Placental Transfer and Fetal Elimination of Morphine-3-β-glucuronide in the Pregnant Baboon

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ABSTRACT:

The glucuronide metabolites of several widely used drugs are detected in fetal plasma after maternal drug administration. However, the disposition of these metabolites is poorly understood and clinical concerns have been raised about accumulation of active metabolites in the fetus. For this reason, morphine-3-β-glucuronide (M3G), an active metabolite of morphine, was studied to provide quantitative data on disposition. Maternal, fetal, and bidirectional placental clearances of M3G were measured in three pregnant baboons. During maternal infusion of M3G to steady-state, the glucuronide metabolite readily appeared in fetal plasma achieving a mean ± S.D. fetal-to-maternal concentration ratio of 0.79 ± 0.04. In paired maternal and fetal infusions, steady-state clearances were 53 ± 3 (maternal), 1.5 ± 0.5 (maternal-to-fetal), 2.6 ± 0.1 (fetal-to-maternal), and −0.70 ± 0.6 ml · min⁻¹ (fetal). These clearance values support bidirectional transfer of M3G across the placenta and indicate negligible direct clearance from the fetus. The clearance of M3G across the placenta is more than 20-fold less than that of morphine. Despite this low index of permeability, placental transfer contributes significantly to the glucuronide pool in the fetus. Placental transfer emerges as the major clearance pathway for the glucuronide from the fetus and suggests a component of active efflux. What is more, the results do not support the concept of sequestration in the fetal intestine as a significant route of clearance. Together these results clarify the distribution and clearance of glucuronides in the pregnant primate and facilitate prediction of fetal exposure to active metabolites.

Glucuronidation is a major drug elimination pathway. The glucuronide metabolites of several drugs have been detected in fetal plasma after maternal drug administration, raising the question of their origin. It has been shown that the sheep and primate fetus can contribute to the presence of glucuronide metabolites through metabolism (Wang et al., 1986, 1990; Olsen et al., 1988; Garland et al., 2005, 2006). In sheep, glucuronide metabolites have extremely low placental permeability suggesting little contribution from the mother (Wang et al., 1985; Olsen et al., 1988). However, the contribution from placental transfer has not been determined in primates. The information that is available suggests that the placental transfer is more permeable than that of the sheep. The similarity of fetal and maternal metabolite concentrations under steady-state conditions after maternal drug or metabolite administration in human and nonhuman primates supports a considerable transplacental contribution from the mother to the fetal metabolite pool (Gerdin et al., 1990a,b; Garland et al., 1998a,b,c, 2001).

The limited consideration given to metabolite disposition in pregnancy may be accounted for by the fact that the metabolites of many drugs are inactive and the commonly held concept that fetal metabolism has little impact on overall drug disposition. However, the glucuronide metabolites of morphine, morphine-3-β-glucuronide (M3G) and morphine-6-β-glucuronide (M6G) have demonstrated distinct activities that may contribute to the effects of the parent drug (Labella et al., 1979; Pasternak et al., 1987; Skarke et al., 2005). Furthermore, recent studies in the pregnant baboon demonstrate that fetal metabolism not only significantly decreases fetal drug concentrations, but also can result in concentrations of metabolites that exceed those in the mother (Garland et al., 2001). The extensive parallels between human and nonhuman primates suggest that studies of fetal metabolism and exposure to metabolites in baboons are relevant to the human fetus (Garland et al., 1996b, 1998a; Caspersen et al., 2007).

Olsen et al. (1988) first raised the possibility that drug metabolites in the fetus could exceed those in the mother owing to formation by the fetus and limited clearance from the fetus. In prior studies, the fetal baboon was shown to metabolize morphine to M3G and M6G (Garland et al., 2005). As in the adult, M3G was the primary metabolite and accounted for approximately 30% of the clearance of morphine from the fetus. This finding suggested that the formation of

ABBREVIATIONS: M3G, morphine-3-β-glucuronide; M6G, morphine-6-β-glucuronide; HPLC, high-performance liquid chromatography; CI, clearance; P1M, Model using data from paired infusions with single maternal compartment and single fetal compartment; P2M, Model using data from paired infusions with central and tissue maternal compartments and single fetal compartment; P2MNFC, Model using data from paired infusions with central and tissue maternal compartments and single fetal compartment with no direct fetal clearance; S1CPT, Model using data from single infusion with one compartment; S2CPT, Model using data from single infusion with central and tissue compartments; MRP, multidrug resistance-associated protein; BCRP, breast cancer resistance protein.
metabolite by the fetus was not negligible (Garland et al., 2005, 2006). In addition, when M3G and M6G were infused directly to the fetus, they were eliminated at a relatively low clearance rate (Garland et al., 2005). Although the mechanism of elimination was not delineated, it was concluded that clearance most likely occurred across the placenta.

The extent to which metabolite concentration in the fetus exceeds the concentration in the mother at steady state during maternal administration can be understood by examining the mathematical expression of the fetal/maternal metabolite ratio derived from the equations of the two-compartment model at steady state (Garland et al., 1996a). However, to predict fetal metabolite concentration in relation to the maternal metabolite concentration, it is necessary to have reliable estimates for placental transfer and direct fetal metabolite clearance, in addition to the currently available data on fetal metabolism. Estimates of clearance parameters for the metabolite can be obtained by the same methodology that has been used for drugs combining data from both maternal and fetal infusions of the compound in the same animal (Szetö et al., 1982b; Garland et al., 1998b). This methodology allows for the calculation of placental clearances in both directions and direct fetal clearance.

