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Pharmacokinetics of Fluoxetine in Pregnant Baboons (*Papio* spp.)

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Fluoxetine is used to treat a number of psychiatric conditions in humans and behavioral problems in animals. Its use in pregnancy must balance maternal benefit with potential risk to the fetus. Knowledge of adult and fetal drug disposition can assist clinicians in selecting therapy that minimizes adverse effects to the fetus. Nonhuman primate models are used frequently in drug dose-translation studies, and pregnancy in baboons has many similarities to human pregnancy. Accordingly, pharmacokinetic analysis of a series of fluoxetine and norfluoxetine administrations to pregnant baboons was performed. The mean maternal baboon steady-state clearance of fluoxetine (42 mL/min/kg) was considerably higher than that in humans. Norfluoxetine, the major active metabolite, had a higher metabolite-to-drug ratio (8.7) than that found in humans, particularly with oral dosing. These results are consistent with more extensive metabolism in baboons than in humans and leads to a higher clearance than would be expected from allometric scaling. Fetal-to-maternal fluoxetine and norfluoxetine ratios under steady-state conditions were similar to those in humans, with fetal concentrations of fluoxetine 42% and norfluoxetine 47% of maternal concentrations. The fetal clearance of fluoxetine (303 ± 176 mL/min) and norfluoxetine (450 mL/min) exceeded reported placental blood flow. Understanding these species-associated differences in metabolism is a prerequisite to extrapolating data between species. Nonetheless, nonhuman primates are likely to remain valuable models for pharmacokinetic studies during pregnancy, particularly those directed toward fetal neurodevelopmental effects. Our results also are applicable to determining appropriate dosing of nonhuman primates in clinical settings.

Abbreviation: SSRI, selective serotonin reuptake inhibitor.

Selective serotonin reuptake inhibitors (SSRI) are the mainstay of antidepressant therapy in human medicine and are becoming increasingly used in veterinary medicine, particularly for treating self-injurious and stereotypic behavior in nonhuman primates.[14,57] In human medicine, SSRI often are used to treat depression in pregnant women, but the decision is complex.[26,48,56] Treating maternal depression is expected to reduce the incidence of suicide and foster a stronger and healthier maternal–infant interaction, thereby also directly benefiting the infant.[10,22,62] Overall, SSRI have a good safety record and have become first-line agents for the treatment of depression during pregnancy.[2,6,11,34] Clinical experience has reported some mild functional behavioral changes in young children exposed to SSRI during pregnancy, a possible association with some cases of persistent pulmonary hypertension of the newborn, and little (if any) anatomic teratogenesis.[6,11,34] Rodent studies, in contrast, demonstrate that fluoxetine has the potential to alter fine brain structure in the somatosensory cortex, particularly during the period corresponding to synaptogenesis, leading to effects on behavior, measured as increased anxiety, in adult animals.[5,45,48] The physician and patient must determine whether the potential benefits of the drug justify the potential risk to the fetus.[12] The ideal agent for use during pregnancy would treat maternal disease yet minimize fetal exposure.[52] The unique relationship between the maternal and fetal circulation affords a situation where the balance between placental transfer and fetal metabolism may render particular agents more advantageous than others.

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Drug metabolism by the fetus can reduce fetal drug concentrations below those in the mother and lead to metabolite concentrations exceeding those in the mother under steady-state conditions.[20,33,61] Because many of the metabolites of SSRI are active, metabolism for some agents may actually increase fetal exposure to the drug effects. Norfluoxetine, a major metabolite of fluoxetine, behaves similarly to fluoxetine with regard to potency and selectivity of serotonin uptake inhibition.[16] Although metabolism of fluoxetine to the active metabolite norfluoxetine was not detected in fetal sheep, the cytochrome P450 isoforms responsible are present in the human fetus, albeit at concentrations below those of the adult.[33,44,63] The pregnant baboon model has greater similarities to humans than do other nonprimate models in terms of placental structure and usually with respect to pharmacokinetics, including metabolism. The use of baboons also permits the study of neurodevelopment in a closely related and relevant species.[58]

The objective of the current study was to use a validated model of fetal drug exposure to design preliminary studies to examine commonalities between species and assess the hypothesis that fetal concentrations of fluoxetine and norfluoxetine differ in their relationship to the respective maternal concentration and that these differences can be explained in terms of fetal metabolism or placental transfer. Specifically, we expected the fetal fluoxetine concentration to be lower and the norfluoxetine concentration to be higher than their respective concentrations in the mother due to formation of norfluoxetine by the fetus. The chronically catheterized fetal baboon model has proven useful to study fetal disposition of drugs and was used in the current study to assess the relationship between maternal and fetal fluoxetine and norfluoxetine plasma concentrations under steady-state conditions, the ability of the fetus to metabolize

fluoxetine to norfluoxetine, and the bioavailability of orally administered fluoxetine.[18,19,21] Together, these pharmacokinetic studies in nonhuman primates enhance our understanding of drug disposition during pregnancy and provide information relevant to the use of fluoxetine in baboons.

Materials and Methods

Study population. Pregnant baboons (*Papio* species) from the Columbia University breeding colony were used in this study. Animals were maintained in accordance with all National Institutes of Health policies, US Department of Agriculture regulations, and AAALAC standards for the care and use of laboratory animals. The research protocol was approved by Columbia University's IACUC. The Deputy Administrator of US Department of Agriculture's Animal and Plant Health Inspection Service, Animal Care, granted an exemption for multiple survival surgeries. Gestational age was determined by using the midpoint of timed matings as the estimated day of conception (± 3 d, term approximately 175 d). Ultrasonography was performed at 70 to 110 d to confirm singleton pregnancy and placental location. Lights in the colony were cycled (lights on, 0700; lights off, 1900), and feeding times (0800 and 1400) were constant.

