Comparative bioavailability of fluoxetine after transdermal and oral administration to healthy cats

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Objective—To determine bioavailability, pharmacokinetics, and safety for transdermal (TD) and oral administration of fluoxetine hydrochloride to healthy cats.

Animals—12 healthy mixed-breed sexually intact 1- to 4-year-old purpose-bred cats.

Procedure—A single-dose pharmacokinetic study involving 3 groups of 4 cats each was conducted in parallel. Fluoxetine in a formulation of pluronic lecithin organogel (PLO gel) was applied to the hairless portion of the pinnae of cats at 2 dosages (5 or 10 mg/kg), or it was administered orally in capsules at a dosage of 1 mg/kg. Plasma samples were obtained and submitted for liquid chromatography-mass spectrometry-mass spectrometry analysis of fluoxetine and its active metabolite, norfluoxetine.

Results—Peak fluoxetine concentration (C_{max}) was lower and time to C_{max} longer for TD administration versus oral administration. Relative bioavailability of each dose administered via the TD route was 10% of the value for oral administration of the drug. Mean plasma elimination half-life after oral administration was 47 and 55 hours for fluoxetine and norfluoxetine, respectively.

Conclusions and Clinical Relevance—This study provides evidence that fluoxetine in a 15% (wt:vol) PLO gel formulation can be absorbed through the skin of cats into the systemic circulation. However, the relative bioavailability for TD administration is approximately only 10% of that for the oral route of administration. (*Am J Vet Res* 2003;64:994–998)

Fluoxetine hydrochloride[a] is a **selective serotonin-reuptake inhibitor (SSRI)** approved for the treatment of humans with depression and other psychiatric disorders. It inhibits uptake of serotonin by blocking the presynaptic reuptake process.[1] Fluoxetine is metabolized primarily by *N*-demethylation to its metabolite, norfluoxetine, which is equally active and selective in inhibiting serotonin uptake in rat synaptosomes.[2]

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Because of the size of commercially manufactured tablets or capsules and the poor palatability of the liquid formulation, administration of fluoxetine in a **transdermal (TD)** formulation could be clinically useful to achieve adequate plasma concentrations in cats. Transdermal gels are products that can potentially improve the ability of medication to penetrate the epidermis and enter the systemic circulationwhen mixed with a drug.[3] Fluoxetine can be formulated in a **pluronic lecithin organogel (PLO gel)**. To our knowledge, there is currently no information available in the public domain regarding the pharmacokinetics of fluoxetine or norfluoxetine in cats when administered orally or via TD formulation; however, information does exist for industry-sponsored experiments on oral administration in cats.[b]

Therefore, the objective of the study reported here was to compare pharmacokinetics of fluoxetine and its primary metabolite, norfluoxetine, in healthy cats after a single dose of fluoxetine administered orally and via a TD formulation. In a preliminary study, TD administration at a rate of 1 mg/kg failed to achieve detectable plasma concentrations of fluoxetine or norfluoxetine. For this reason, dosages of 5 and 10 mg/kg were chosen for TD administration in the study reported here. A second objective of the study was to evaluate the dose of fluoxetine that, when administered as a TD preparation, would approximate the dosage reported[4] for oral administration (1 mg/kg, PO, q 24 h) used in cats with behavior problems. The final objective of the study was to characterize adverse reactions observed during use of a PLO gel-fluoxetine formulation.

Materials and Methods

Animals—Twelve healthy mixed-breed adult cats were used in the study. Cats were sexually intact males and females ranging from 1 to 4 years of age and weighing 2 to 5 kg. All cats were fully vaccinated against panleukopenia virus, calici virus, viral rhinotracheitis virus, and rabies. All cats had negative results when tested for FeLV and FIV. Results of CBC and serum biochemical analyses were within acceptable limits prior to onset of the study. Cats were fed a dry commercial diet ad libitum throughout the study period. Cats were delivered to the animal facility and allowed a 1-week acclimation period before the day of treatment. All procedures were approved by the Institutional Animal Care and Use Committee of the College of Veterinary Medicine at Purdue University.