The objective of the present study was to identify the major routes of elimination of the primary morphine metabolite, M3G, from the fetus, determine the contribution of placental transfer to the presence of the metabolite in the fetus, and quantify the contributions of placental and nonplacental clearance pathways. This study is part of a comprehensive evaluation of the disposition and effect of drugs and their metabolites in the pregnant baboon using morphine as a model agent.

Materials and Methods

Animal Model. The animals were studied using a system of individualized backpack and tether. This system, along with the methods for maintenance, breeding, preconditioning, anesthesia, surgery, and postoperative care, were described in detail in a previous report and are summarized here (Stark et al., 1989). Animals were maintained in accordance with all regulations for the care and use of laboratory animals by the National Institutes of Health, U.S. Department of Agriculture, and American Association for the Accreditation of Laboratory Animal Science. Research protocols were approved by the Institutional Animal Care and Use Committee at Columbia University.

Breeding colony. A breeding colony of baboons (Papio species) housed in the Institute of Comparative Medicine, Columbia University, New York, was available for study. Gestational age was calculated from the date of conception. This date was estimated as the midpoint of the period of timed mating (±3 days). Term gestation (~175 days) was estimated from the average length of pregnancy in the colony. Ultrasound was performed at 70 to 110 days to confirm singleton pregnancy, fetal size, and placental location. Lights in the colony room were cycled (7:00 AM on and 7:00 PM off) and feeding times (8:00 AM and 4:00 PM) were constant.

Surgery. Surgery was scheduled between 120 and 145 days. Approximately 2 weeks before surgery, the animal was fitted to a backpack and sham-tethered. This allowed proper adjustment of the backpack straps and assessment of acceptance of the animal to the tether system. Only those animals who readily adapted to the system were studied. Surgery was performed under general anesthesia (isoflurane and nitrous oxide) using sterile surgical techniques. Vascular catheters were placed in the maternal femoral artery and vein and in the fetal carotid artery and jugular vein. An amniotic fluid catheter was sutured to the neck region. Electrophysiologic electrodes and a tracheal catheter were also placed to monitor fetal well-being and drug effects. The uterus was closed in two layers taking care to appose amniotic membranes. After closure of the rectus sheath and before skin closure, the catheters and electrodes were tunneled under the skin to the midscapular region of the mother where they exited the skin to the backpack.

Tether system. The backpack housed the pressure transducers and the catheter and electrical connectors. It was attached to a stainless steel tether cable that connected to a freely rotating pole on the top of the cage. The catheters passed through this cable and were connected to peristaltic infusion pumps and solutions that were attached to the pole (P720; Instech Laboratories, Inc., Plymouth Meeting, PA). The maternal and fetal vascular catheters were continuously infused with normal saline containing heparin (1 U · ml⁻¹) at rates of 5 and 2 ml · h⁻¹, respectively, to keep catheters patent. Catheters were accessible at the top of the cage to obtain blood and amniotic fluid samples. An electrical swivel at the top of the pole allowed the whole apparatus to turn freely with the activity of the animal while providing electrical power for pumps and signal transmission of physiological data.

Postoperative analgesia and monitoring of pregnancy. Postoperative analgesia was supplied with a continuous infusion of morphine sulfate (50–150 µg · kg⁻¹ · h⁻¹) to the mother. This was reduced stepwise as the mother resumed normal activity in the 2 to 4 days after surgery. No tocolytic agents were used. Cefazolin was administered daily for 3 days to the mother and into the amniotic fluid and then only as indicated. The mothers were maintained in their home cages near other familiar animals. A minimum of 6 days after surgery and at least 48 h after cessation of postoperative morphine was allowed for stabilization before the start of infusion protocols.

Mothers were monitored for signs of labor assessed by maternal behavior and pressure recordings from the amniotic fluid catheter (Cobe low-level pressure transducers; Cobe, Boulder CO; Hewlett Packard 8805-B Amplifier; Hewlett Packard, San Diego, CA). Physiologic stability of the fetus was assessed by fetal heart rate, blood pressure, arterial acid base status, and blood gas values (Hewlett Packard 3040A heart rate monitor; Hewlett Packard; Radiometer ABL735 co-oximeter, Radiometer America, Westlake, OH). This animal model allowed the pharmacokinetic studies to be performed during the last trimester of a stable pregnancy without the need for anesthesia or undue restraint.

Pharmacokinetic Protocol. Drug doses and preparation. M3G was obtained from the National Institute of Drug Abuse, Division of Neuroscience and Behavioral Research (Bethesda, MD). All M3G solutions were prepared from a 2 mg · ml⁻¹ stock solution with normal saline and stored at −70°C until use. The planned doses were 2.5 mg · h⁻¹ · 15 kg⁻¹ to the mother and 0.1 mg · h⁻¹ to the fetus. These doses were based on the plasma concentrations achieved in a nonpregnant animal for the mother and a dose kinetic study for the fetus (Garland et al., 2005). The goal was to obtain similar maternal concentrations across animals during maternal infusion and to obtain fetal concentrations during fetal infusions similar to those in the fetus during infusion of morphine to the mother. Thus, issues of potentially saturating fetal elimination processes and confounding clearance calculations were avoided, although a previous study had indicated linear dose kinetics for M3G in the fetus (Garland et al., 2005).