Surgical procedures and tethering system. The animals were studied by using an individualized backpack and tether system. This system, along with the methods for maintenance, breeding, preconditioning, anesthesia, surgery, and postoperative care, have previously been described in detail[58] and are summarized here. All baboons used had adapted readily to the backpack and tether. Surgery was done under general anesthesia (isoflurane and nitrous oxide) and by using sterile technique. Within the constraints of surgical scheduling, the timing of fetal surgery and cannula placement was selected to conduct studies at similar gestational ages, have sufficient time to perform the series of infusions, and increase the likelihood of catheter function.

Vascular catheters were placed in a maternal artery and vein and in the fetal carotid artery and jugular vein. An amniotic fluid catheter was attached on the fetal neck. EEG and ECG electrodes were placed to monitor fetal drug effects and wellbeing. The uterus was closed, and all catheters and electrodes were tunneled to the midscapular region of the mother, where they exited to the backpack. The backpack housed the pressure transducers and catheter and electrical connectors and was attached to a tether cable connected to a freely rotating pole at the top of the cage. Peristaltic infusion pumps and solutions were fixed to the pole. An electrical swivel allowed the whole apparatus to turn with the activity of the animal while it provided electrical power to the pumps and for signal transmission of physiologic data. The maternal and fetal vascular catheters were continuously infused with heparinized normal saline to maintain patency. Catheters were accessible at the top of the cage to obtain blood samples. Postoperative analgesia was administered via continuous infusion of morphine sulfate (50 to 150 µg/kg/h) to the mother. The analgesic dose was tapered postoperatively over 2 to 4 d as the mother resumed normal activity. Cefazolin (1 g daily) was administered preoperatively, with 2 additional doses given over the next 24 h. The mothers were returned to their home cages near other familiar animals.

After a minimum of 7 d after surgery and 48 h after cessation of morphine sulfate, a physiologic baseline was obtained for a period of at least 2 d before starting the infusion protocols. Postsurgical labor resulting in preterm delivery was the main complication associated with the surgery. Mothers were monitored for signs of labor, with daily assessments of behavior and pressure recordings from the amniotic fluid catheter. The physiologic stability of the fetus was assessed according to fetal heart rate, blood pressure, and arterial acid base and blood gas values. Liveborn infants were weighed at birth. This model allowed the pharmacokinetic and pharmacodynamic studies to be performed over the last trimester of a stable pregnancy without the need for anesthesia.

Drug administration. The data presented here are from a series of drug administration protocols designed to obtain preliminary pharmacokinetic and physiologic data to help establish doses for future studies. These protocols included: 1) fluoxetine infused into the fetus; 2) a norfluoxetine bolus administered to the fetus to assess fetal clearance of the metabolite; 3) an oral bioavailability study to assist in design of long-term oral exposure study; 4) maternal administration of fluoxetine by continuous infusion to assess disposition between mother and fetus under steady-state conditions; and 5) the feasibility of chronic oral dosing was assessed in a single baboon.

The goal for the maternal dose was to achieve concentrations measured in clinical studies (196 to 460 ng/mL). The goal for the fetal dose was to achieve fetal concentrations comparable to those seen in the fetus during maternal infusion. The midrange of the daily human dose (that is, 50 mg; range, 20 to 80 mg) translates to approximately 20 mg in baboons by using a body surface area drug-dose translation formula.[38,50] Applying this formula to data from sheep also suggested that a dose of 20 mg was appropriate for the baboon.[33] Therefore, the fluoxetine doses we selected initially were 10 to 20 mg daily for adults and approximately 5 mg daily for fetuses. The fluoxetine doses initially selected in this study are within the same range as those used in baboons and rhesus macaques treated for self-injurious behavior.[14,31] Doses were increased as measured concentrations became available. For all administrations, fluoxetine (Spectrum Pharmaceuticals, Henderson, NV) or norfluoxetine (Sigma Aldrich, St Louis, MO) were weighed, dissolved in sterile water, and then brought to required volume with normal saline. Heparin was added for continuous infusions. Solutions were passed through 0.22-µm filters into sterile infusion bags (or sterile syringes for bolus studies). Solutions were stored at −20 °C until required and then thawed at room temperature. For oral administration, the drug dose was added to an orange-flavored beverage that the baboons readily consumed. A calibrated peristaltic infusion pump (P720, Instech Laboratories, Plymouth Meeting, PA) was used for continuous infusions. Although the pumps were calibrated, the dose delivered (mg/d) was based on the actual amount of drug delivered. Blood was placed in microtainer tubes containing heparin and plasma separator (BD, Biosciences, San Jose, CA). After centrifugation of the sample, the plasma was transferred to 1.5 mL plastic vials and stored at −20 °C within 30 min of collection.

Fetal fluoxetine infusion We expected that fetal infusion of fluoxetine would produce the most consistent drug concentrations in the fetus to provide the comparative physiologic data for assessing neurobehavioral effects of fluoxetine on the fetus.[43,59] Prior to infusion, initial samples were obtained from the fetal and maternal arterial and amniotic fluid catheters. Then the dead space of the fetal venous line (2.5 mL) was primed with the drug solution, which was infused at the rate of 2 mL/h by using the peristaltic infusion pump. The initial dose selected was 4.8 mg/d. All fetal infusions were by the jugular vein catheter, with fetal samples collected from the carotid arterial catheter.[20] Eventually, this dose increased as high as 15 mg/d. This dose had seemed high, particularly as was being given intravenously, but subsequent plasma concentrations were still lower than

ideal for measurement of concentrations in maternal samples. Samples of maternal and fetal blood and amniotic fluid were obtained at 1, 3, 6, 24, 48, 72, 75, and 78 h after the start of infusion. When the hematocrit of the fetus was low, some samples were omitted. Due to the limited blood volume of fetuses, samples were not collected during the elimination phase.

