STEREOSELECTIVE PHARMACOKINETICS OF FLUOXETINE AND NORFLUOXETINE ENANTIOMERS IN PREGNANT SHEEP

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ABSTRACT:
We examined the stereoselective disposition of fluoxetine (FX) and its metabolite norfluoxetine (NFX) in five pregnant sheep. Racemic FX was administered i.v. to the ewe (50 mg) and the fetus (10 mg) on separate occasions. Maternal and fetal blood, maternal urine, and fetal amniotic and tracheal fluid samples were collected for 72 h. FX and NFX isomers were quantified by gas chromatography–mass spectrometry. They rapidly crossed the placenta [maternal to fetal area under the plasma concentration versus time curve (AUC) ratios 0.59 and 0.65, respectively]. There was significant FX stereoselectivity with S/R FX AUC ratios averaging 1.65 ± 0.33 and 1.73 ± 0.29 in ewe and fetus, respectively, after maternal dosing. The maternal clearance and volume of distribution were also higher for (R)-fluoxetine than for (S)-fluoxetine. FX, NFX, and their glucuronides were present in maternal urine but accounted for only 3.4% of maternal drug elimination. In contrast, NFX was not detected in the fetus after fetal FX administration, which is consistent with the absence of measurable fetal nonplacental clearance of the drug and the lack of NFX formation in fetal hepatic microsomal incubations. There was also no fetal production of FX and NFX glucuronides in vivo. Both FX and NFX were extensively and stereoselectively bound in maternal and fetal plasma, with the free fraction S/R FX ratio averaging 0.46 ± 0.06 and 0.58 ± 0.10 in ewe and fetus, respectively. Thus, FX exhibits extensive stereoselective disposition, which is likely due to differential plasma protein binding of the FX isomers, and there is no detectable fetal formation of NFX, FX, and NFX glucuronides.

Currently there are five serotonin reuptake inhibitors (SSRIs) available worldwide with fluoxetine (Prozac; Eli Lilly & Co., Indianapolis, IN) being the first SSRI introduced in the North American market in 1987 (Catterson and Preskorn, 1996; Hiemke and Härtter, 2000). More than a decade after its introduction, fluoxetine (FX) still remains one of the most frequently prescribed antidepressants of this class for the treatment of depressive, obsessive-compulsive, and eating disorders. FX is a racemate of two optical isomers, both exhibiting selective serotonin reuptake inhibition with about equal potencies in vitro and in vivo (Gram, 1994). However, the S-enantiomer of FX has a slightly longer duration of action than the R-enantiomer in vivo (Wong et al., 1990). In the human, the main metabolites of fluoxetine are norfluoxetine (NFX), FX, NFX conjugates, and hippuric acid, which together may account for up to 95% of the administered dose (DeVane, 1999). The plasma concentration of NFX is comparable with that of FX at steady state (Hwang et al., 1980). Although the metabolite of SFX, S-norfluoxetine (SNFX) is an equally effective serotonineruptake inhibitor as SFX, R-norfluoxetine (RNFX) is a weak inhibitor (Fuller et al., 1992). Therefore, it appears that the longer duration of action of SFX in vivo is due in part to the liberation of its more potent metabolite, SNFX (Fuller et al., 1992).

FX and other SSRIs are not approved for use in human pregnancy. Despite this, they are in common use for the treatment of depression and other affective disorders during gestation (Newport et al., 2001). However, there is very limited information on the pharmacokinetics of fluoxetine in human/animal pregnancy. Case reports (Spencer, 1993; Mhanna et al., 1997) on fluoxetine and norfluoxetine exposure at birth and immediately postpartum indicate that both the parent drug and its active metabolite readily cross the placenta. Several prospective studies examining birth outcomes from SSRI-exposed pregnancies have reported no significant increase in morphological teratogenic risk associated with maternal FX therapy in the first trimester (Pastuszak et al., 1993; Goldstein, 1995; Chambers et al., 1996; Nulman et al., 1997). In contrast, third trimester exposure is associated with reduced birth weight and postnatal weight gain and an increased incidence of

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preterm delivery, admission to a special nursery, and poor neonatal adaptation (Chambers et al., 1996, 1999; Cohen et al., 2000; Nordeng et al., 2001). Recently, we and our collaborators have reported altered facial and heart rate responses to painful medical procedures in infants exposed to FX and other SSRIs in utero (Oberlander et al., 2002). However, there are no reported effects of the drug on infant/child global IQ and language development (Nulman et al., 1997; Kulin et al., 1998). On the other hand, prenatal exposure to fluoxetine in animal models does suggest long-term effects on endocrine and behavioral function (Di Pasquale et al., 1994; Vorhees et al., 1994; Cabrera-Vera and Battaglia, 1998). Studies of the placental transfer of fluoxetine in animals appear limited to a single study in rats, which assessed fetal exposure after administration of radiolabeled FX to the dam. Results of these autoradiographic studies suggest that FX and/or its metabolites readily cross the placenta to result in extensive fetal tissue distribution (Pohland et al., 1989).

To date there is very limited information on the extent of placental transfer and subsequent fetal exposure to fluoxetine and its pharmacologically active metabolite norfluoxetine. In addition, the stereoselective disposition of FX during pregnancy has not been studied, and there is no information on the ability of the fetus to metabolize the drug. The primary objective of the current study was to examine maternal and fetal stereoselective disposition of FX and NFX in the chronically instrumented ewe and fetus after both maternal and fetal administration of racemic FX. A further objective was to compare the rate of fluoxetine demethylation in maternal and fetal hepatic microsomes studies in vitro. It is very feasible to chronically implant catheters in the maternal and fetal vasculature and other fluid compartments of pregnant sheep, which permits serial sampling of blood and other fluids. In addition, the fetal lamb and human are similar in terms of physiologic functions (Harding and Bocking, 2001). Thus, pregnant sheep have been commonly used to study maternal-fetal drug disposition and effects (Rurak et al., 1991).

