saline (0.9% NaCl) solution (2 U/ml) into the catheter every 6 hours. Before administration of drug and 72 hours and 7 days after drug administration, cats were sedated again, and a urinary catheter was passed to empty the bladder and collect urine samples.

Food was withheld for 12 hours before and 24 hours after IV and oral administration of drug. For IV administration, troglitazone[c] was dissolved in medical grade **dimethyl sulfoxide (DMSO)**[d] and diluted with 0.9% NaCl solution and fresh feline plasma to yield a 15% DMSO:42.5% NaCl:42.5% plasma solution. Only 3 cats were administered troglitazone (5 mg/kg of body weight), IV, during a 12-minute infusion period via a catheter[e] inserted in the cephalic vein, whereas all 5 cats were administered troglitazone tablets[f] (40 mg/kg, PO) with 5 ml of a semiliquid food.[g] Two of 3 cats were sedated during IV administration. After drug administration, cats were housed in metabolic cages.

Blood samples for drug analysis were collected from the jugular vein catheter prior to drug administration 0 (immediately after administration), 0.33, 0.5, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 18, 24, 48, and 72 hours and 7 days after IV administration as well as 0.17, 0.25, 0.33, 0.5, 0.67, 1, 1.33, 1.67, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 18, 24, 48, and 72 hours and 7 days after oral administration. A 1.5-ml volume of heparinized blood was collected from the catheter into a syringe. This procedure ensured the catheter was cleared of heparinized saline solution. Blood samples (2 ml) were collected for use in determining the concentration of troglitazone, and the 1.5-ml volume of heparinized blood was reinjected into the catheter. Blood samples were transferred into EDTA-containing tubes, and harvested plasma was stored at –20 C until assayed. Urine was collected for 72 hours and stored at –20 C.

Assay of drug concentrations—Troglitazone concentrations in plasma and urine were determined, using a slightly modified **high-performance liquid chromatography (HPLC)** method.[15] Briefly, internal standard (0.02 ml of 9-acetylanthracene[h] at 5 mg/ml in 90:10 ethyl acetate[i]:n-hexane[j]) and PIC-A[k] (0.1 ml) were added to each plasma or urine sample (0.1 ml), followed by addition of 3 ml of ethyl acetate:n-hexane (90:10). Samples were shaken for 20 minutes and centrifuged at 1,220 × g for 5 minutes. The organic layer was separated, evaporated to dryness at 40 C under a gentle stream of nitrogen gas, and reconstituted in 300 µl of reagent grade DMSO.[l] Fifty microliters was injected into the HPLC system. Analysis was by reverse-phase chromatography on an octyldecylsilane column (5 µm, 300 × 6 mm [ID]).[m] The mobile phase consisted of a 62:38:0.5 solution of acetonitrile[n]:water:phosphoric acid[o] at a flow rate of 1.2 ml/min. The eluent was monitored by use of UV absorbance at 230 nm.

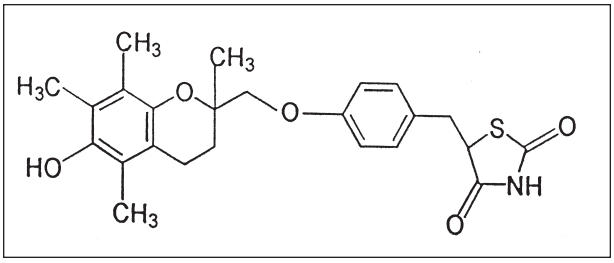

Figure 1—Chemical structure of troglitazone.