Fetal norfluoxetine bolus. Administration of the metabolite norfluoxetine directly to the fetus permitted the evaluation of the clearance of the metabolite from the fetus. Bolus administration was used because for the amount of norfluoxetine required to obtain readily measurable concentrations, using an extended continuous infusion was not an option. The dose selected was 4 mg and similar to that of fluoxetine. Timed selected samples (reflecting the small fetal blood volume) were obtained to delineate the plasma concentration–time curve.

Maternal bioavailabilty. Bioavailability in the mothers was assessed by administering the same dose (20 mg) of fluoxetine intravenously and orally at least 48 h apart and at least 72 h from cessation of a prior drug administration. The order of administration was assigned randomly. This same dose was given in a preliminary positron emission tomographic study using a labeled ligand for the serotonin transporter and showed completed displacement of the ligand from the placental tissues where it had previously shown extensive binding. This study was to be performed prior to the maternal infusion whenever possible or in the postpartum period if necessary. A baseline sample was obtained to ensure that no measurable drug remained in the system. Samples were timed to afford as complete a picture of the concentration–time course as possible yet remain within safe limits of daily blood sample volumes for mother and fetus. In one baboon, a single daily oral dose (20 mg) of fluoxetine was administered for 6 wk. In this animal, samples were collected by venipuncture under ketamine sedation every 2 wk just prior to the morning dose.

Maternal infusion. Infusion of fluoxetine to the mother permits evaluation of fetal exposure to fluoxetine and norfluoxetine in a clinically relevant paradigm. Although fluoxetine clearly is not given by the intravenous route in the clinical arena, steady-state concentrations are maintained with oral daily dosing, albeit with peaks and troughs. In the animal model, continuous intravenous infusion provides the most reliable and stable method of comparing maternal and fetal drug and metabolite concentrations.[17] In addition to assessment of fetal exposure and maternal clearance, placental clearances of fluoxetine can be estimated when data are combined with those from fetal drug infusion in the same animal. Because the half-life of fluoxetine in baboons was unknown and is 1 to 4 d in humans,[47,54] drug infusions were planned to continue as long as possible until stopping at delivery. This design required that the maternal infusion be last in the series. The drug dose was planned to be increased at weekly intervals unless side effects became apparent. No side effects were noted during the study. A baseline sample was obtained prior to infusion of fluoxetine and then, beginning 24 h after initiation, samples of maternal and fetal blood and amniotic fluid were obtained every 24 h.

HPLC. The assay was done by a core lab in the Analytical Psychopharmacology Division, the New York State Psychiatric Institute. Plasma fluoxetine and its metabolite, norfluoxetine, were quantified by using HPLC with fluorescence detection after precolumn derivatization with dansyl chloride. The method is based on a published procedure for fluoxetine and norfluoxetine with some minor changes.[55,60] Plasma containing an internal standard was alkalinized and extracted with 20% ethyl acetate in n-heptane. After mixing and centrifugation, the organic phase was back-extracted with diluted HCl. After mixing and centrifugation, the organic phase was aspirated and the aqueous phase dried. The residue was derivatized with 1% dansyl chloride, evaporated to dryness, and reconstituted with mobile phase. Chromatography was done by using a Supelcosil LC-18 column (Sigma Aldrich, St Louis, MO) and a mobile phase of 15:10:75 (phosphase buffer:methanol:acetonitrile) at a flow rate of 2.0 mL/min. The eluent was monitored by using a fluorescence detector with the excitation wavelength set at 235 nm and a cutoff filter at 470 nm. For human plasma, the calibration curve is linear between 800 and 10 ng/mL, and the minimal quantifiable limit is approximately 5 ng/mL.[60] The total chromatographic analysis time was less than 15 min with the within-day imprecision error not exceeding 4.3% for fluoxetine and 3.8% for norfluoxetine.

Pharmacokinetic and statistical analyses. Mean steady-state concentrations were determined after inspection of concentration–time curves to assess when steady state was achieved. Within-animal means were determined for use in overall means (± 1 SD) when more than one dose was given to an individual baboon. Steady-state fetal:maternal and metabolite:drug ratios were calculated for individual steady-state pairs when available, and then a mean was determined for each animal. Maternal and fetal clearances were calculated by dividing the infusion rate by the respective mean steady-state concentrations. Maternal and fetal clearances were normalized to body weight. The fetal weights at the time of the infusion were extrapolated from a trendline using data from previous baboon studies and birth weights when available. A Student one-sample *t* test (Statistical Analysis System, SAS Institute, Cary, NC) was used to test the hypothesis that both the fluoxetine and norfluoxetine fetal:maternal ratios differed from 1.0 ($\alpha = 0.05$).

For bolus studies, WinNonlin (Pharsight, Certara, St Louis, MO) was used to generate pharmacokinetic parameters for bolus studies. In addition to computing AUC values, concentration–time values were fit to 1-, 2-, and 3-compartment models. A user-defined model was used to estimate parameters for drug metabolite. Selection of the model with best fit was determined according to visualization, SE of parameter estimates, correlation coefficients (R), and Akaike and Schwartz criteria. Maternal bioavailability was calculated by dividing AUC after oral administration by that obtained after the same intravenous dose.