Materials and Methods

Animals and Surgical Preparation. The study was approved by the University of British Columbia Animal Care Committee, and the procedures performed on sheep conformed to the guidelines of the Canadian Council on Animal Care. The detailed surgical procedures used have been described previously (Kwan et al., 1995). Briefly, five pregnant Dorset Suffolk cross-bred ewes, with a maternal body weight of 69.8 ± 16.4 kg (mean ± S.D.), were surgically prepared at 117 to 126 days of gestation (123 ± 3 days, term = 145 days). Surgery was performed aseptically under halothane (1–2%) and nitrous oxide -/H11006°C. Similarly, surgery was performed using a cellulose dialysis membrane (12-kDa cut-off; Sigma-Aldrich) for 4 h at 39°C.  Different drug-free plasma samples were spiked, incubated, and dialyzed at 39°C. Pooled experimental maternal and fetal plasma samples (ex vivo) were also dialyzed in a similar manner.

Study of Hepatic Micromes in Vitro. Maternal and fetal hepatic microsomes were prepared from two pregnant ewes (135 and 139 days' gestation) afterinduction of anesthesia with i.v. sodium pentobarbital (120 mg/kg). A single slice of the maternal liver and the whole fetal liver were rapidly obtained from the animals and thoroughly washed free of blood with ice-cold 0.05 M Tris buffer (pH 7.4) containing 1.15% KCl. Microsomes were prepared using a standard differential ultracentrifugation technique at 4°C (Lu and Levin, 1972) and were stored at −70°C until use. For assessing fluoxetine demethylation by the microsomal preparations, 3 mg/ml of microsomal protein was incubated at 39°C in 0.15 M Tris buffer (pH 7.4) containing 1.15% KCl. Microsomes were prepared using a standard differential ultracentrifugation technique at 4°C (Lu and Levin, 1972) and were stored at −70°C until use. For assessing fluoxetine demethylation by the microsomal preparations, 3 mg/ml of microsomal protein was incubated at 39°C in 0.15 M Tris buffer (pH 7.4) containing 1.15% KCl. Microsomes were prepared using a standard differential ultracentrifugation technique at 4°C (Lu and Levin, 1972) and were stored at −70°C until use. For assessing fluoxetine demethylation by the microsomal preparations, 3 mg/ml of microsomal protein was incubated at 39°C in 0.15 M Tris buffer (pH 7.4) containing 1.15% KCl. Microsomes were prepared using a standard differential ultracentrifugation technique at 4°C (Lu and Levin, 1972) and were stored at −70°C until use.
Pharmacokinetic Analysis. Maternal and fetal total body clearances (CL), mean residence time (MRT), and steady-state volumes of distribution of the parent drug (Vdss) were calculated by standard pharmacokinetic procedures:

\[ CL_{\text{parent}} = \frac{\text{Dose}}{\text{AUC}_{\text{parent}}} \]  
\[ \text{MRT}_{\text{parent}} = \frac{\text{AUMC}_{\text{parent}}^{-\infty}}{\text{AUC}_{\text{parent}}} \times \frac{{\tau}}{2} \]  
\[ (V_{\text{dss}})_{\text{parent}} = (CL_{\text{TB}})_{\text{parent}} \cdot \text{MRT}_{\text{parent}} \]

where \( \text{AUC}_{\text{parent}}^{\infty} \), \( \text{AUMC}_{\text{parent}}^{\infty} \), and \( \tau \) are the area under the curve, area under the first moment curve calculated by the linear trapezoidal rule, and the infusion duration, respectively. The terminal elimination half-life (t1/2p) of the parent drug and as the metabolite was obtained from a noncompartmental fitting of the data using the nonlinear least-squares regression software WinNonlin (Pharsight, Mountain View, CA). Renal clearances values for parent drug and metabolite in the ewe were calculated by dividing the total amount of the drug or metabolite excreted in urine by the respective maternal plasma AUC.

The maternal and fetal placental and nonplacental clearance values for the fluoxetine isomers were calculated using the method described for labetalol by Yeleswaram et al. (1993). Maternal placental clearance (CLmp) was estimated from the data using the nonlinear least-squares regression software WinNonlin (Pharsight, Mountain View, CA). Renal clearances values for parent drug and metabolite in the ewe were calculated by dividing the total amount of the drug or metabolite excreted in urine by the respective maternal plasma AUC.

Statistical Analysis. Statistical analysis was performed on the pharmacokinetic parameters using paired and unpaired t tests and two-way analysis of variance for repeated measurements with an appropriate post hoc test, if necessary. Data were considered to be significantly different when \( p < 0.05 \). Microsoft Excel (Microsoft, Redmond, WA), GraphPad Prism 3.0 (GraphPad Software Inc., San Diego, CA), and SigmaPlot version 4.01 (SPSS Inc., Chicago, IL) were used for statistical analysis and graphical presentation. Data values in the text and tables are presented as the mean ± S.D. Fetal weight in utero at the time of experimentation was estimated from the weight at birth and the time interval between the experiment and birth (Kwan et al., 1995).