Preparation of formulations—Powdered fluoxetine hydrochloride[a] (purity, > 99%) was obtained. Capsules and TD gel were prepared by a local pharmacy.[c] Fluoxetine powder was prepared in gelatin capsules designed to deliver 1 mg of fluoxetine/kg. The TD formulation was prepared by mixing fluoxetine powder with PLO gel. Briefly, 9 g of fluoxetine was mixed with a sufficient amount of ethoxy diglycol to form a paste.

Lecithin isopropyl palmitate solution was added to the paste, and the product was mixed well and transferred to a 60-mL syringe. Pluronic F127 (20% [wt:vol]) solution was added to achieve a final volume of 60 mL; the product was mixed well by use of a second syringe and luer-lock adapter such that the resultant mixture formed a gel. Concentration of the TD formulation was 15 mg of fluoxetine/0.1 mL of gel.

Experimental protocol—Cats were randomly assigned to 1 of 3 groups (4 cats/group). One group of cats was given fluoxetine via oral administration at a rate of 1 mg/kg, and the 2 remaining groups were given fluoxetine via TD administration (1 group at 5 mg/kg and the other group at 10 mg/kg). The TD groups received the product by application of the gel formulation to the inner (nonhaired) surface of 1 pinnae in an area ≤ 4 cm^2. The inner pinna was selected on the basis that it is mostly hairless and easy to access. Furthermore, in 1 study,[5] absorption of estradiol gel in women was most effective when the application area was small. Local and systemic toxic effects were monitored for a 14-day period after drug administration.

On the day of fluoxetine administration, a 1.5-mL blood sample was collected from each cat by use of a catheter[d] that had been previously inserted in a jugular or femoral vein (time 0), and fluoxetine was administered immediately thereafter. Blood samples were collected 1, 2, 4, 9, 12, and 24 hours after administration and then daily for a total of 14 days. Samples were collected from the aforementioned catheter in the jugular or femoral vein. Catheters were flushed with 3 mL of heparinized saline (0.9% NaCl) solution prior to collection of each sample. After collection of a sample, catheters were capped and then filled with heparinized saline solution to ensure patency was maintained. Blood samples were collected in heparinized evacuated tubes,[e] and plasma was harvested and frozen at –20°C until analysis.

Measurement of drug concentrations—Plasma samples were analyzed to determine concentrations of fluoxetine and norfluoxetine by use of a **liquid chromatography-mass spectrometry-mass spectrometry (LC-MS-MS)** method[f] validated for the concentration range of 1 to 500 ng/mL. Fluoxetine and norfluoxetine were separated by use of a cyanide hydroxide column.[g] Extraction from plasma was accomplished by use of a solid-phase procedure with a 130-mg, 3-mL solid-phase extraction column.[h] An aliquot (2 mL) of 0.1M potassium phosphate buffer (pH, 6.0) and 25 µL of an internal-standard solution (2.00 µg/mL) were added to 0.5 mL of plasma. Samples were then loaded onto an isolute column, and analytes were eluted with 2% ammonium hydroxide in a solution of methylene chloride:methanol (80:20). The eluant was removed by evaporation, and the material was reconstituted with 100 µL of a solution of methanol:0.05% trifluoroacetic acid (80:20). Samples (25 µL) were injected onto the LC-MS-MS system. Cross-validation accuracy of the assay was ≤ 12.18% for fluoxetine and ≤ 14.36% for norfluoxetine, whereas the cross-validation precision was ≤ 6.85% for fluoxetine and ≤ 10.94% for norfluoxetine.

Pharmacokinetic and statistical analyses—Noncompartmental pharmacokinetic variables were calculated for each cat by use of computer software.[i] Variables calculated included **maximum plasma concentration (C_{max})**, **time when C_{max} was achieved (T_{max})**, **terminal elimination half-life ($t_{1/2}$)**, and integration of the **area under the plasma concentration curve from the time of administration to the last quantifiable time point (AUC_{0-t})**. Value for $t_{1/2}$ was calculated as follows:

$$t_{1/2} = \ln_2/\lambda_z$$

where $\ln_2$ is the natural logarithm (base 2), and λ_z is the elimination exponent calculated by the least sum of squares. Bioavailability for TD administration was determined and compared with bioavailability for oral administration; group means were used to calculate relative bioavailability of the TD formulation.