Results

Animal demographics. Data from 10 baboons were available from the various study protocols (Tables 1 and 2). The mean maternal weight was 15 ± 2.3 kg. Pharmacokinetic studies began, on average, 13 d after the fetal surgeries. The gestational age at surgery ranged from 127 to 145 d (mean, 132 d). Birth weights were available for 4 of the 6 pregnancies and ranged from 540 g to 760 g at gestational ages ranging from 157 to 166 d. Estimated fetal weights at the time of fetal infusion ranged from 371 to 683 g (Table 2). All fetuses exposed to fluoxetine, norfluoxetine, or both compounds had normal heart rate patterns and pH and blood gas values. No differences in preliminary EEG measures were noted. Fetal survival ranged from 19 to 47 d after surgery.

Steady-state conditions. Achieving steady-state conditions is a critical assumption for drawing conclusions on the relationship between the maternal and fetal concentrations. To illustrate steady-state conditions, Figure 1 (baboon 400, maternal infusion) and Figure 2 (baboon 397, fetal infusion) compare the fluoxetine and norfluoxetine concentrations in the mother and fetus throughout the maternal and fetal infusions. The metabolite:drug ratios in both of these infusions remained

Table 1. Demographics, concentration, and clearance data on study animals during maternal administration of fluoxetine

				Maternal plasma (ng/mL)		Fetal plasma (ng/mL)		Maternal clearance	
Animal	Maternal weight (kg)	Gestational age at drug administration (d)	Dose (mg/d)	Fluoxetine	Norfluoxetine	Fluoxetine	Norfluoxetine	Fluoxetine (mL/min)	Normalized to maternal weight (mL/min/kg)
369	20	144	12.3	26.0	52.0			329	16.4
372	15	152	13.3	13.4 ± 2.30	24.2 ± 3.27	7.8 ± 0.84	13.6 ± 2.07	689	46.0
374[a]	12	160	20	not done	not done	not done	not done	(1971)	(164.3)
397	15	156	38.2	29.0	28.0	11.0	14.0	915	61.0
399[a]	15		20	21.3 ± 16.2	143 ± 26.1	not done	not done	(651)	(43.4)
400	17	137	24.8	20.1 ± 1.46	54.8 ± 5.6	5.6 ± 0.79	18.7 ± 1.75	855	50.3
400	17	146	51.8	60.5 ± 10.9	137 ± 36.3	16.8 ± 2.87	47.5 ± 5.26	595	35.0
							Overall[b]	664 ± 212	41.7 ± 16.9

Where appropriate, data are given as mean ± 1 SD.
[a]All animals received the dose via intravenous infusion, except for baboons 374 (IV bolus) and 399 (oral administration). Maternal clearances for these 2 animals are listed in parentheses to show that they were not included in the overall maternal clearance calculation for intravenous infusion.
[b]Means for individual baboons were calculated before determination of the mean across all animals. Animals 374 and 399 were not included in the calculation of the overall mean.

Table 2. Demographics, concentration, and clearance data on study animals during fetal administration of fluoxetine

				Maternal plasma (ng/mL)		Fetal plasma (ng/mL)		Fetal clearance	
Animal	Estimated fetal weight at drug infusion (g)[a]	Gestational age at drug infusion (d)	Dose (mg/d)	Fluoxetine	Norfluoxetine	Fluoxetine	Norfluoxetine	Fluoxetine (mL/min)	Normalized to fetal weight (mL/min/kg)
362	554	143	4.6	not detected	not detected	19.0 ± 1.00	not detected	168	303
367	660	149	1.83	not detected	not detected	7.0 ± 0.82	not detected	182	275
367	683	153	4.08	not done	not done	9.7 ± 5.77	not detected	293	429
372	371	141	5.3	not done	not done	17.2 ± 4.82	4.5 ± 0.71	642	1730
373	421	145	5.7	not done	not done	21.0 ± 3.65	not detected	188	448
394	518	137	15.4	4.7 ± 1.53	4.0	52.3 ± 15.9	5.3 ± 0.58	204	395
397	563	145	15.9	16.1 ± 1.95	29.6 ± 7.2	34.1 ± 13.5	10.3 ± 2.42	324[b]	575[b]
							Overall[c]	303 ± 177	594 ± 511

Where appropriate, data are given as mean ± 1 SD.
[a]Fetal weights were extrapolated from birth weight by using a trendline from present and prior time-mated baboon birth weights (n = 32). When birth weight was unavailable, mean birth weight for gestational age was used.
[b]Norfluoxetine clearance, 450 mL/min; norfluoxetine clearance normalized to fetal weight, 800 mL/min/kg.
[c]Means for individual baboons were calculated before determining the overall mean across all animals.

stable by day 3 after initiation of the infusion, or, as in baboon 400, the dose was increased. Several amniotic fluid concentrations during the infusions were available and were always less than those in the fetus (not shown). Steady-state conditions for fluoxetine were achieved for all compartments by 24 h and for norfluoxetine by 48 to 72 h.