Results

The average maternal body weight was 69.8 ± 16.4 kg, and estimated fetal body weights on the day of maternal and fetal experiments were 2.87 ± 0.83 and 2.75 ± 0.68 kg, respectively. Average gestational age on the day of FX administration was 130.4 ± 2.6 and 129.4 ± 3.6 days for the maternal and fetal experiments, respectively. Mean gestational age and fetal weight at delivery were 141.4 ± 3.0 days and 3.73 ± 0.92 kg, respectively.

Maternal Fluoxetine Administration. Plasma pharmacokinetics. Figure 1 illustrates the maternal arterial plasma concentration versus time profiles obtained for the FX and NFX isomers after maternal racemic FX administration. Plasma concentrations of both FX isomers increased rapidly during the 10-min infusion period and then declined tri-exponentially. The plasma concentration of SFX was significantly higher than that of RFX throughout the experimental period, with the individual plasma S/R concentration ratios ranging from 1.42 to 2.24 (\( p < 0.05 \), two-way analysis of variance for repeated measures). Table 1 presents the calculated maternal pharmacokinetic parameters for the FX and NFX isomers after maternal drug administration. The mean S/R ratio of plasma AUC for FX was 1.65 ± 0.33, resulting in a higher total body clearance of RFX (50.7 ± 12.4 ml/min/kg) than SFX (30.7 ± 3.8 ml/min/kg) (\( p < 0.05 \), paired t test). The steady-state volume of distribution of RFX was ~50% higher than that of the S-isomer. However, no significant stereoselectivity was observed in the apparent terminal elimination half-life for the FX isomers. Maternal plasma concentrations of the NFX isomers increased over the first 2 h and then declined slowly (Fig. 1). Unlike the parent compound, there was no significant difference in the AUC of SNFX and RNFX with the S/R ratio averaging 0.94 ± 0.22. The maternal AUC ratios of the NFX to FX isomer were 0.63 ± 0.23 and 1.10 ± 0.39 for S- and R-isomers, respectively, and these are significantly different. The apparent maternal terminal half-lives for RNFX and SNFX were not significantly different. However, the half-life values (and other pharmacokinetic variables for NFX) after FX dosing likely reflect both elimination and continued metabolite formation and so may not provide totally accurate estimates.

Similar to the maternal profile, fetal plasma concentrations of FX isomers also increased rapidly during the infusion period and then declined exponentially (Fig. 2). The fetal plasma SFX levels remained consistently higher than the corresponding RFX concentrations throughout the experimental period. The calculated fetal pharmacokinetic parameters for FX and NFX are presented in Table 1. The fetal S/R FX AUC ratio averaged 1.73 ± 0.29, which was not significantly different from the corresponding maternal value. The fetal-to-maternal (F/M) AUC ratio was similar for the SFX (0.60 ± 0.15) and RFX (0.58 ± 0.19) isomers. Although the apparent fetal terminal elimination half-life of the FX isomers did not exhibit any significant stereoselectivity, the values measured were significantly longer than those calculated for the mother (9.3 ± 0.3 versus 6.7 ± 0.7 for SFX; 10.8 ± 1.3 versus 6.6 ± 2.5 h for RFX) (\( p < 0.001 \)). The fetal AUC ratios of NFX to FX isomers were 0.72 ± 0.18 and 0.98 ± 0.15 for
Steroselective Pharmacokinetics of Fluoxetine in Pregnancy

Maternal and Fetal Plasma Pharmacokinetic Variables after Maternal Administration of Racemic Fluoxetine (50 mg) in Pregnant Ewes

<table>
<thead>
<tr>
<th>Variable</th>
<th>$R$ isomer</th>
<th>$S$ isomer</th>
<th>S/R ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>126.1 ± 37.6</td>
<td>202.1 ± 46.6*</td>
<td>1.65 ± 0.33**</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>50.7 ± 12.4</td>
<td>30.7 ± 3.8*</td>
<td>0.62 ± 0.10**</td>
</tr>
<tr>
<td>$V_{ss}$ (liter/kg)</td>
<td>9.3 ± 1.3</td>
<td>6.3 ± 1.1*</td>
<td>0.68 ± 0.11**</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>6.6 ± 2.5</td>
<td>6.7 ± 0.7</td>
<td>1.14 ± 0.48</td>
</tr>
</tbody>
</table>

Maternal values

- AUC (μg · h/l): 135.2 ± 50.5
- $t_{1/2}$ (h): 28.2 ± 18.6
- AUC$_{FNX}$/AUC$_{FX}$: 1.10 ± 0.39

Fetal values

- AUC (μg · h/l): 69.8 ± 18.0
- $t_{1/2}$ (h): 10.8 ± 1.3***
- F/M AUC: 0.58 ± 0.19***

\* Significantly different from the $R$ isomer value; **, ratio is significantly different from unity; ***, significantly different from the corresponding maternal value.

Data are presented as mean ± S.D. ($n = 5$).

S- and R-isomers, respectively. These are similar to the maternal values and are significantly different. As observed in maternal plasma, there was no significant difference in the AUC values for SNFX and RNFX, with the S/R ratio averaging 1.25 ± 0.29. The F/M AUC ratios were 0.76 ± 0.33 for SNFX and 0.53 ± 0.13 for RNFX and were not significantly different. Although the mean F/M AUC ratio for NF (0.65) was higher than the mean value for FX (0.59), this difference was also not statistically significant. The apparent fetal terminal elimination half-lives of the NFX isomers showed significant stereoselectivity (S/R ratio of 0.66 ± 0.05, $p < 0.001$); however, they were not significantly different from the maternal values.