Data for C_{max}, T_{max}, AUC_{0-t}, and $t_{1/2}$ were statistically analyzed. Results were logarithmically transformed, and group data were compared by use of a 1-way ANOVA. Multiple comparisons among groups for each variable were performed by use of the Tukey method. We did not include a multiplicity adjustment for analysis of multiple-response variables. We calculated 95% **confidence intervals (CIs)** for the difference between means on the logarithmic scale and back-transformed them to provide CIs on ratios of mean results for comparison of each possible pair of the 3 groups. The 3 CIs were adjusted by use of the Tukey method to yield a simultaneous 95% CI for each variable. When the ratio of mean results equals 1, then there is no difference. A 95% CI on a ratio of means that does not include a value of 1 indicates a significant difference at a value of $P = 0.05$. Results of this parametric statistical analysis in which we assumed a normal distribution for the logarithmically transformed values were substantiated by application of the nonparametric Wilcoxon test.

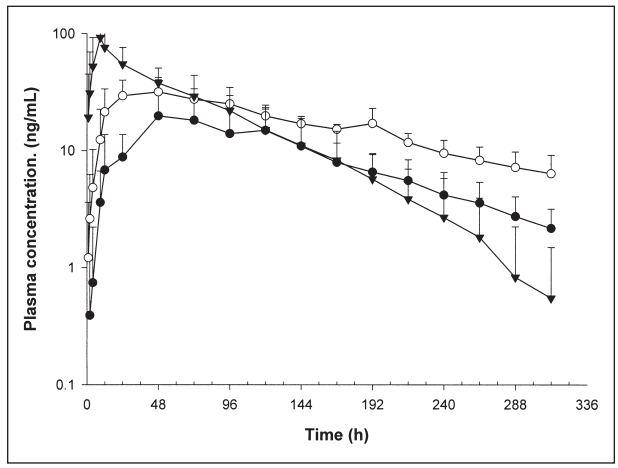

Figure 1—Semilogarithmic plot of mean ± SD plasma concentrations of fluoxetine after oral (1 mg/kg [inverted triangle]) and transdermal (5 mg/kg [solid circle]; 10 mg/kg [open circle]) administration of fluoxetine to 3 groups of cats (n = 4 cats/group). Time 0 = Prior to dosing.

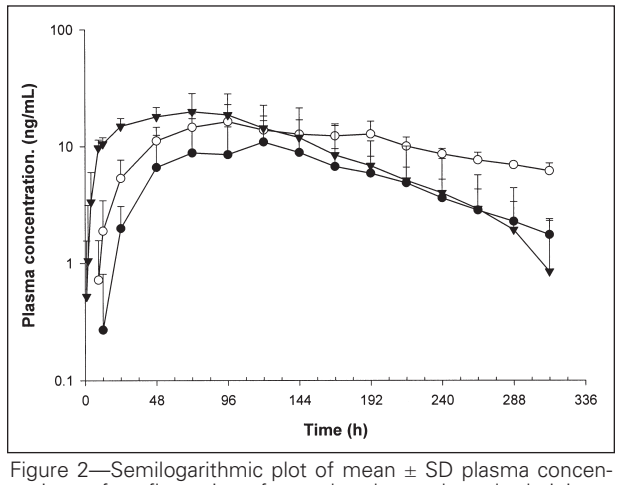

Figure 2—Semilogarithmic plot of mean ± SD plasma concentrations of norfluoxetine after oral and transdermal administration of fluoxetine to 3 groups of cats. *See* Figure 1 for key.