Maternal and fetal disposition during maternal infusion of fluoxetine. Four pregnant animals received infusions of fluoxetine (Table 1, Figure 1). Maternal fluoxetine doses ranged from 12 to 52 mg daily and achieved plasma concentrations ranging from 13 to 60 ng/mL, with one animal receiving 2 different doses. The concentration appeared to rise proportionally with the dose. The mean maternal steady-state clearance of fluoxetine was 664 mL/min (range, 329 to 915 mL/min) and was dose independent. When normalized to maternal body weight at the time of infusion, the maternal steady-state clearance ranged from 16.4 to 61 mL/min/kg (Table 1). Animal weight did not account for any of the variability in maternal clearance. Overall, fluoxetine concentrations were much lower than anticipated from species dose translation. To achieve the lower end of the target concentration, a dose of 200 mg daily or 13 mg/kg—more than 10-fold the predicted dose based on the body surface area—would have been required. Maternal norfluoxetine concentrations were 80% higher than were maternal fluoxetine concentrations. In the 3 baboons for which fetal samples were available, fetal concentrations of fluoxetine were well below maternal concentrations, with the fetal:maternal ratios ranging from 0.28 to 0.59 (mean ± 1SD, 0.42 ± 0.16) of maternal concentrations. Similarly, the fetal norfluoxetine concentrations were all lower in the fetus than in the mother, with ratios ranging from 0.34 to 0.56 (0.47 ± 0.11). The fetal:maternal steady-state ratios for both fluoxetine ($P < 0.03$) and norfluoxetine ($P < 0.02$) differed from 1. The metabolite:drug ratios for the mother and fetus during the maternal infusion were 1.8 ± 0.77 and 2.1 ±0.99, respectively.

Fetal clearance of fluoxetine and norfluoxetine. Data were obtained from 6 fetal infusions of fluoxetine (Table 2, Figure 2). Fetal fluoxetine doses ranged from 4 to 16 mg daily and achieved plasma concentrations ranging from 7 to 52 ng/mL. As in the mother, the concentration in fetal plasma seemed to

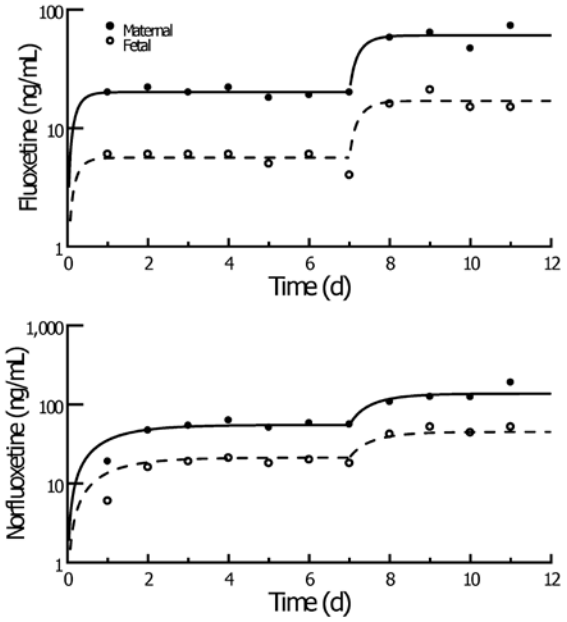

Figure 1. Maternal fluoxetine infusion (baboon 400). Fluoxetine and norfluoxetine concentrations in maternal and fetal plasma during constant infusion of fluoxetine to the mother at 25 and 52 mg/d. Curves are based on mean steady-state concentrations. The solid (maternal) curves are generated from function plots similar to a one-compartment infusion model. Clearances from Table 1 were used for fluoxetine; equation parameters that generated steady-state levels equaling the mean steady-state concentration were used for norfluoxetine. The dashed (fetal) curves represent the maternal function multiplied by the fetal:maternal ratio.

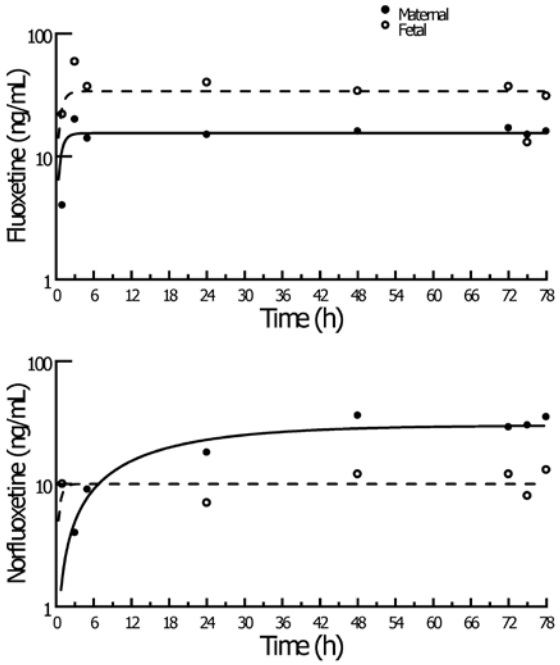

Figure 2. Fetal fluoxetine infusion (baboon 397). Fluoxetine and norfluoxetine concentrations in fetal and maternal plasma during constant infusion of fluoxetine to the fetus at 16 mg/d. Curves are based on mean steady-state concentrations. Dashed (fetal) curves are generated from function plots similar to a one-compartmental model. Clearances from Table 2 were used for fluoxetine; equation parameters that generated steady-state levels equaling the mean steady-state concentration were used for norfluoxetine. The solid (maternal) curve for fluoxetine represents fetal function divided by the fetal:maternal ratio. The maternal norfluoxetine curve was generated by fitting the data to a one-compartmental infusion model.

rise proportionally with dose such that clearances appeared to be dose independent. The fetal steady-state clearance of fluoxetine was quite variable and ranged from 168 to 642 mL/min. When normalized to estimated fetal body weight at the time of infusion, fetal steady-state clearance ranged from 275 to 1730 mL/min/kg (Table 2). Animal weight did not account for any of the variability in fetal clearance. Neither dosage nor gestational age appeared to affect clearance values. At the initial fetal fluoxetine doses, neither fluoxetine nor norfluoxetine could be measured in the mother nor could norfluoxetine be measured in the fetus. At the increased dose of approximately 15 mg/d, norfluoxetine was detectable, albeit at low concentrations, in both the mother and fetus. Although only 2 baboons yielded data, maternal concentrations of norfluoxetine were not less than those in the fetus. Both the fetal:maternal concentration ratios of norfluoxetine (0.56 and 0.35) and the metabolite:drug ratios (0.11 and 0.29) in the fetus during the fetal infusion were similar to those during the maternal infusion . The maternal metabolite:drug ratio during the fetal infusion was 1.9. Together, these data suggest little formation of metabolite by the fetus.