The infusion solutions were prepared in a single batch. The maternal solution was prepared first by diluting the stock solution with normal saline to a concentration of 0.5 mg · ml⁻¹. An aliquot of this solution was diluted 1:10 to prepare the fetal solution (0.05 mg · ml⁻¹). These solutions were used to maintain variability between maternal and fetal doses based on dose preparation. Heparin (sodium injection, USP, 5000 U · ml⁻¹; American Pharmaceutical Partners, Schaumburg, IL) was added during preparation to achieve a concentration of 0.5 U · ml⁻¹ for the fetal solution and 1.0 U/ml⁻¹ for the maternal solution. The solutions were filtered through 0.22-µm, 25-mm MCE filters (Thermo Fisher Scientific, Pittsburgh, PA) into sterile Viaflex infusion bags (Baxter, Dearfield, IL). The infusion bags were wrapped in aluminum foil to protect them from light and stored at −20°C until they were ready to be used on the animal.

Infusion and sampling protocols. All infusions were administered through the maternal superficial femoral or fetal internal jugular venous catheters, and all samples were obtained through maternal superficial femoral and fetal carotid arterial catheters. The order of the infusions was randomized to control for gestational age and time since surgery. The two infusions (maternal and fetal) were scheduled approximately 1 week apart. The bag of infusion solution was thawed overnight at room temperature, and the bag was weighed before being installed. After a baseline sample, the infusion was started at the appropriate rate using a precalibrated peristaltic infusion pump (P720; Instech Laboratories, Inc.). The maternal solution would deliver 2.5 mg · h⁻¹ at 5 ml · h⁻¹. Rate adjustments were made for each animal on the basis of their weight so the mother would receive 2.5 mg · 15 kg⁻¹ · h⁻¹. The fetal infusion would deliver 0.1 mg · h⁻¹ when infused at 2 ml · h⁻¹. These rates of infusion were...
used as the drug solutions replaced the normal saline infusions used to maintain vascular patency.

The duration of infusion was 52 h with samples obtained at 6, 24, 30, 48, 50, and 52 h from the beginning of the infusion in both the mother and fetus. The drug solution was covered with an opaque bag throughout. The infusion was stopped, and timed samples were obtained from the infusion recipient (mother or fetus) during the elimination phase. For the mother these were at 15, 30, 45, 60, 90, 120, and 180 min after the infusion was stopped. For the fetus, samples were obtained at 15, 30, 45, 90, 120, and 180 min after the infusion was stopped. Maternal samples were not taken during fetal elimination as the concentrations would be too low to be of value and fetal samples were not taken after maternal infusion to minimize the volume of blood obtained from the fetus.

The infusion solution bag was reweighed after the infusion. The difference between the weight of the solution bag from the beginning and end of the infusion divided by the total time of the infusion was used to determine a final infusion rate. Although all pumps were precalibrated to deliver 5 or 2 ml · h⁻¹, some differences in rates were noted once the pumps were infusing to the animal, presumably attributable to changes in the resistance to flow.

Blood samples were obtained by drawing back the dead space of the catheter plus 0.5 to 1 ml of blood for the fetus and a minimum of 3.0 ml of blood for the mother. The sample volume of 0.6 ml was then obtained for drug analyses. The extra blood withdrawn was returned, and the line was flushed with 3 ml of normal saline, which was sufficient to clear the line. Blood was placed in heparinized microtainer tubes (BD, Franklin Lakes, NJ), gently mixed, and then separated by centrifugation. Plasma was transferred to plastic vials and stored at −70°C until analysis. A sample of infusion solution was also obtained.

**HPLC Method for Analysis of M3G in Plasma Samples.** The HPLC method used was similar to that reported previously for measuring morphine, M3G, and M6G except that only fluorometric detection was used as M3G is not electrochemically active (Garland et al., 2005, 2006). The method uses an automated Waters Alliance 2695 HPLC system (Waters) with fluorometric and/or coulochemical detection.

**Extraction procedure for plasma samples.** Samples were prepared using solid-phase extraction columns (1-ml Bond Elute C18 50 mg columns; Varian, Inc., Sugarland, TX) on a VISIprep-DL solid-phase vacuum manifold (model 5-7250) followed by sample evaporation under nitrogen with a Visidyry drying attachment (model 5-7124; Supelco Bellefonte, PA). The columns were conditioned under vacuum with two 1-ml washes of methanol (HPLC grade; Mallinkrodt; Thermo Fisher Scientific), 1 ml of purified water (Milli-Q NanoPure Water Filtration System; Millipore, Bedford, MA), and 1 ml of 0.01M ammonium bicarbonate buffer (pH 9.3, HPLC grade; Mallinkrodt; Fisher Scientific). The solid-phase extraction columns were then loaded by gravity with 200 μl of ammonium bicarbonate buffer (pH 9.3) followed by 100 μl of sample plasma or, for standards, 100 μl of blank pooled plasma with 20 μl of 5% standard. 100 μl of the internal standard nalorphine HCl (800 ng/ml; Sigma-Aldrich, St. Louis, MO), and finally an additional 200 μl of ammonium bicarbonate buffer (pH 9.3). After equilibration, the columns were washed with 1 ml of 0.01M ammonium bicarbonate (pH 9.3) and dried under vacuum. The samples were eluted with two 150-μl washes of methanol (HPLC grade; Thermo Fisher Scientific), dried under nitrogen, and reconstituted in 200 μl of mobile phase. All samples were extracted in duplicate.