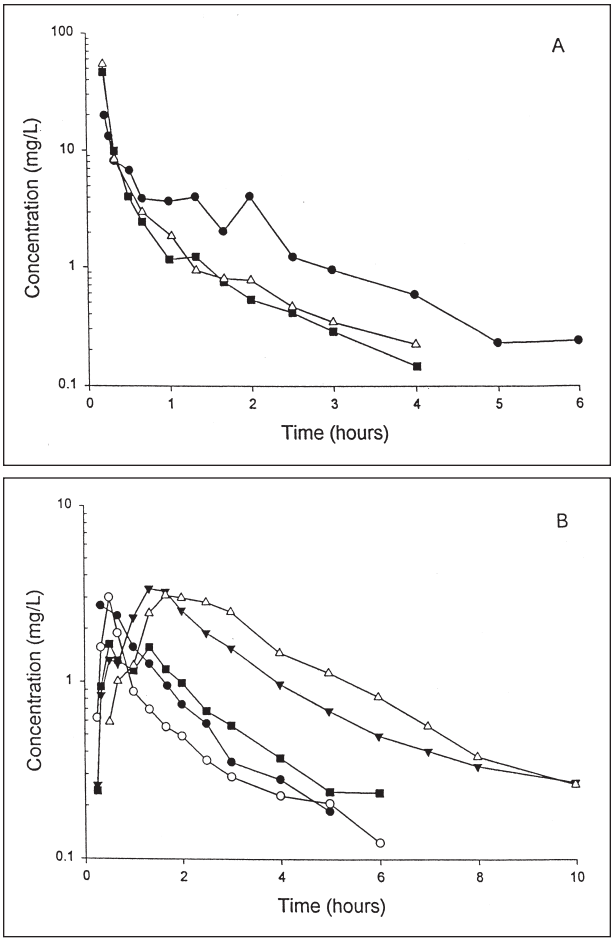

Figure 2—Plasma concentrations of troglitazone after IV administration (5 mg/kg of body weight) to 3 cats (A) and after oral administration (40 mg/kg) to 5 cats (B). Each symbol represents values for 1 cat. For oral administration, one cat (●) incorrectly received a dose of troglitazone calculated at a lower dosage (20 mg/kg).

Calibration standards for troglitazone ranged from 0.1 to 100 mg/L. The lower limit of quantitation was 0.2 mg/L. The HPLC method yielded satisfactory precision and accuracy with intra- and interday coefficients of variation of < 0.5% and 18%, respectively, for all drug concentrations.

Data analyses—Area-moment analysis was used to calculate pharmacokinetic variables for troglitazone after IV and oral administration. The **area under the plasma concentration-time curve (AUC)** and the **area under the first nonnormalized moment curve (AUMC)** were determined by Lagrange polynomial interpolation and integration from time zero to the last measured sample time with extrapolation to infinity, using the terminal phase slope.[16] Harmonic mean

Table 1—Pharmacokinetic variables of troglitazone following intravenous administration (5 mg/kg of body weight) and oral administration (40 mg/kg) to healthy cats

Variable	Range	Mean	SD
Intravenous (n = 3)			
AUC (mg • h/L)	51,188–62,401	56,023	5,764
CL_T (L/h/kg)	0.28–0.36	0.33	0.042
VD_{SS} (L/kg)	0.13–0.40	0.23	0.15
$t_{1/2}$ (h)	1.0–1.2	1.1	0.1
MRT (h)	0.4–1.2	0.7	0.4
Oral (n = 5)			
T_{max} (h)	0.33–1.67	0.87	0.60
C_{max} (mg/L)*	1.63–3.38	2.78	0.78
K_a (/h)†	0.26–1.28	0.70	0.52
AUC (mg • h/L)*	13,842–46,490	26,651	15,808
F (%)†	3.5–9.1	6.9	3.0
$t_{1/2}$ (h)	1.1–3.2	1.9	0.9

*Mean and SD determined from values for 4 cats, because 1 cat received a lower dose of troglitazone (20 mg/kg). †Mean and SD determined from values for only 3 cats.

AUC = Area under the plasma concentration-time curve. CL_T = Total clearance. VD_{SS} = Steady-state volume of distribution. $t_{1/2}$ = Terminal phase half-life. MRT = Mean residence time. T_{max} = Time to maximum concentration. C_{max} = Maximum concentration. K_a = First-order absorption rate constant. F = Oral bioavailability.

values for **half-life ($t_{1/2}$)** and pseudo SD were calculated by the method of Lam et al.[17] **Total clearance (CL_T)** was calculated as $dose_{IV}/AUC_{IV}$, **mean residence time (MRT)** was calculated as AUMC/AUC, and **steady-state volume of distribution (VD_{SS})** was calculated as $CL_T \bullet RT_{IV}$. **Oral bioavailability (F)** was calculated as $(AUC_{PO}/AUC_{IV}) \times (dose_{IV}/dose_{PO})$, and the first-order absorption rate constant after oral administration was calculated as $1/(MRT_{IV} - MRT_{PO})$. The maximum concentration and time to maximum concentration were observed directly from plasma concentration versus time data.

Results

A technical error resulted in 1 cat (the cat that had not been sedated for IV administration) receiving a lower oral dosage (20 mg of troglitazone/kg) than the remaining 4 cats. Values for this cat were not used to determine mean ± SD for AUC or maximum concentration.

Transient hemolysis was detected after IV administration, but it resolved within 24 hours. Changes in PCV before and 72 hours after drug administration did not differ between cats when they received the drug orally versus receiving it IV.

Plasma concentrations of troglitazone as a function of time were plotted for samples obtained after IV administration of 5 mg of troglitazone/kg (**Fig 2A**) and oral administration of 40 mg of troglitazone/kg (**Fig 2B**). Pharmacokinetic variables for troglitazone in specific cats, as well as mean values, were determined (**Table 1**). The relatively high variability in the disposition of troglitazone observed in these cats has been reported in other species, including humans.[1]

Discussion

Troglitazone is an orally administered insulin-sensitizing agent that may be of use in the treatment of cats with DM. The purpose of the study reported here was to characterize the pharmacokinetics of troglitazone in healthy cats after IV and oral administration.