Amniotic fluid and tracheal fluids. The amniotic fluid concentration of the FX and NFX isomers increased over the first 12 to 18 h of the experiments and then decreased slowly (Fig. 3). These concentrations were similar to or slightly lower than their respective fetal plasma concentrations, and no significant accumulation of either the FX or NFX isomers was observed. Unlike the fetal plasma concentration profiles (which showed a higher concentration of SFX over RFX), there were no significant differences between the amniotic fluid concentrations of the FX or NFX isomers. Fetal tracheal fluid concentrations of the FX isomers were slightly higher than those measured in amniotic fluid (Fig. 4). Similar to the amniotic fluid, there were no significant differences in the concentrations of the FX or NFX isomers. Glucuronide or sulfate conjugates of FX or NFX were not detected in the amniotic fluid samples.

Fetal urinary pharmacokinetics. The renal clearance, cumulative amount excreted, and percentage of administered dose excreted as FX, NFX, and their corresponding glucuronides in maternal urine are provided in Table 2. Although both parent drug and metabolite as well as their conjugates were present in maternal urine, their total contribution to overall maternal drug elimination was minimal (3.4% of the administered dose). Representative cumulative urinary excretion plots of the FX and NFX isomers and their corresponding glucuronide conjugates are shown in Figs. 5 and 6, respectively. The FX and NFX isomers did not reach plateau levels by the end of the experimental period, resulting in some underestimation of their cumulative excretion. This was also the case with the FX and NFX isomer glucuronides. Neither the cumulative amount nor the renal clearance of the FX and NFX isomers and their glucuronide conjugates exhibited any significant stereoselectivity. Additional urine collection may have provided a more accurate estimate of urinary pharmacokinetic parameters; however, further urine collection was not performed due to ethical and practical concerns regarding prolonged use of a Foley catheter in the ewes. Sulfate conjugates could not be detected in these urine samples.

Blood gas and glucose/lactate status. During the maternal experiments, the control period fetal femoral arterial pH, $pCO_2$, $pO_2$, O$_2$ saturation, and hemoglobin, lactate, and glucose concentrations were 7.362 ± 0.014, 49.3 ± 5.7 mm Hg, 22.5 ± 2.1 mm Hg, 50.7 ± 2.9%, 10.6 ± 0.5 g/dl, 1.14 ± 0.04, and 0.46 ± 0.04 mM, respectively. There were no significant changes in pH, $pCO_2$, and glucose concentration during the experimental period. However, fetal O$_2$ saturation decreased significantly from 22.5 ± 2.1 mm Hg to a minimum of 17.0 ± 1.41 mm Hg at 10 min into the infusion and thereafter returned to control levels at 6 h (20.7 ± 1.15). Similarly, fetal O$_2$ saturation decreased...
significantly from 50.7 ± 2.9% to a minimum value of 36.1 ± 6.0% by 15 min after initiating the infusion and then returned to normal values at 9 h (51.0 ± 8.0%). Fetal arterial blood lactate concentration increased significantly from 1.14 ± 0.06 mM (predose) to a maximum of 1.27 ± 0.30 mM and subsequently returned to control values at 9 h (1.18 ± 0.08 mM).

**Fetal Fluoxetine Administration.** *Plasma pharmacokinetics.* The fetal femoral arterial plasma concentration versus time profiles for FX and NFX isomers after fetal racemic FX administration are depicted in Fig. 7. Similar to the maternal profiles observed after maternal FX dosing, plasma concentrations of both FX isomers increased rapidly during the infusion period and then declined tri-exponentially, with SFX concentrations being significantly higher than those of RFX throughout the experimental period. Unlike with maternal FX administration, the NFX isomers were not detected in any of the fetal plasma samples. Table 3 presents the calculated fetal pharmacokinetic parameters for the FX isomers after fetal drug administration. The S/R ratio of plasma AUC for FX was 2.20 ± 0.11, resulting in a significantly higher total body clearance (based on estimated fetal weight) of RFX (167.3 ± 45.8 ml/min/kg) than that of SFX (76.7 ± 21.7 ml/min/kg). These fetal values are significantly higher (*p* < 0.01) than the corresponding estimates of maternal RFX and SFX total body clearances obtained in the maternal dosing experiments (50.7 ± 12.4 and 30.7 ± 3.8 ml/min/kg, respectively; Table 1). The *V*₄₅₀ for RFX (38.7 ± 17.6 l/kg) was significantly higher than that of SFX (17.4 ± 8.4 l/kg), resulting in an S/R ratio of 0.45 ± 0.06, and again, these values are higher than the corresponding maternal *V*₄₅₀ estimates (9.3 ± 1.3 and 6.3 ± 1.1 l/kg; Table 1). However, no significant stereoselectivity was observed in the apparent terminal elimination half-life for the FX isomers.

Similar to maternal FX administration, maternal plasma concentrations of the FX isomers after fetal infusion increased rapidly initially and then declined exponentially (Fig. 8). Maternal SFX concentrations were also observed to be significantly higher than those of RFX after fetal FX dosing. Unlike maternal FX administration, measurable levels of the NFX isomers were only observed in two of the five ewes. Table 3 presents the calculated maternal pharmacokinetic parameters for FX and NFX. The maternal S/R FX AUC ratio was 1.73 ± 0.29, which is similar to the maternal S/R AUC ratio calculated after maternal dosing (1.65 ± 0.33; Table 1) but is significantly lower than...
the fetal S/R ratio (2.20 ± 0.11). No significant differences were observed for either FX- or NFX-isomer apparent elimination half-lives.