Table 1—Mean ± SD values for pharmacokinetic variables of fluoxetine and norfluoxetine after oral and transdermal administration of fluoxetine hydrochloride to 3 groups of cats (n = 4 cats/group)

Compound	Variable	1 mg/kg Oral	5 mg/kg Transdermal	10 mg/kg Transdermal
Fluoxetine				
	C_{max} (ng/mL)	94.8 ± 34.3[a]	23.3 ± 20.2[b]	32.9 ± 9.1[b]
	T_{max} (h)	6.5 ± 2.9[a]	51.0 ± 28.4[b]	39.0 ± 26.6[b]
	AUC_{0-t} ([µg • h]/mL)	5.4 ± 2.4	2.9 ± 1.8	5.4 ± 0.5
	$t_{1/2}$ (h)	46.8 ± 6.5[a]	87.0 ± 34.1[a,b]*	126.5 ± 53.4[b]*
	Bioavailability (%)	100	10.7†	10.0†
Norfluoxetine				
	C_{max} (ng/mL)	20.8 ± 8.2	9.8 ± 7.9	16.3 ± 6.4
	T_{max} (h)	72.0 ± 19.6	108.0 ± 31.0	96.0 ± 0.0
	AUC_{0-t} ([µg • h]/mL)	3.0 ± 1.6	1.6 ± 1.0	3.3 ± 0.8
	$t_{1/2}$ (h)	55.3 ± 7.6[a]	86.6 ± 20.6[a,b]	130.0 ± 32.2[b]

*Transdermal terminal-elimination half-life ($t_{1/2}$) values are apparent $t_{1/2}$ values and do not represent true elimination of the drug. †Relative bioavailability on the assumption that the orally administered dose is totally absorbed.
[a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$).
C_{max} = Maximum concentration in plasma. T_{max} = Time to reach maximum concentration in plasma. AUC_{0-t} = Area under the plasma concentration-time curve from the time of administration to the last quantifiable time point.

Results

Fluoxetine administration was evident in all cats in the study. However, none of the cats in the study had local or systemic toxic effects after the administration of fluoxetine-PLO gel during the 14-day study period.

Semilogarithmic plots of the concentrations of fluoxetine and norfluoxetine after oral and TD administration were created (**Fig 1 and 2**). Values were calculated for pharmacokinetic variables for fluoxetine and norfluoxetine after oral and TD administration of fluoxetine, and statistical analysis of mean values was performed (**Table 1**). A more detailed statistical analysis of the fluoxetine results was conducted, and graphs were drawn of the 95% CIs of ratios of mean results for comparison of each pair of administrations (**Fig 3**). Graphs for norfluoxetine yielded similar results.

Cats orally administered fluoxetine had significantly higher C_{max} values, compared with values for cats given fluoxetine via TD administration. In addition, T_{max} was significantly longer for both TD groups, compared with the value for the oral-administration group. We did not detect significant differences among administration groups when comparing bioavailability (ie, AUCs). However, evaluation of the CIs and mean results (Table 1) indicated closest equivalence between TD administration at a rate of 10 mg/kg and oral administration at a rate of 1 mg/kg. For the 5- and 10-mg/kg TD-administration doses, mean relative bioavailability was approximately 10%, compared with bioavailability for oral administration.

Mean $t_{1/2}$ of fluoxetine after oral administration was 46.8 hours, whereas mean $t_{1/2}$ of its active metabolite, norfluoxetine, was 55.3 hours. For TD administration, apparent mean $t_{1/2}$ of fluoxetine was 87.0 (5 mg/kg) and 126.5 hours (10 mg/kg), whereas apparent mean $t_{1/2}$ of norfluoxetine was 86.6 (5 mg/kg) and 130.0 hours (10 mg/kg). We did not detect a significant difference in the apparent mean $t_{1/2}$ for fluoxetine and norfluoxetine between the 1 mg/kg oral administration and the 10 mg/kg TD administration.