After IV administration, troglitazone concentrations in plasma declined rapidly (Fig 2A), with a mean terminal phase $t_{½}$ of 1.1 ± 0.1 hours. The CL_T of troglitazone was 0.33 ± 0.04 L/h/kg. Similar to other species,[15] CL_T of troglitazone was moderate relative to hepatic blood flow (1.4 L/h/kg)[18,19] in cats. Indeed, the CL_T value obtained experimentally in these cats is similar to the predicted value (0.55 L/h/kg) calculated on the basis of body weight.[15]

Troglitazone was not detected in urine, indicating that unchanged troglitazone is not eliminated appreciably by renal excretion in cats. This is consistent with findings in other species, including humans, in which < 5% of the drug was eliminated unchanged in the urine.[20] Thus, elimination of troglitazone in cats is likely the result of metabolism and biliary excretion of the drug. Troglitazone reportedly is eliminated predominately by biliary excretion in mice, rats, monkeys,[20] and, presumably, humans. Troglitazone also is metabolized to yield sulfate and glucuronide conjugates.[15,20] A pharmacologically active oxidized quinone metabolite, which is further metabolized to a sulfate conjugate, also is formed.[20] Sulfate conjugation of troglitazone predominates in humans, rats, and dogs, whereas glucuronide conjugation predominates in mice and monkeys. Compared to other domestic animals, cats are relatively deficient in their ability to conjugate xenobiotics with glucuronic acid, and most conjugates formed by cats are sulfates.[21]

The VD_{SS} of troglitazone was 0.23 ± 0.15 L/kg, suggesting that distribution of the drug was limited to extracellular spaces.[22] The VD_{SS} in cats was lower than VD_{SS} values (approx 0.8 L/kg) reported in mice, rats, dogs, and monkeys.[15] Studies in humans and other animals have documented that the drug is highly protein bound. Extensive plasma protein binding of troglitazone may limit the distribution of the drug in cats.

The terminal phase $t_{½}$ of troglitazone in cats (1.1 hours) in our study was within the range of $t_{½}$ values (0.67 to 17 hours) reported in controlled studies on animals and clinical studies in humans.[15,23] Smaller species generally have shorter $t_{½}$ values for the drug.

Absorption of troglitazone in cats after oral administration was rapid but incomplete. Peak concentrations of the drug were observed approximately 1 hour after oral administration. Mean F of troglitazone in cats was 6.9% and ranged from 3.5 to 9.1% (Table 1). Oral bioavailability of troglitazone in cats is comparable to the F of 10 to 26% reported for mice, rats, and monkeys.[15,20] The low F of troglitazone appears to be attributable to the low aqueous solubility of the drug.

The overall disposition of troglitazone in cats was similar to that observed in other species, including humans. The drug has low and variable F. The CL_T of the drug is moderate. Little, if any, unchanged troglitazone is excreted in urine; thus, metabolism and biliary excretion play major roles in elimination of the drug.

On the basis of pharmacokinetics of troglitazone determined in healthy cats, and on the basis of pharmacodynamics of the drug in other animals and humans, a dosage regimen of 20 to 40 mg of troglitazone/kg administered orally once or twice per day to cats will produce plasma concentrations of the insulin-sensitizing agent similar to concentrations document-

ed to be effective in humans. Additional studies are needed to determine efficacy for this drug when used in the treatment of diabetic cats as well as tolerance associated with long-term administration of troglitazone. On the basis of reported toxic effects in human beings, it is recommended that use of this drug be avoided in cats with evidence of hepatic dysfunction.

[a]I-Cath, Charter Med Inc, Lakewood, NJ.
[b]Telazol, Fort Dodge Laboratories, Fort Dodge, Iowa.
[c]Troglitazone, Parke-Davis, Ann Arbor, Mich.
[d]Domoso medical grade, Fort Dodge Laboratories Inc, Fort Dodge, Iowa.
[e]Sovereign, Sherwood Medical, Tullamore, Ireland.
[f]Rezulin, Warner-Lambert, Morris Plains, NJ.
[g]a/d, Hill's Pet Products Inc, Topeka, Kan.
[h]9-acetyl anthracene 95% grade, Fisher Chemicals, Fairlawn, NJ.
[i]Ethyl acetate HPLC grade, Fisher Chemicals, Fairlawn, NJ.
[j]n-Hexane 95% HPLC grade, Fisher Chemicals, Fairlawn, NJ.
[k]PIC A, Waters Corp, Milford, Mass.
[l]Dimethyl sulfoxide (DMSO) reagent grade, Fisher Chemicals, Fairlawn, NJ.
[m]YMC octyldecylsilane column (5 μm, 300 × 6 mm [ID]), YMC Co, Wilmington, NC.
[n]Acetonitirile HPLC grade, JT Baker, Phillipsburg, NJ.
[o]o-Phosphoric acid 85% HPLC grade, Fisher Chemicals, Fairlawn, NJ.

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