Amniotic and tracheal fluid excretion. Similar to the fetal plasma drug concentration profiles, NFX isomers were not detected in either amniotic or tracheal fluid. The amniotic fluid concentrations of the FX isomers increased over the first hour and decreased slowly thereafter, reaching the assay limit of quantitation at 36 h. Postinfusion amniotic fluid FX concentrations were about 5- to 10-fold lower than fetal plasma levels, and no significant accumulation was observed. Unlike the fetal plasma concentration profiles (which show higher concentrations of SFX over RFX), there was no significant difference between the levels of the FX isomers in amniotic fluid. As observed with maternal FX dosing, glucuronicide or sulfate conjugates were also not detectable in amniotic fluid samples after fetal drug administration. FX isomer concentrations in tracheal fluid were ∼10-fold higher than those observed in amniotic fluid. Tracheal fluid FX isomer concentrations were very similar to those measured in the fetal plasma; however, unlike plasma, no significant stereoselectivity was observed.

Maternal urinary pharmacokinetics. Similar to maternal FX dosing, the total urinary excretion of FX, NFX, and their glucuronides was low and accounted for only 0.75% of maternal drug elimination (data not shown). However, unlike maternal FX dosing, the cumulative amount of the FX isomers exhibited significant stereoselectivity (S>R), with the S/R ratio averaging 1.63 ± 0.23. There was, however, no difference in either the renal clearance of the FX isomers (S/R ratio of 0.98 ± 0.27), which was normalized to maternal AUC or the cumulative amount of the NFX isomers (S/R ratio of 1.09 ± 0.57). As in the maternal dosing experiments, sulfate conjugates could not be detected.

Blood gas and glucose/lactate status. During the fetal experiments, the control period fetal femoral arterial pH, pCO₂, pO₂, O₂ saturation, and hemoglobin, lactate, and glucose concentrations were 7.369 ± 0.001, 49.0 ± 1.1 mm Hg, 23.0 ± 2.0 mm Hg, 56.9 ± 6.8%, 10.9 ± 0.5 g/dl, 1.03 ± 0.21, and 0.46 ± 0.11 mM, respectively. There were no significant changes in pH and pCO₂ during the experimental period. However, as with maternal drug administration, fetal pO₂ decreased significantly from the control value to 14.5 ± 1.5 mm Hg at 1 h and then returned to control levels at 9 h (22.0 ± 2.9). Similarly, fetal O₂ saturation decreased from 56.9 ± 6.8% to 32.7 ± 3.0% at 15 min, and returned to normal values at 9 h (52.0 ± 6.7%). Fetal arterial blood lactate concentration increased from 1.03 ± 0.21 mM to reach a maximum of 2.33 ± 1.56 mM at 30 min and subsequently returned to the control value at 9 h (1.39 ± 0.81 mM). Blood glucose concentration also increased from 0.46 ± 0.11 mM to a maximum of 0.75 ± 0.16 mM at 1 h and thereafter returned to control levels at 24 h (0.54 ± 0.18 mM).

Plasma protein binding. Table 4 shows a summary of in vitro and ex vivo plasma protein binding of the FX and NFX isomers. FX and NFX are both highly bound by ovine plasma proteins (i.e., bound fraction ∼95%). The FX free fraction in maternal plasma exhibited significant stereoselectivity both in vitro and ex vivo, with the S/R ratio averaging 0.46 ± 0.09 and 0.46 ± 0.06, respectively. In vitro, the free fractions of RNFX tended to be higher than those for SNFX, but this difference was not statistically significant. In the in vitro fetal determinations, the free fractions of both FX and NFX isomers were significantly higher than in the ewes, i.e., the binding was less. Similar to maternal plasma, there was a significant difference in the free fraction of FX isomers with the levels of R-isomer being ∼2-fold higher than the S-isomer. There was also a tendency for the RNFX free fraction to be higher than that for SNFX in vitro, but this difference was not statistically significant. Fetal NFX binding could not be determined ex vivo because of the lack of this metabolite in fetal plasma during fetal drug administration. There was no significant concentration dependence for the free fraction of any of the isomers over the observed maternal plasma concentration range, and this was also the case in the fetus.

Maternal and fetal placental and nonplacental clearances of RFX and SFX. The mean values for placental and nonplacental clearance of the FX isomers in the ewe and fetus, which were calculated using eqs. 4 through 7, are given in Table 5. The table gives values based upon total plasma drug concentrations, which were calculated for each animal and also for unbound drug levels. These latter values were determined using the average values based on total drug concentration and the maternal and fetal plasma protein drug binding data in Table 4. In terms of the clearances based on total drug concentrations, fetal total clearance of both RFX (167.3 ± 45.8 ml/min · kg) and SFX (76.3 ± 21.7 ml/min · kg) were significantly higher than the corresponding maternal values (51.1 ± 12.7 and 30.9 ± 7.4 ml/min · kg). However, in the fetus, placental clearance comprised 89.4 ± 36.9 and 94.0 ± 37.3% of total clearance for RFX and SFX, respectively, whereas in the ewe it was nonplacental clearance that accounted for the bulk of total clearance, averaging 92.2 ± 3.8 and 93.5 ± 4.0% for RFX and SFX, respectively. The fetal nonplacental clearance values (RFX, 17.2 ± 56.1 and SFX, 4.9 ± 28.4 ml/min · kg) were not significantly different from zero, and in some cases, the values were <0. Table 5 also gives the S/R ratios for all the clearance values except CL₁, and in every case, the ratio based on total drug concentration was significantly <1, suggesting stereoselective differences in the placental transfer of the FX isomers and also in nonplacental clearance of the drug in the ewe. However, when the clearance values were estimated on the basis of unbound drug concentration, these stereoselective differences largely disappeared, and this was also the case for the CL₁ - CL₉, difference.