After the fetal fluoxetine infusion, fetus 397 received a bolus intravenous dose of norfluoxetine to determine fetal norfluoxetine clearance (Figure 3). Samples collected prior to norfluoxetine administration had no detectable fluoxetine or norfluoxetine. No fluoxetine was detected in any of the subsequent samples either. Data fit a one-compartmental model (R, 0.98; SE, 5.8; Akaike criterion, 33; Schwartz criterion, 33). The clearance estimate obtained by using a one-compartmental model was 450 mL/min (CV, 18%), was similar to that obtained from the AUC (357 mL/min; Table 2, Figure 3). The volume of distribution was 50 L (CV, 11%).

Oral bioavailability. Because fluoxetine typically is administered orally in clinical practice, assessment of maternal bioavailability after oral administration to pregnant baboons was attempted. After a single oral dose of 20 mg, equal to that often administered to human patients, fluoxetine concentrations in baboons were barely quantifiable (Figure 4). A 2-compartmental model was the best fit for the intravenous bolus dose given to baboon 374 (R, 0.996; SE, 2.6; Akaike criterion, 56; Swartz criterion, 58). The clearance was 1971 mL/min (CV, 35%), and the volume of distribution was 587 L (CV, 40%). The bioavailability of fluoxetine when comparing the AUC for the oral and intravenous routes was 0.2 in this animal. The norfluoxetine concentration profiles were fairly similar. Therefore, although overall bioavailability seems decreased, there also appears to be significant first-pass metabolism to norfluoxetine. This first-pass metabolism does not account for all the decreased bioavailability, otherwise the norfluoxetine AUC during oral administration would be higher than the AUC during intravenous administration, which was not the case.

The same oral dose of fluoxetine (20 mg) was given daily to animal 399. At 2, 4, and 6 wk, trough concentrations were similar to those seen after continuous intravenous administration. The norfluoxetine-to-fluoxetine ratio was 8.7 or more than 4-fold higher than during the steady-state infusion. The clearance after oral administration was 651 mL/min. This value seems inconsistent with the reduced bioavailability that was suggested after single-dose administration but may reflect delayed absorption not captured during the terminal phase (Figure 4). Neither fluoxetine nor norfluoxetine was detectable in fetal plasma after

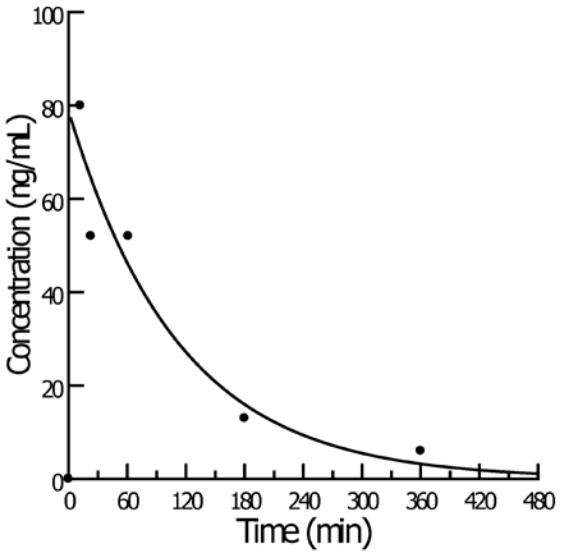

Figure 3. Fetal norfluoxetine bolus (baboon 397). Norfluoxetine (4 mg) was administered to the fetus over 4 min. Shown is the one-compartmental model (R = 0.98).

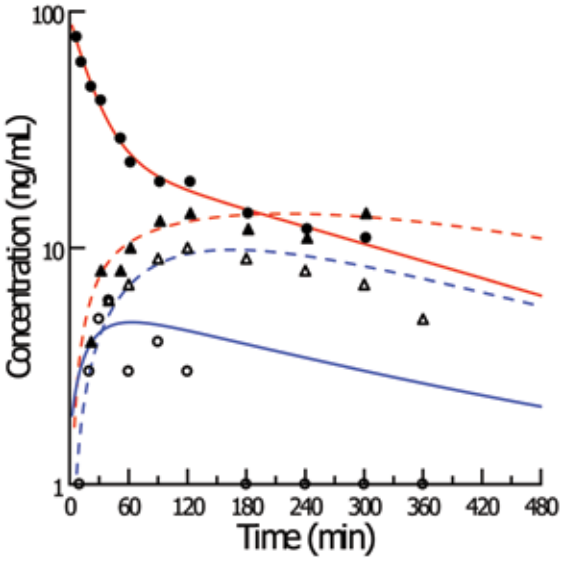

Figure 4. Bioavailability of fluoxetine (baboon 374). This pregnant baboon received an intravenous bolus dose (20 mg) of fluoxetine followed 48 h later by a single oral dose (20 mg). The curve for the intravenous bolus dose represents a 2-compartmental model (R = 0.996). Values for oral data were low, and values in trace amounts were plotted as 1. Data were fitted by using the model parameters generated from the intravenous data and estimating the absorption rate constant plus a bioavailability fraction. Due to the poor fit, the AUC value was calculated manually to give a bioavailability of 20%. Metabolite concentrations were modeled in a similar manner, by using the intravenous and oral data as the input function and estimating the rate of formation and metabolite clearances. The ● symbol represents the fluoxetine concentration, whereas the ○ symbol represents the norfluoxetine concentration after intravenous (red) administration of fluoxetine. The ▲ symbol represents the fluoxetine concentration, whereas Δ represents the norfluoxetine concentration after oral (blue) administration of fluoxetine.

oral administration but were measurable during the intravenous dose administration.