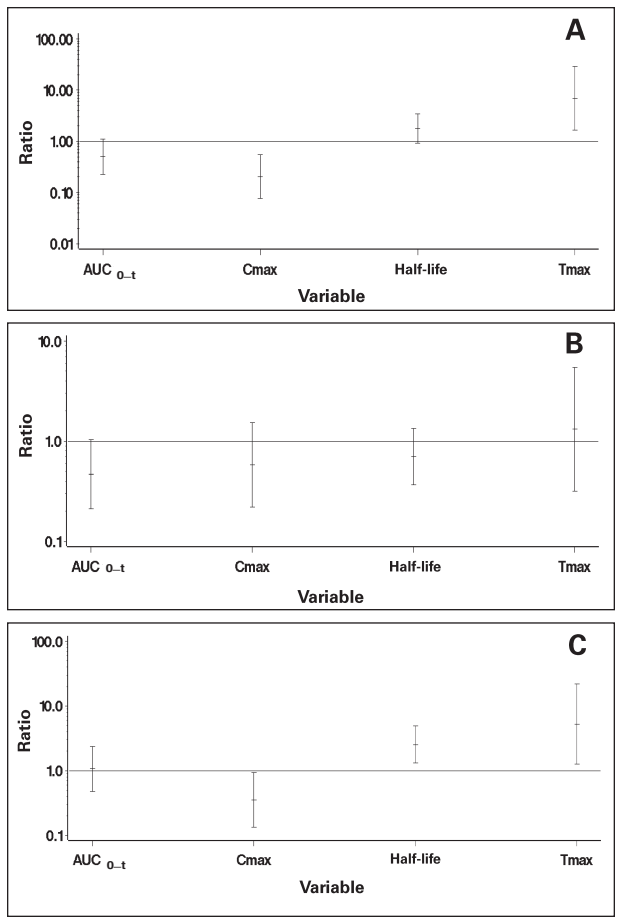

Figure 3—Graphs of the 95% confidence intervals of the ratio of mean pharmacokinetic results when comparing each pair of fluoxetine administrations. A—Transdermal administration (5 mg/kg):oral administration (l mg/kg). B—Transdermal administration (5 mg/kg): transdermal administration (10 mg/kg). C—Transdermal administration (10 mg/kg): oral administration (1 mg/kg). AUC_{0-t}= Area under the plasma concentration-time curve from the time of administration to the last quantifiable point.C_{max} = Maximum concentrationin plasma.T_{max} Time to reach maximum concentration.

Discussion

The use of psychotropic medications has become increasingly more common in veterinary medicine. Combined with behavioral and environmental modification, the use of various types of anxiolytics and anti-

depressants has resulted in increasing success when treating animals with various behavioral disorders.[6,7] Patients with problem behaviors, such as compulsive disorder, separation anxiety, aggression, and phobic conditions, have been helped through the conscientious use of pharmaceuticals.[5]

The 3 primary classes of psychotropic medications used in the treatment of animals with behavior disorders are benzodiazepines, tricyclic antidepressants, and SSRIs. Fluoxetine was 1 of the first SSRIs used in animals. In cats, it has been used for the treatment of hyperesthesia, intermale aggression, wool sucking or fabric chewing, psychogenic alopecia, and territorial aggression.[4] In 1 study,[8] fluoxetine treatment significantly reduced the rate of urine marking in cats when administered at a daily dose of 1 mg/kg.

Most psychotropic pharmaceuticals prescribed by veterinarians are not formulated for use in domestic animals and can be difficult to administer. Accurate and successful dosing of cats with standard-strength products can be especially challenging because of their small size, agility, and finicky nature. This often necessitates the use of products created to provide forms more easily usable for dosing. Specially sized capsules and flavored liquids (often with poor palatability) have been the most common approaches to the problem of inappropriate commercial formulations.

Another alternative to manipulating conventional formulations that are administered orally is the use of gels for TD administration. Specific properties of the dermal structure and physiologic processes play a role in the manner by which various drugs and carrier formulations are absorbed through the dermis and reach the circulation.[9] There are several types of gels available for TD administration, but the most commonly used at this time is PLO gel. This product combines a lipid-soluble fragment (pluronics or poloxamers) with a hydrophilic fraction (lecithin or isopropyl palmitate). Consequently, it is suitable for a wide variety of pharmaceuticals. Advantages of TD administration include ease of application and avoidance of the first-pass effect of hepatic metabolism, thereby improving the bioavailability of the drug at lower dosages (assuming that cutaneous first-pass metabolism is less than that of hepatic first-pass metabolism). Disadvantages include the need for preparation of a product, skin irritation, insufficient application caused by an animal rubbing the medication off the applied area, and lack of accurate pharmacokinetic data.