Fluoxetine demethylation by maternal and fetal hepatic microsomes. In the maternal hepatic microsomal preparations from the two ewes, the norfluoxetine formation rates were 26.8 and 21.3 pmol/mg protein/30 min for SFNX and 28.4 and 23.5 pmol/mg protein/30 min for RNFX. The S/R ratios were 0.94 and 0.91. In contrast, with the fetal microsomes, no norfluoxetine formation was detected with either preparation.
Fetal and maternal plasma pharmacokinetic variables after fetal administration of racemic fluoxetine (10 mg) in pregnant ewes (mean values ± S.D., n = 5)

<table>
<thead>
<tr>
<th>Variable</th>
<th>R isomer</th>
<th>S isomer</th>
<th>SR ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fetal values</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoxetine AUC (µg · h/l)</td>
<td>219.0 ± 127.1</td>
<td>483.3 ± 283.6*</td>
<td>2.20 ± 0.11**</td>
</tr>
<tr>
<td>CL (ml/min · kg)</td>
<td>167.3 ± 45.8</td>
<td>76.7 ± 21.7*</td>
<td>0.48 ± 0.05**</td>
</tr>
<tr>
<td>Vdss (l/kg)</td>
<td>38.7 ± 17.6</td>
<td>17.4 ± 8.4*</td>
<td>0.45 ± 0.06**</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>7.4 ± 2.9</td>
<td>7.3 ± 3.2</td>
<td>0.98 ± 0.14</td>
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<tr>
<td>Norfluoxetine AUC (µg · h/l)</td>
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<td>not quantifiable</td>
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</tr>
<tr>
<td>Maternal values</td>
<td></td>
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</tr>
<tr>
<td>Fluoxetine AUC (µg · h/l)</td>
<td>21.2 ± 5.1</td>
<td>56.6 ± 13.1*</td>
<td>1.73 ± 0.29***</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>10.8 ± 1.3</td>
<td>9.3 ± 0.3***</td>
<td>0.93 ± 0.19</td>
</tr>
<tr>
<td>M/F AUC</td>
<td>0.12 ± 0.06**</td>
<td>0.10 ± 0.05**</td>
<td>0.79 ± 0.17</td>
</tr>
<tr>
<td>Norfluoxetine AUC (µg · h/l)</td>
<td>34.5</td>
<td>31.0</td>
<td>0.92</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>23.1</td>
<td>19.5</td>
<td>0.56</td>
</tr>
</tbody>
</table>

* Significantly different from the R isomer value.
** Ratio is significantly different from unity.
*** Significantly different from the corresponding fetal value.
† n = 2 because of undetectable NFX in three ewes.

Discussion

Placental Transfer of Fluoxetine and Norfluoxetine. There was rapid maternal to fetal placental transfer of both fluoxetine and norfluoxetine after maternal administration, with the F/M AUC ratios averaging ~0.59 and 0.65, respectively. Placental drug transfer after acute maternal administration seems largely dependent upon the drug’s lipophilicity (Rurak et al., 1991). FX is highly lipophilic (log octanol/water partition coefficient = 4.05). However, its F/M AUC ratio is lower than the values for diphenhydramine (0.85) and metoclopramide (0.82), both of which have substantially lower partition coefficient values of 3.1 and 1.1, respectively (Yoo et al., 1986; Riggs et al., 1988). The reduced fetal exposure to fluoxetine, compared with these two drugs, is likely due to the high plasma protein binding of the drug, which can also reduce maternal-fetal drug transfer (Rurak et al., 1991). The FX F/M AUC ratio in sheep is also lower than the F/M drug concentration ratio of ~1 that we have recently determined at delivery in pregnant women treated with the drug for clinical depression (Kim et al., 1999). This difference is unlikely to be solely due to the structural and functional differences between the sheep and human placenta, since a lower placental permeability in the former species is largely restricted to hydrophilic compounds (Faber and Thornburg, 1983). Rather it may be due to long-term drug administration in humans, compared with acute administration in the current study. In the former situation, fetal drug elimination becomes important in determining fetal exposure (Rurak et al., 1991). If the human fetus has a limited ability to metabolize FX, as appears to be the case with the fetal lamb (see below), this could explain the high fetal-maternal plasma drug concentration ratio. In line with this is our study involving 8-day i.v. FX infusion in pregnant sheep (Chien et al., 1999); the fetal/maternal drug concentration ratio of 0.75 is higher than the F/M AUC ratio in the current study. It is still lower than the corresponding value in the human (~1), and this could be due to a lesser degree of difference between maternal and fetal plasma protein binding of FX in the latter species compared with sheep. However, plasma protein binding of FX does not yet appear to have been determined in the human fetus.