Discussion

Baboons generally are considered useful models for pharmacologic studies and have proven valuable in the study of drugs in pregnancy. Similarities in placental structure and fetal physiology permit drug pharmacokinetics from baboons to be translated to human application. Species differences in adult clearance of fluoxetine, highlighted in this study, is an important consideration in selecting clinical doses of fluoxetine for baboons as well as for designing studies of prenatal fluoxetine exposure in baboons and other nonhuman primates.

In baboons, the extensive clearance of fluoxetine led to much lower concentrations than expected. The mean baboon fluoxetine clearance (42 mL/min/kg during intravenous infusion) exceeds the clearance values reported for humans (10 mL/min/kg), sheep (18 mL/min/kg), and mice (25 mL/min/kg).[25,29,33] Species-specific differences in these enzyme isoforms may contribute to this disparity. In adult humans, fluoxetine is metabolized to norfluoxetine by the metabolic pathway of cytochrome P450 enzymes CYP2D6, CYP3A4, CYP2C9, and CYP2C19.[23,24,35,39] Humans are characterized as extensive or poor metabolizers of drugs, according to genetic polymorphisms that lead to differences in CYP2D6 activity. These differences have a substantial effect on the disposition of fluoxetine and norfluoxetine in humans.[25] There is some evidence to support that CYP2D6 activity in baboons may exceed that in humans[42] and perhaps explains the increased norfluoxetine-to-fluoxetine ratio observed after oral administration (8.7) compared with that found in humans (0.79).[25,33] Although fluoxetine inhibits CYP2D6 metabolism of dextromethorphan, it is unclear whether fluoxetine alters its own metabolism and contributes to the prolonged time needed to achieve steady-state conditions in humans and the drug's slow elimination.[3] In sheep, steady-state conditions appear to be achieved by 7 to 8 d, which is in keeping with a half-life of 24 h for the metabolite.[33,41] Sustained infusion was obtained in only one baboon, but no evidence for decreasing clearance with time was apparent.

Although the metabolic pathway of cytochrome P450 enzymes is generally considered to be the major player in the metabolism of fluoxetine, only 10% to 15% of radiolabeled fluoxetine orally administered to adult humans is retrieved over time as the active components (fluoxetine and norfluoxetine), thus making it possible that other metabolic pathways account for direct clearance.[37] In adults, glucuronide conjugates of both fluoxetine and norfluoxetine comprise approximately 15% of retrieved radioactivity. The glucuronosyltransferase system is another potential metabolic pathway. The glucuronosyltransferases that metabolize fluoxetine and norfluoxetine have not been identified but may also contribute to species-specific differences in clearance. The glucuronosyltransferase enzyme families for humans, baboons, and macaques are closely related, although baboons and macaques have more diversified glucuronosyltransferase subpopulations than do humans.[1] This difference is particularly true for the human UGT2B7-like isoforms, which are known to be central in drug metabolism and contribute to the increased clearance of morphine in baboons.[20] The identity of the glucuronosyltransferase isoform specific for fluoxetine or norfluoxetine would help to clarify their role in the observed increased clearance of the drug compared with that in humans.

Although fluoxetine clearances in baboons were similar for the intravenous and oral routes of administration, the norfluoxetine:fluoxetine ratio was 3 times higher in the orally dosed baboon, suggesting a high first-pass metabolism or cytochrome P450 induction after prolonged administration. Comparing the intravenous and oral administration routes revealed that the bioavailability of fluoxetine in baboons seemed reduced. However, the peak oral concentrations for fluoxetine and norfluoxetine found in this study were similar to those in

humans, suggesting similar distribution characteristics. The slow-elimination clearance in humans allows for a build-up of the plasma concentrations over several weeks, whereas the shorter half-life of fluoxetine in baboons prevents this delay and allows steady-state conditions to be achieved in 1 to 2 d. Clinical experience in nonhuman primates has recognized that higher doses are required when environmental enrichment and manipulation fail to correct the adverse behavior.[14,15] Self-injurious and stereotypic behavior in macaques is associated with low serotonin metabolite concentrations in cerebrospinal fluid; consequently SSRI, specifically fluoxetine, are increasingly being used to control aggression and impulsive behavior in animals.[40] In a study assessing the pharmacokinetics of fluoxetine in rhesus macaques, increased doses of fluoxetine (10 mg/kg; 7 to 9 kg) were administered chronically to achieve serum concentrations similar to that of humans.[54] This dosage is similar to that predicted from the baboon data, although the value is more than 10-fold that in humans. However, at doses of 2 mg/kg, stereotypic behavior in macaques greatly declined.[14] Allometric scaling generally accounts for species-associated differences in body mass but fails to address species-specific differences in metabolism.[66] High intrinsic hepatic or intestinal clearance (or both) will increase overall clearance and first-pass metabolism, thus likely explaining the interspecies differences.