**Chromatographic conditions.** The separation was performed isocratically on a Waters Alliance 2695 HPLC system using a Spherisorb C18 column (ODS2, 3 μm, 4.6 × 100 mm i.d.; Waters) at ambient temperature with a flow rate of 1.5 ml/min. The mobile phase was 10 mM sodium phosphate monobasic (Thermo Fisher Scientific), 1.5 mM SDS (ultraPUR; Invitrogen, Carlsbad, CA), and 24% acetonitrile (HPLC grade; Thermo Fisher Scientific) adjusted to pH 2.1 with o-phosphoric acid 85% (HPLC grade; Thermo Fisher Scientific). After injection of 50 μl of sample, the eluting compounds were measured by fluorescence detection (excitation 210 nm and emission 340 nm) on a Linear model LC303 detector (Linear Instruments, ESA, Bedford, MA). The lower limit of detection was taken as the lowest point on the standard curve, which was 5.0 ng · ml⁻¹ for M3G. Values for a control sample included in each extraction had a mean value of 260 ng · ml⁻¹ and coefficient of variation of 12.3% in 25 extractions over a period of 4 years.

**Pharmacokinetic and Statistical Analyses.** Determination of steady state.

### Table 1

<table>
<thead>
<tr>
<th>Animal</th>
<th>Weight (kg)</th>
<th>Event</th>
<th>Gestational Age</th>
<th>Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>13.5</td>
<td>Surgery</td>
<td>Delivery</td>
<td>Maternal</td>
</tr>
<tr>
<td>M</td>
<td>14.5</td>
<td>132</td>
<td>165</td>
<td>153</td>
</tr>
<tr>
<td>F</td>
<td>15.5</td>
<td>126</td>
<td>145</td>
<td>139</td>
</tr>
</tbody>
</table>

M3G concentrations at each time point during M3G infusion were visually inspected and the early time points were expressed as a percentage of the mean values from samples taken 48 to 52 h after the onset of infusion. The mean of appropriate samples was then used to define a mean steady-state concentration for each animal and infusion route.

**Steady-state clearances.** The total (ClM and ClFt), placental (ClMf and ClFM), and direct or nonplacental (ClFb and ClFt) clearances of M3G for the maternal and fetal compartments were determined by the method of Szeto et al. (1982) using the following equations (note: c is used for steady-state concentrations of metabolite during maternal infusion and c̅/c̅̄ during fetal infusion; R̄M and R̄F are the rates of infusion to the mother and fetus).

ClM̄ = c̄M̄ - c̄F̄ - (c̄ / c̄M̄)

ClM = ClM̄ - (c̄M̄ / c̄M)

ClF = ClF̄ - (c̄F̄ / c̄F)

Cl̄M = Cl̄M - Cl̄MF

Cl̄F = Cl̄F - Cl̄FM

### Compartamental analyses.** Concentration-time values during and after infusion for each route of infusion in each animal were visually inspected and fit to one-, two-, or three-compartment models, as appropriate, using WinNonlin (Pharsight, Mountain View, CA). Then the data from the paired infusions were fit to user-defined models that simultaneously fit the data from the two infusions. Selection for the model with the best fit was determined by visualization, standard errors of parameter estimates, correlation coefficients, and Akaike and Schwartz criteria.

**Statistical analyses.** Model data are presented as parameter estimate ± S.E. Data for all animals are presented as mean ± S.D. The small number of animals precludes reasonable statistical comparisons, although differences between maternal and fetal concentrations, concentration ratios, and placental clearances were compared by paired t test or one-sample t test; α = 0.05 was considered significant. All statistical analyses were done using the Systat statistical package (Systat Software, Inc., Point Richmond, CA).

### Results

Data for paired maternal and fetal infusions of M3G from three pregnant animals were analyzed. The gestational ages of the animals at surgery, infusion, and delivery are provided in Table 1. Fetuses had normal blood gas values and normal heart rate patterns as estimated from prior research (Daniel et al., 1992). All fetuses survived for at least 7 days beyond the time of study.

**Steady-State Concentrations.** The concentration-time curves graphically represented in Fig. 1 suggest that the steady state for M3G is achieved in both maternal and fetal compartments by 24 h if not by...
6 h. However, the variance in maternal concentrations during the maternal infusion in two animals suggested that the rate of infusion of metabolite varied, making it difficult to define steady state numerically. For this reason, only the 48- to 52-h concentration time points were used to define steady-state concentrations.

The calculated rates of drug delivery and mean steady-state values from 48 to 52 h are provided in Table 2. During maternal infusion, fetal steady-state concentrations are similar to but slightly less than maternal concentrations ($p < 0.05$). The similarity between fetal and maternal concentrations supports transfer of the metabolite across the placenta. On the other hand, the difference, reflected in the fetal/maternal concentration ratio being less than 1 (Table 2; Fig. 2), implies either a direct clearance pathway on the fetal side of the placenta or the fact that fetal-to-maternal clearance exceeds maternal-to-fetal clearance. During fetal infusion, maternal concentrations are well below those in the fetus. The difference between fetal and maternal concentrations during fetal infusion reflects the high maternal clearance and/or low placental clearance. The stability in fetal-to-maternal ratios during both maternal and fetal infusions provides further support that the steady state was achieved relatively early (Fig. 2).

**Noncompartmental Steady-State Model.** The clearances calculated from the mean steady-state concentrations are presented in Table 3. As expected, the maternal clearance was by far the highest. With regard to the placental clearances, the fetal-to-maternal clearance was consistently higher than the maternal-to-fetal clearance ($p < 0.06$). This difference suggests the presence of active efflux of metabolite from the fetus. Overall, less than 4% of the dose of M3G administered to the mother crossed the placenta. The calculated direct fetal clearances gave values less than 0 that support no direct loss of metabolite from the fetus other than to the mother.