Fluoxetine Pharmacokinetics in the Ewe and Fetus. The average maternal CL of racemic FX averaged 41.5 ml/min · kg. In the human, FX clearance differs in poor and extensive metabolizers of model substrates for cytochrome P450 2D6 (CYP2D6) (Hamelin et al., 1996; Fjordside et al., 1999). However, in both cases, the CL values of ~2.5 ml/min · kg in poor metabolizers and ~10 ml/min · kg in extensive metabolizers) are considerably lower than the sheep value. Likewise, the human t1/2 values for FX (~1–4 days) and NFX (~7–15 days) (Hiemke and Härtter, 2000) are much longer than the values of 6.7 and 23 h in pregnant sheep. In contrast, the maternal Vdss of FX in sheep (~7.8 l/kg) is below the range (14–100 l/kg) reported in the human (Hiemke and Härtter, 2000). These differences could in part be due to variations in drug administration regimens. FX clearance decreases and t1/2 increases in humans with chronic drug dosing (Catterson and Preskorn, 1996; Hiemke and Härtter, 2000), and this appears due to FX-induced inhibition of CYP2D6 (Alfaro et al., 2000). We have observed a similar decrease in FX clearance in sheep during an 8-day i.v. infusion protocol (Chien et al., 1999). However, the differences in the sheep and human data could also be due to pregnancy, since the values determined in the current study of pregnant sheep for CL (41.5 ml/min · kg), t1/2 (6.7 h), and Vdss (7.8 l/kg) are different from estimates we have made in nonpregnant ewes (CL, 28.3 ml/min · kg; t1/2, 18.0 h; Vdss, 15.1 l/kg) (Kim et al., 1996). The nonpregnant values are closer to those obtained in human subjects.
Similar changes may occur in human pregnancy, since there is an induction of CYP2D6 and increased dose requirements of antidepressants (Wisner et al., 1993; Wadelius et al., 1997).

Racemic fluoxetine CL (122 ml/min · kg) and Vdss (28.1 l/kg) in the fetus are significantly higher than in the ewe. As shown in Table 5, this total CL is largely contributed by fetal to maternal placental transfer. The impact on the fetus is likely minimal, since similar changes in fetal oxygenation regularly occur as a consequence of antepartum uterine contractions and fetal somatic activity (Harding et al., 2002). We have also found FX administration results in an initial transient increase in plasma serotonin levels (J. Morrison, C. Chien, K. W. Riggs, and D. W. Rurak, unpublished data). Serotonin is a potent uterine vasoconstrictor (Clark et al., 1980); thus, it is likely that FX elicited inhibition of serotonin reuptake by platelets increased plasma serotonin levels, leading to decreased uterine blood flow and fetal oxygen delivery. The impact on the fetus is likely minimal, since similar changes in fetal oxygenation regularly occur as a consequence of antepartum uterine contractions and fetal somatic activity (Harding et al., 1983).

Fluoxetine Effects on Fetal Blood Gas and Acid-Base Status. With both maternal and fetal FX administration, there were transient decreases in fetal oxygenation and blood pH and increases in blood lactate level. We have recently observed similar transient changes over the first ~6 h of an 8-day maternal i.v. FX infusion protocol, and these were associated with a decrease in uterine blood flow (Morrison et al., 2002). We have also found FX administration results in an initial transient increase in plasma serotonin levels (J. Morrison, C. Chien, K. W. Riggs, and D. W. Rurak, unpublished data). Serotonin is a potent uterine vasoconstrictor (Clark et al., 1980); thus, it is likely that FX elicited inhibition of serotonin reuptake by platelets increased plasma serotonin levels, leading to decreased uterine blood flow and fetal oxygen delivery. The impact on the fetus is likely minimal, since similar changes in fetal oxygenation regularly occur as a consequence of antepartum uterine contractions and fetal somatic activity (Harding et al., 1983).

Fluoxetine Metabolism in the Ewe and Fetus. In humans, urinary excretion of fluoxetine, fluoxetine glucuronide, norfluoxetine, norfluoxetine glucuronide, and hippuric acid account for ~50% of a single dose of 14C-fluoxetine (Lemberger et al., 1985; Bergstrom et al., 1988). In addition, there is urinary excretion of \( \text{p-trifluoromethylphenol} \), a dealkylated metabolite in rat and human (Urichuk et al., 1997). In the current study, maternal urinary excretion of FX, NFX, and their glucuronides accounted for only 3.4% of the dose. The maternal renal clearance values for FX and NFX were correspondingly low (Table 2), which is similar to the situation in humans (Lemberger et al., 1985). As the cumulative urinary excretion curves of these compounds did not reach a plateau level, we may have slightly underestimated their role in FX elimination. Nonetheless, the data strongly suggest that the renal excretion of these compounds contributes much less to overall FX elimination in sheep than in the human.
hippuric acid excretion was not determined, since estimation of the amount resulting from FX metabolism would require a labeled form of the drug. Recently, we detected trifluoromethylphenol in urine from adult sheep after FX administration but have not yet assessed its quantitative importance (C. Chien, D. W. Rurak, K. W. Riggs, unpublished data). Thus, the metabolic profile of FX in adult sheep appears qualitatively, but perhaps not quantitatively, similar to that in the human. However, the specific cytochromes P450 responsible for FX metabolism in the sheep remain to be determined. Although CYP2B and 3A have been identified in sheep liver (Galtieri and Alvinerie, 1996), information on other P450 isoforms is lacking.

In contrast to the ewe, there appears to be little or no metabolism of FX in the fetus. Thus, in the fetal FX administration experiments, NFX was present in the ewe but not the fetus. Moreover, CL_{f_{ew}} for RFX and SFX (Table 5) are not significantly different from zero, indicating no measurable nonplacental elimination of the drug in the fetus. In the in vitro hepatic microsomal incubations, there was no NFX production by fetal microsomes. Thus, the NFX in the fetus during maternal FX administration (Fig. 2) was likely formed in the ewe and diffused across the placenta. There is virtually no information available in the literature for the types of cytochrome P450 present in the ovine fetus. However, CYP2C9, 2C19, and 2D6, which appear to be involved in the N-deethylation of FX (Stevens et al., 1993; Hamelin et al., 1996; von Motlke et al., 1997; Fjordside et al., 1999), are minimally expressed in the human fetal liver and develop in the early neonatal period (Ring et al., 1999). Our data suggest that this may also be the case in sheep.