Despite differences in adult metabolism, the fetal:maternal concentration ratios are similar among species.[4,25,26,33] The fetal:maternal ratios of fluoxetine and norfluoxetine obtained during continuous maternal infusions to baboons are similar to those in humans (0.61) and sheep (0.59), suggesting similarities in fetal disposition.[26,27,33,49,57] Although the human samples were obtained at a time of physiologic instability (birth) and after oral dosing, they likely are a fair representation of fetal exposure, given the slow clearance, high volume of distribution, and long half-life of fluoxetine in humans.[26] Processes that lead to fetal concentrations that are less than those in the mother are differential ionization and protein binding, fetal metabolism, and active placental efflux transport.[36] pH differences have a relatively small effect on increasing the fetal:maternal ratio, given that fluoxetine is a weak base (pKa 8.7); however fluoxetine is highly protein-bound (94% in adults),[37] and this differential protein binding likely contributes to the lower concentrations in the fetus.[6,53] Fluoxetine binds to both albumin and α1-acid glycoprotein, with likely greater affinity for α1-acid glycoprotein because of the drug's basic properties.[29] In general, protein binding is less in the fetus than in the mother, although fetal concentrations of these proteins can be quite variable.[28] A study that measured fluoxetine and norfluoxetine protein binding in both ewes and their fetuses confirmed decreased binding in the fetus (92% compared with 94%).[33] Although the differences in protein binding between fetal and maternal plasma perhaps account for the observed fetal:maternal ratio at steady-state conditions, direct fetal clearance mechanisms should be considered.

Fetal clearance, perhaps the current study's most robust measure (Table 2), strikingly exceeds reported measures of fetal placental blood flow among various species, including nonhuman primates, ranging from 104 to 212 mL/min/kg.[7-9,13,51] Placental clearance of fluoxetine should not exceed placental blood flow, unless there is an active transport process, but even then, clearance would be restricted to some extent by blood flow. Sequestration is highly unlikely, considering the high permeability of fluoxetine. Overall, metabolism—rather than active placental transport—may account for the high fetal clearance of both fluoxetine and norfluoxetine that was observed in this study.

The critical importance of fetal metabolism is underscored by the fact that although protein binding and ionization can alter total drug concentration, only metabolism by the fetus or active efflux transport by the placenta can reduce active drug concentrations. The cytochrome P450 isoforms that metabolize fluoxetine are detectable in human fetal liver; however, these enzymes generally exhibit at most 10% of the adult activity at birth.[24] Norfluoxetine was detected during direct infusion of fluoxetine to fetal baboons, but this was not the case in the sheep study.[33] Moreover, on incubating fluoxetine with ovine fetal hepatic microsomes, the same investigators found no norfluoxetine formation.[33] Human studies have reported similar concentrations of fluoxetine and norfluoxetine in cord serum at delivery and in infants 48 h after birth, suggesting slow elimination of the drug.[26,32] However, on close inspection of the data, the norfluoxetine concentrations actually increased, whereas the fluoxetine level was unchanged. It is difficult to determine specific clearances during the first few days of life due to the dynamic changes that are occurring in both the distribution and elimination mechanisms in newborns. A low level of activity might lead to some norfluoxetine formation by the fetus, although the extent to which the norfluoxetine that we noted in our baboons came from fetal metabolism or crossed the placenta from the maternal circulation still needs to be determined. More importantly, although fetal metabolism of fluoxetine to norfluoxetine could explain a fetal:maternal ratio of 0.55 to 0.65 for fluoxetine during maternal infusion, it does not explain a similar fetal:maternal ratio for norfluoxetine. An alternative metabolic pathway such as glucuronidation could account for both. Although not directly measured in the current study, fetal glucuronidation of drugs has previously been quantified in fetal baboons.[18,20,21] Neither fluoxetine nor norfluoxetine glucuronides were found in ovine amniotic fluid, suggesting little or no fetal glucuronidation in sheep.[1,33,46] However, approximately 10% and 6.4%, respectively, of fetal clearance in the sheep model was nonplacental when both the R and S enantiomers are considered, suggesting an unmeasured metabolic clearance. Glucuronide metabolites were not analyzed in this baboon study, nor could fetal nonplacental clearance be quantified. Additional research is required to delineate whether metabolic clearance pathways contribute to the clearance of fluoxetine and norfluoxetine from fetal baboons.

Active efflux transport from fetus to the mother across the placenta is the remaining notable mechanism that could account for the difference in the observed fetal:maternal steady-state concentration ratios in baboons. Fluoxetine is lipophilic, with an octanol–water partition coefficient of 1.8, and as such is expected to have relatively high placental clearance.[64] Even though a wide variety of placental drug transporters are present in the placenta, currently there are no specific data supporting that they transport SSRI.[30] Fluoxetine is considered to be a potent competitive inhibitor of the human ABCB1 transporter in the placenta and may lead to decreased activity of the placental p-glycoprotein barrier during pregnancy.[65] The majority of evidence suggests that fluoxetine is passively transferred across the placenta.

The key findings of the current study are the high clearance of fluoxetine from adult baboons and the high fetal clearance of the drug and its measured metabolite. The adult baboon fluoxetine clearance suggests a significant difference in drug metabolism compared with that in humans, despite known genetic similarities. Future studies can still use the baboon model but require a clear understanding of the metabolic differences between baboons and humans. The baboon fetal clearance was not easily explained by the data, and additional research is necessary

to examine fetal elimination mechanisms. Species-associated differences should be taken into account when designing drug studies and when prescribing drugs for clinical veterinary use.

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