**Compartmental Models.** A stepwise approach was taken to develop a compartmentally based model of maternal-fetal distribution and clearance (Fig. 3). The models were used to estimate the volumes of the maternal and fetal compartments, to provide information on elimination half-life of M3G from the fetus, and to refine estimates of clearances. To address the issue of the variability in maternal infusion rate early on, a subset of the data set was analyzed from 48 to 54 h in addition to the entire data set. Parameter estimates were similar for both data sets; however, the limited data set was associated with smaller errors. Parameter estimates for stepwise modeling of the 48- to 54-h data set are presented in Table 4 and are discussed below.

Inspection of the elimination phases of the maternal and fetal data sets suggested that a one-compartment model would be sufficient to describe the system in most cases (Fig. 1). The maternal and fetal data sets were independently fit to one-, two-, and three-compartment models using a constant infusion input and clearance parameters (WinNonlin). Initial parameters were program-defined. Several weighting schemes were applied, but none improved estimations. Reported results used uniform weighting with Gauss-Newton minimization. Estimates were derived for one- and two-compartment models because there were insufficient late time points available to discern a third compartment. These models are referred to as S1CPT and S2CPT designated as maternal or fetal to reflect single infusion with one or two compartments (Fig. 3; Table 4). In general, there was reasonable fit to both one- and two-compartment models. For the maternal infusion, the maternal samples slightly favored the two-compartment model; although standard errors for the volumes of the two maternal compartments and the tissue clearance were moderately large. Although not constrained by the model, the clearance from the central to the deep component was not different from the clearance from the deep to the central compartment. This finding suggested passive exchange between these two compartments. For the fetal infusion, the one-compartment model provided the most reliable fit across animals, although the data from animal 275 were perhaps better described by two compartments.

Elimination half-lives were generated from the one- and two-compartment models for the mother and fetus to provide additional support for the time to reach steady state (Table 5). The maximum estimate for half-life from the fetus was less than 2.5 h, suggesting that no more than 12 h are needed to approximate steady state.

On the basis of these initial results with compartmental modeling, two user-defined maternal-fetal models were set up in WinNonlin that simultaneously fit the maternal and fetal concentrations during the paired maternal and fetal infusions for each pregnancy (Fig. 3). The first model was a maternal one-compartment–fetal one-compartment model (P1M) and the second model was a maternal two-compartment–fetal one-compartment model (P2M). Maternal-fetal exchange was through the central maternal compartment. Both of these models included a direct fetal clearance component. For exchange between the maternal central and deep compartments, a single clearance parameter was used to minimize the overall number of parameters required. This is in keeping with passive diffusion as suggested above. Again, after evaluation of different weighting schemes, uniform weighting and Gauss-Newton minimization were used. Initial parameters were based on the estimates from the independent one- and two-compartment models and the steady-state clearances. Various initial conditions were explored to assess the robustness of the model. For instance, the initial premise of no difference in the bidirectional clearances across the placenta and the presence of a direct fetal clearance component to account for the fetal-to-maternal difference in concentrations were used as initial conditions with minimal effect on final estimates. The model with a maternal tissue compartment provided the best fit (Fig. 4); however, error in the estimates of the size of the maternal compartments was large (Table

![Fig. 1. Steady-state M3G infusions to the mother and fetus. Individual M3G concentration-time plots in maternal (●●●) and fetal (▲▲▲) plasma for each animal during continuous infusion of M3G to the mother (left hand column) and fetus (right hand column). Animal 267, top row; animal 273, middle row; animal 275, bottom row.](image)
DISPOSITION OF M3G IN PREGNANCY

TABLE 2

Dose and steady-state concentrations of M3G

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose</th>
<th>Concentration during Maternal Infusion</th>
<th>Concentration during Fetal Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>F</td>
<td>F/M Ratio</td>
</tr>
<tr>
<td></td>
<td>mg h⁻¹</td>
<td>ng ml⁻¹</td>
<td></td>
</tr>
<tr>
<td>267</td>
<td>2.23</td>
<td>0.105</td>
<td>692 ± 34</td>
</tr>
<tr>
<td>273</td>
<td>2.02</td>
<td>0.086</td>
<td>625; 697*</td>
</tr>
<tr>
<td>275</td>
<td>2.35</td>
<td>0.112</td>
<td>813 ± 35</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>2.30 ± 0.2</td>
<td>0.11 ± 0.02</td>
<td>685 ± 22</td>
</tr>
</tbody>
</table>

M, maternal; F, fetal.
*Two values only.

4). Observed concentrations differed from predicted concentrations by 2% at steady state to 10 to 20% at very early and very late time points. Simulations were run using the parameters from animal 267 with varying amounts (2–10%) of normally distributed random error added to assess the effect of number of data points. Parameters were then estimated by applying the models to these new data sets. Increasing the data points significantly reduced error.

Models incorporating data from both infusions allow separation of direct fetal clearance from fetal placental clearance. The estimates for fetal-to-maternal placental clearance determined from the paired infusions were remarkably similar to those for overall fetal clearance determined from the single infusions (Table 4). Furthermore, the estimates for direct fetal clearance determined from the paired infusions were negligible. To further assess the validity of absence of direct fetal clearance, simulations with parameter estimates for animal 267 were rerun with the fetal-to-maternal placental clearance reduced to equal the maternal-to-fetal clearance and direct fetal clearance added to equal the amount of the reduction. With 10% error added to simulated data, limiting time points to the times of observed data, and setting initial conditions to no direct fetal clearance so that all fetal clearance was placental, the model gave estimates that were not different from those used in the simulation. This result supports the fact that the model can distinguish between direct fetal clearance and active placental transport despite the limited number of time points in the data set.