The study reported here provides proof that fluoxetine can be absorbed when delivered via TD administration in a PLO gel. All 8 cats given fluoxetine via TD administration in this study absorbed fluoxetine and produced the metabolite, norfluoxetine. Fluoxetine was also absorbed after oral administration, and norfluoxetine was also produced. However, the drug concentration-versus-time patterns for TD administration indicated a delayed absorption rate and an apparent prolongation of the absorption phase, resulting in an apparent increase in the $t_{1/2}$ (ie, flip-flop kinetics), when compared with patterns for oral administration of fluoxetine. In this study, calculated $t_{1/2}$ values for fluoxetine and norfluoxetine after oral administration were approximately 2 days.

Analysis of these results suggests that fluoxetine is absorbed more slowly when administered as a TD preparation than when administered orally. This delay may result from the fact that the amount of fluoxetine that moves across the skin is independent of concentration (zero-order kinetics).[10] In addition, considerable variation in the rate of absorption was seen among cats, as revealed by a higher SD for T_{max} in the TD-administration groups. One possibility for the cause of this variation is that differences in the epidermis (ie, the physical barrier) among cats may have caused differences in the rate and amount of drug absorbed. Cutaneous blood flow to the pinnae, thickness of the dermis, surface area of skin to which gel is applied, and the complement of metabolic enzymes within the pinnae could all contribute to variations in the rate and amount of fluoxetine absorbed among animals.[11]

Analysis of AUC data for the study reported here suggests that a 10-fold higher dose of fluoxetine was necessary for TD administration to achieve a similar concentration (ie, AUC) to that achieved with the orally administered dose. Thus, equivalency between oral and TD administration for a particular product cannot be assumed unless supporting data exist for that product. Our study documented that an assumption of dose equivalency between formulations for TD and oral administration would result in insufficient concentrations of fluoxetine.

In this study, dermal irritation was not observed after single-dose TD administration. However, we conducted a preliminary study in which we administered repeated daily doses (data not shown) to duplicate the dosing regimen anecdotally used by veterinarians. For the repeat-dose study, fluoxetine was given via TD administration at a rate of 10 mg/kg. That preliminary study was terminated, because many cats had evidence of substantial dermal irritation after several days of treatment. Additional studies are needed to determine whether the irritation was an effect of the PLO gel, fluoxetine, or the combination of the 2 in the prepared formulation. Dermal reactions have been reported in a study[12] in which multiple doses of methimazole in PLO gel were administered.

Analysis of results of the study reported here supports the idea that before a drug is prepared in an alternate dosing formulation, comparative pharmacokinetics between the alternate and traditional routes of administration should be determined to assure accurate administration. Although this information is available for some medications in humans,[13-17] such data are sorely lacking in the veterinary literature for all products currently applied by the TD route. The ultimate responsibility for the outcome when treating a patient with any medication, regardless of its form, remains with clinicians. As a result, great care must be taken, and full disclosure and education of the client should take place before dispensing products that require TD administration.

[a]Prozac, Eli Lilly Co, Indianapolis, Ind.
[b]Elanco Animal Health, Eli Lilly Co, Indianapolis, Ind: Unpublished data, 2002.
[c]Family PharmaCare, West Lafayette, Ind.

[d]Arrow 20-gauge, 12-cm catheters, Arrow International, Reading, Pa.
[e]Vacutainer, Becton-Dickinson, Rutherford, NJ.
[f]Validated bioanalytical assay, Prevalere Life Sciences Inc, Whitesboro, NY.
[g]Waters Nova-Pak CN-HP (3.9 × 75 mm), Waters Corp, Milford, Mass.
[h]Isolute SPE/Columns (HCX 130 mg, 3 mL), Argonaut Technologies Inc, Foster City, Calif.
[i]WinNonlin Professional, version 3.1, Pharsight Corp, Mountain View, Calif.

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