The fetal lamb also appears to lack the phase II metabolic pathways for FX, since glucuronide and sulfate conjugates of FX and NFX were not detected in amniotic fluid. However, glucuronide conjugates of other drugs are produced by the sheep fetus, including acetaminophen (Wang et al., 1985), morphine (Olsen et al., 1988), ritodrine (Wright et al., 1991), labetalol (Yeleswaram et al., 1993), valproic acid (Kumar et al., 2000), and indomethacin (Krishna et al., 2002). Since the glucuronide conjugates are hydrophilic, they do not readily cross the sheep placenta and, if formed in the fetus, accumulate in amniotic and allantoic fluids (Rurak et al., 1991). Consequently, the absence of FX and NFX glucuronides in amniotic fluid provides strong evidence against significant glucuronidation in the fetus. The reason for this deficiency is not yet apparent. However, the glucuronide conjugates of the drugs listed above involve ether linkages, whereas FX glucuronidation involves an amine linkage. In addition, although morphine-3-glucuronide is formed in the fetal lamb, morphine-6-glucuronide is not (Olsen et al., 1988), whereas both conjugates are synthesized in adult sheep (Milne et al., 1993). These findings suggest that although some UDP-glucuronosyltransferase isoforms are present in fetal lamb, others including those used by FX and NFX are not.

Stereoselective Pharmacokinetics. The present study provides the first evidence for stereoselective FX disposition during pregnancy. In both the ewe and fetus, the R-isomer had a lower AUC and higher plasma free fraction, CL, and V_{d_{ss}} compared with (S)-fluoxetine. The presence of these stereoselective differences in the fetus with fetal FX administration indicates that the stereoselective processes are operative in fetal as well as in adult sheep. FX elimination occurs mainly by the hepatic route, with limited renal clearance (Table 2). Moreover, total FX clearance is low, and for such a drug, hepatic clearance depends mainly upon hepatic intrinsic clearance of the free (unbound) drug and the free drug fraction (Wilkinson and Shand, 1975). As shown in Table 4, the free fraction for RFX in both the ewe and fetus is significantly higher than the values for SFX. However, with both isomers and with NFX, plasma protein binding in the ewe is higher in the fetus and similar to that reported for the human (Lemberger et al., 1985). The significantly lower binding in the fetus is likely due to the lower concentrations of albumin and other plasma proteins (Kwan et al., 1995). When the ex vivo free fraction values in Table 4 are used with the total drug RFX and SFX CL values in Tables 1 and 4, the resulting free drug CL values in the ewe (53.3 versus 70.7 l/h/kg) and fetus (96.2 versus 70.5 l/h/kg) are much closer compared with the total drug CL estimates. This is also the case for the estimates of maternal and fetal placental and nonplacental clearance based on free drug concentrations, compared with those based on total drug levels (Table 5). In addition, as illustrated in Figs. 1, 2, and 7, the difference in FX isomer concentrations was present immediately after drug injection, and the initial S/R ratio did not change appreciably throughout the rest of the sampling period. Moreover, the V_{d_{ss}} values that are different between the FX isomers. Overall, these findings suggest that differential plasma protein binding of FX isomers could contribute to the stereoselective disposition of the FX isomers after acute drug administration in pregnant sheep.

Fjordside et al. (1999) have reported stereoselective FX disposition in humans after a single oral dose but only in poor metabolizers of paroxetine, i.e., with a deficiency in CYP2D6. Also, there are differences in racemic fluoxetine pharmacokinetics between extensive and poor metabolizers of debrisoquin (Hamelin et al., 1996). Recently, we have found stereoselective disposition of FX in maternal, fetal, and infant plasma during chronic fluoxetine therapy in pregnant and postpartum women (Kim et al., 1999). Moreover, the magnitude of the S/R plasma concentration ratio increases with the duration of therapy and is inversely related to maternal FX CL. Furthermore, FX inhibits CYP2D6 over an 8-day drug administration period (Alfaro et al., 2000), and the drug is a substrate for this enzyme. Thus, in the human, stereoselective FX distribution appears to occur only in subjects with low or absent CYP2D6 activity, when other presumably stereoselective metabolic pathways come into play. This is different from the current results in pregnant sheep. However, as was discussed above, we have recently observed a decrease in maternal FX clearance in pregnant sheep during an 8-day drug infusion protocol (Chien et al., 1999), and this is associated with a progressive rise in the maternal plasma S/R FX concentration ratio. These findings suggest that stereoselective metabolic factors may come into play in sheep with chronic FX dosing, similar to the apparent situation in the human. In contrast, with acute drug administration, it is the differential protein binding of the FX isomers that is more important. Whether this factor is also important in the human has not yet been determined.

In summary, these studies demonstrate that there is rapid placental transfer of both FX and NFX to the fetal lamb after maternal drug (racemic FX) administration resulting in significant fetal exposure. FX undergoes extensive stereoselective disposition resulting in higher levels of the SFX isomer in both maternal and fetal plasma; this appears to be largely due to differential plasma protein binding of the FX isomers. In contrast, stereoselective disposition was not observed for NFX. It also appears that both phase I and II metabolic pathways for FX are lacking in the fetus.

References