On the basis of this finding, a final model was set up that eliminated the direct fetal clearance component (P2MNFC). This model yielded a more robust estimate of placental clearances and permitted more reasonable comparison of the bidirectional placental clearances, supporting the fact that fetal-to-maternal clearance is greater than maternal-to-fetal clearance (p < 0.05). This comparison is presented as the ratio of the fetal and maternal placental clearances in Table 4. The support for the presence of an active clearance process from the fetus to the mother is strengthened by the finding that this clearance ratio is greater than 1.

Discussion

The objective of this research was to identify the major routes of elimination of glucuronide metabolites from the fetus, determine the contribution of placental transfer to the presence of the metabolite in the fetus, and quantify the contributions of placental and nonplacental pathways for metabolite clearance. Under steady-state conditions during maternal infusion, the concentrations of M3G in the fetus closely approximate those in the mother and, as such, demonstrate significant transfer of this major glucuronide metabolite of morphine across the primate placenta. The results from the paired maternal and fetal infusions support the conclusion that the small but consistent difference between fetal and maternal concentrations seen during maternal infusion are due to enhanced efflux of the glucuronide from the fetus and not to direct clearance mechanisms from the fetus. The addition of this new information enhances the ability of clinically relevant pharmacokinetic models to predict fetal exposure to drugs and their metabolites during maternal therapy.

The appearance of morphine glucuronide in fetal plasma after maternal administration provides clear evidence that glucuronide metabolites can cross the primate placenta and result in significant fetal concentrations. Fetal levels reach a plateau at concentrations that are 70 to 90% of maternal concentrations by 24 h. The fetal-to-maternal clearance calculated for M3G (1.5 ml min⁻¹) is considerably lower than clearances of the parent drug morphine and of zidovudine (56 and 30 ml min⁻¹, respectively), the two other drugs that have been evaluated in this animal model (Garland et al., 1998b, 2001). The lower clearance of the glucuronide metabolite no doubt reflects the low lipid solubility of this relatively large polar molecule. It is important to note that although the placental clearance of the glucuronide is low, the half-life in the fetus is not overly prolonged. This finding is also a reflection of low lipid solubility of the metabolite, leading to a lower volume of distribution. No values are available for volume of distribution and clearance of M3G in humans; however, morphine-6-glucuronide has been studied in adult humans. Values for clearance and volume of distribution standardized to weight (2.2 l min⁻¹ kg⁻¹ and 0.12 l kg⁻¹) are similar to those in the adult baboon (Lotsch et al., 1998).

The finding of a lower concentration of M3G in the fetus than in the

TABLE 3

Clearances calculated using steady-state model

<table>
<thead>
<tr>
<th>Animal</th>
<th>Clₘₐₜ</th>
<th>Clₙₜ</th>
<th>Clₘₕₜ</th>
<th>Clₙₕₜ</th>
<th>Clₙₚₜ</th>
<th>Clₙₚₜ</th>
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<tbody>
<tr>
<td></td>
<td>ml min⁻¹</td>
<td></td>
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<tr>
<td>267</td>
<td>55.7</td>
<td>2.08</td>
<td>1.74</td>
<td>2.55</td>
<td>54.0</td>
<td>−0.47</td>
</tr>
<tr>
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<td>2.42</td>
<td>1.86</td>
<td>2.69</td>
<td>51.2</td>
<td>−0.27</td>
</tr>
<tr>
<td>275</td>
<td>50.1</td>
<td>1.18</td>
<td>0.88</td>
<td>2.53</td>
<td>49.2</td>
<td>−1.3</td>
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<tr>
<td>Mean</td>
<td>52.9</td>
<td>1.89</td>
<td>1.49</td>
<td>2.59</td>
<td>51.5</td>
<td>−0.7</td>
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<tr>
<td>SD</td>
<td>2.9</td>
<td>0.64</td>
<td>0.53</td>
<td>0.09</td>
<td>2.4</td>
<td>0.6</td>
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</table>
mother under steady-state conditions is robust despite technical problems with the stability of the maternal infusion rate in two animals (Figs. 1 and 2). Under steady-state conditions, it is expected that the concentrations of a compound in the mother and fetus will be equal when the compound administered to the mother is passively transferred across the placenta, and there is no direct clearance from the fetus (Szeto et al., 1982a; Wang et al., 1986; Garland et al., 2005). Hence, the finding of a fetal/maternal concentration ratio less than 1 for M3G leads to the conclusion that total fetal clearance (fetal-to-maternal clearance plus direct fetal clearance) is in excess of the maternal-to-fetal clearance.

The question then becomes how much of the excess clearance is direct fetal clearance and how much is active transport from the fetus to the mother. The clearance estimates from both noncompartmental steady-state analyses and from compartmental pharmacokinetic modeling suggest that this excess can be accounted for entirely by active transport from the fetus to the mother and that there is no measurable contribution from other direct fetal clearance mechanisms.

It is possible that variance in the dose of M3G caused by initially inconsistent infusion rates could influence the estimates for both fetal-to-maternal clearance and direct fetal clearance. Examination of the steady-state equations (eqs. 1–6) indicates that maternal-to-fetal clearance is independent of the maternal dose because the fetal-to-maternal concentration ratio during maternal infusion is dose independent. The remainder of the data used to calculate maternal-to-fetal clearance is from fetal infusions as is the calculation of total fetal clearance. Fetal placental clearance, on the other hand, is directly proportional to the maternal infusion rate and thus the distribution of the total fetal clearance between placental or direct fetal clearance remains at issue.

Examination of the individual data sets allows further clarification. For animal 273 (Table 2; Fig. 1), the mean maternal infusion rate would underestimate the actual dose being received at the time of measurement of steady-state concentrations because the maternal concentrations at 48 h were higher than those obtained from 6 to 24 h. In turn, this would underestimate the fetal placental clearance. However, fetal placental clearance cannot exceed total fetal clearance. Thus, the conclusion that there is no direct fetal clearance remains likely for this animal. In contrast, for animal 275 the mean infusion rate would overestimate total fetal clearance and might obscure a direct fetal clearance component. To address the issue of variability of maternal infusion rate in this animal a secondary analysis of the data was performed. A parameter that would correct for a linear change in dose was included in the model. With this correction of infusion rate, the model estimates of fetal-to-maternal clearance remained greater than maternal-to-fetal clearance with no direct fetal clearance. This secondary exploration of the data increases confidence in the finding that there is no direct fetal clearance and that the difference in maternal and fetal concentrations is due to an active transport process from the fetus to the mother.

The finding of an absence of any direct fetal clearance of the metabolite is quite striking and was unexpected. In the human, drug metabolites are known to be present in amniotic fluid and in fetal and newborn meconium. Based on the impermeable nature of drug metabolites, it had been assumed that metabolites are excreted in fetal urine and cleared by fetal swallowing with subsequent sequestration...
in the intestinal tract until passage of meconium after birth. In the current mass balance studies, the absence of evidence for direct fetal clearance would indicate that any metabolite excreted into the amniotic fluid was swallowed and reabsorbed intact and/or directly transferred across the amnion and chorion into the maternal circulation. The implication of the present study is that there is no support for a direct fetal clearance pathway such as deconjugation of M3G to the maternal and fetal compartments. When it occurs, transfer of drug between the maternal and fetal compartments, when it occurs, is primarily at the level of the placenta (Tuntland et al., 1998). Furthermore, exchange seemed to occur in both directions and more transfer occurring at the level of the placenta (Tuntland et al., 1998).

The finding of enhanced efflux of the glucuronide from the fetus was not wholly unexpected. With consideration of the chemical structure of glucuronides, paracellular transport mechanisms, transfer through syncytial pores, or the assistance of transport carrier proteins would be required for passage through lipid membranes (Faber et al., 1992). Although the mechanisms by which glucuronide metabolites cross the placenta are unclear, it is known that transporters are abundant in the placenta, and several may have an affinity for glucuronides (St-Pierre et al., 2000; Pascolo et al., 2003; Maliepaard et al., 2001; Nagashige et al., 2003). MRP2 has affinity toward M3G, P-glycoprotein does not, and the affinity of BCRP toward other glucuronides has been shown (Xie et al., 2000; Adachi et al., 2005; Zamek-Gliszczynski et al., 2006; van de Wetering et al., 2007). Multidrug resistance 3 and MRP1 are present on the basolateral surface of syncytiotrophoblast and fetal capillary endothelium; however, their affinity toward M3G is unknown (Nagashige et al., 2003; Esseenko et al., 2006). MRP3, located predominantly in fetal capillary endothelium transports M3G (St-Pierre et al., 2000; van de Wetering et al., 2007). Less is known about transporters that allow access of M3G to the interior of the endothelial and trophoblastic cells. Organic acid transport peptides seem the most likely candidates and are known to be expressed in the placenta (Nishino et al., 1999; Nishimura and Naito, 2005). Notably, fetal clearance of M3G across the placenta is not saturable at levels expected clinically (Garland et al., 2005). From this study it is reasonable to propose that placental passage of glucuronides has at least a component of active transport superimposed on other transfer mechanisms. Active transport could also occur at the level of the amnion, chorion, and decidua, although it is unlikely to have a significant impact. For zidovudine, transfer at the level of the amnion was estimated to be less than 3% of the transfer occurring at the level of the placenta (Tuntland et al., 1998). Furthermore, exchange seemed to occur in both directions and more likely reflected passive diffusion.

It is reasonable to assume that metabolite transfer between the maternal and fetal compartments, when it occurs, is primarily at the level of the placenta. The placenta is highly specialized for transfer of substances between the mother and fetus. However, in the model “placental” clearances would include any transfer of drug between the maternal and fetal compartments. For example, drug that crosses directly from the mother to the amniotic fluid is then swallowed

| Table 4 |
| Comparison of volume of distribution and clearance estimates from compartmental pharmacokinetic models |

<table>
<thead>
<tr>
<th>Animal</th>
<th>SIcpt</th>
<th>S2cpt</th>
<th>P1m</th>
<th>P2m</th>
<th>P2mnfc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td>3100 ± 330</td>
<td>1610 ± 500</td>
<td>2980 ± 240</td>
<td>1600 ± 450</td>
<td>1600 ± 440</td>
</tr>
<tr>
<td>273</td>
<td>2660 ± 430</td>
<td>920 ± 400</td>
<td>2500 ± 260</td>
<td>900 ± 300</td>
<td>893 ± 290</td>
</tr>
<tr>
<td>275</td>
<td>3030 ± 410</td>
<td>1220 ± 270</td>
<td>2950 ± 460</td>
<td>1220 ± 780</td>
<td>1210 ± 760</td>
</tr>
<tr>
<td>Fetal</td>
<td>267</td>
<td>267 ± 13</td>
<td>266 ± 690</td>
<td>265 ± 18</td>
<td>265 ± 12</td>
</tr>
<tr>
<td>273</td>
<td>368 ± 8</td>
<td>368 ± 39</td>
<td>367 ± 20</td>
<td>367 ± 19</td>
<td></td>
</tr>
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<td>275</td>
<td>131 ± 16</td>
<td>52.0 ± 1.5</td>
<td>130 ± 11</td>
<td>131 ± 10</td>
<td>131 ± 10</td>
</tr>
</tbody>
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**Values are means ± S.E.**

| Table 5 |
| Elimination half-lives |

<table>
<thead>
<tr>
<th>Animal</th>
<th>SIcpt</th>
<th>S2cpt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal</td>
<td>0.65 ± 0.06</td>
<td>1.0 ± 0.2</td>
</tr>
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<td>273</td>
<td>0.57 ± 0.07</td>
<td>1.0 ± 0.2</td>
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<tr>
<td>275</td>
<td>0.70 ± 0.08</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>0.64 ± 0.06</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Fetal</td>
<td>1.5 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>273</td>
<td>1.8 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>275</td>
<td>1.3 ± 0.1</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>1.6 ± 0.3</td>
<td>2.0 ± 0.6</td>
</tr>
</tbody>
</table>
and absorbed by the fetus would be represented in ClFM. On the other hand, drug excreted in fetal urine and then crossing directly from amniotic fluid to the maternal circulation would be included in ClFM. Direct fetal clearances (ClFD) would reflect any secondary metabolic processes occurring in the fetus or placenta (such as β-glucuronidase activity leading to deconjugation) and any excretion of metabolite in fetal urine that fails to reappear in either the fetal or maternal circulation. The latter includes sequestration of the metabolite in the fetal intestinal tract, diffusion directly into the maternal gut or bladder, and vaginal amniotic fluid leak.

There are limited reports on the permeability of the placenta toward glucuronide metabolites, although transfer had been suspected on the basis of the levels of glucuronide metabolites found in the fetus after parent drug administration (Gerdin et al., 1990b; Garland et al., 1998b, 2001). M3G administered to pregnant rhesus monkeys was detected in fetal plasma by percutaneous fetal sampling (Gerdin et al.,

![Model fitting of paired infusion data](image-url)
DISPOSITION OF M3G IN PREGNANCY

The structural differences between the primate and sheep placenta may account for some of these observed differences in placental permeability (Schröder, 1995; Enders and Carter, 2004). The sheep has an epitheliochorial placenta with four distinct membranes intervening between the fetal and maternal circulations, whereas the maternal spiral arteries of the primate hemomonochorial placenta end directly in lacunae where maternal blood bathes the fetal syncytiotrophoblast that is almost fused with the walls of the fetal villous capillaries to form two closely opposed layers. Although each placental type evolved to minimize the diffusional distance, the permeability for hydrophilic substances in the sheep is markedly reduced for a substance with a molecular radius greater than 0.45 nm (Faber et al., 1992; Enders and Carter, 2004). The guinea pig placenta is also hemomonochorial in nature, and permeability similar to that of the primate placenta might be expected. However, it is difficult to draw direct comparisons with the data that are available. Olsen et al. (1989) did find that the permeability for M3G was in keeping with an inverse relationship with molecular weight. However, very little transfer of M3G was evident.

The overall goal of this research is to build a reliable and usable model of drug and metabolite distribution in pregnancy. The steady-state approach provides a reliable estimate of clearance from a limited number of blood samples, which is a critical factor for studies of the fetus. The use of clearance parameters is also useful as they provide a measure of physiological processes. In the present study, fetal-maternal and maternal-fetal clearances are likely measures of placental function. For the metabolite, direct maternal clearance most likely reflects maternal renal clearance. The steady-state model has been extensively used in the pregnant sheep model to examine aspects of placental transfer and fetal metabolism (Szeto et al., 1982a; Wang et al., 1986; Yeleswaram et al., 1993; Krishna et al., 2002). Primate studies are more limited and have mainly been directed toward addressing the disposition of anti-human immunodeficiency virus medications in pregnancy (Odincs et al., 1996; Garland et al., 1998b; Tuntland et al., 1998). Predictive models require delineation and acquisition of estimates for the major factors contributing to disposition. With information on a small number of model drugs, it is likely that comparative in vitro studies of fetal metabolism and placental transfer (passive and/or active) will be predictive of fetal disposition.

More complex compartmental models can be developed; however, the ability to discriminate between models becomes more difficult as the number of parameters and the estimates of error increase. Using a stepwise approach as exemplified in this study allows inclusion and exclusion of parameters at each step, keeping the number of parameters and hence error to a minimum. The ability to both infer and sample compartments of interest, in this case the mother and fetus, defines the transfer between the two compartments. Although, the term compartment is still applied to the maternal and fetal space, the transfer between these two spaces is independent of additional compartments in either the mother or fetus. As such, measurements of transfer between compartments are noncompartmental although the specific pathway may not be defined. This methodology could be extended for confirmation of the absence of direct fetal clearance by comparing concentrations in the fetus during infusions to the fetus with fetal concentrations obtained during infusions into the fetal intestinal and/or amniotic cavity.

In conclusion, the permeability of the placenta for M3G is low in keeping with the physicochemical properties of the compound. However, the rate of placental transfer is sufficient for clinically relevant concentrations of M3G to be present in the fetus. Furthermore, the permeability is sufficiently limited that when the metabolite is produced by the fetus, fetal concentrations of the metabolite are likely to exceed those in the mother. Finally, the placenta appears to be the primary route for clearance of the glucuronide metabolites from the fetus with little, if any, sequestration in deep fetal compartments. This new knowledge on the disposition of glucuronide metabolites will be applied to metabolite concentrations obtained in the mother and fetus during drug infusion for the development of pharmacokinetic models relevant to primate pregnancy.

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References


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