Diphenhydramine Disposition in the Sheep Maternal–Placental–Fetal Unit: Determinants of Plasma Drug Concentrations in the Mother and the Fetus†

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Received July 16, 1999. Final revised manuscript received September 28, 1999. Accepted for publication October 5, 1999.

Abstract ☐ The objective of this study was to identify the important factors that determine plasma concentrations of diphenhydramine (DPHM) in the mother and the fetus after maternal as well as fetal steady-state drug administration. Inter-relationships were evaluated between maternal and fetal placental and nonplacental clearances, plasma protein binding, and steady-state plasma concentrations of DPHM among data obtained from 18 pregnant sheep during late gestation. The major determinant of plasma DPHM concentrations in the mother after maternal as well as fetal administration appears to be maternal plasma protein binding and maternal nonplacental clearance. In contrast, the major determinant of fetal plasma DPHM concentrations after maternal drug administration was the extent of fetal first-pass hepatic drug uptake from the umbilical vein. However, after fetal drug administration, the fetal plasma concentrations were related to the extent of fetal plasma protein binding and fetal placental and nonplacental clearances. The index of fetal-to-maternal placental drug transfer after fetal drug administration (steady-state maternal-to-fetal plasma concentration ratio) was related to steady-state fetal plasma unbound fraction and fetal placental and nonplacental clearance. However, this index was not related to the magnitude of the factors operating on the maternal side of the placenta such as maternal plasma protein binding and maternal nonplacental clearance. This might indicate a lack of complete equilibration of the unbound drug concentrations on the two sides of the placenta at the exchange site.

Introduction

Although the kinetics of placental transfer of most drugs appear to follow the principles of simple diffusion across biological membranes, the extent and time course of fetal drug exposure are not related solely to the ease of placental drug transfer. Instead these are the result of a complex interplay between the kinetics of placental drug transfer as well as many other factors related to maternal and fetal components of the pregnant unit. These include the relative extent of maternal and fetal plasma protein binding of the drug, the efficiency of maternal and fetal drug elimination via metabolism or renal excretion, and recirculation of the drug between amniotic and allantoic fluid compartments and the fetal circulation.[1,2] The measurement of area under the fetal plasma concentration vs time curve (AUC) or steady-state concentration after maternal drug administration, although a clinically useful index of the extent of fetal drug exposure, does not provide any information about the different factors determining its magnitude. The computation of maternal and fetal placental and nonplacental clearances according to a two-compartment pharmacokinetic model after separate maternal and fetal steady-state drug administration provides a more detailed insight into various factors determining fetal drug exposure (Figure 1).[3] This pharmacokinetic modeling essentially partitions the complex array of these pharmacokinetic factors into three main categories, i.e., factors related to the placenta (maternal and fetal placental clearance), the mother (maternal nonplacental clearance), and the fetus (fetal nonplacental clearance). Thus, it is possible to separately examine the effect of various physicochemical (e.g., drug lipophilicity and pK_a, etc.), and maternal and fetal biological variables (e.g., plasma protein binding, placental blood flows, drug metabolism capacity) on these three categories of pharmacokinetic factors and the resultant effects on fetal drug exposure. This makes it feasible to determine the relative importance of each pharmacokinetic variable in determining fetal exposure to a particular drug, and to make comparisons among different drugs in terms of the most important factor(s). However, a detailed analysis of the importance of various placental, maternal, and fetal pharmacokinetic factors in determining fetal drug exposure has rarely been performed for any drug.

Diphenhydramine or 2-(diphenylmethoxy)-*N*,*N*-dimethylamine (DPHM) is a potent histamine H1-receptor antagonist. It has been widely used during human pregnancy for the treatment of nausea and vomiting, insomnia, allergic rhinitis, and common coughs and colds. Previous studies in our laboratory, using chronically instrumented pregnant sheep, demonstrated that DPHM readily crosses the ovine placenta and is eliminated from the fetus via both placental and nonplacental routes.[4] In continuation of these studies, we have utilized DPHM as a model high-clearance drug that undergoes rapid and extensive placental transfer, to examine the factors affecting different aspects of maternal–fetal drug disposition of this class of compounds. This includes the study of comparative maternal–fetal drug

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† Abbreviations: DPHM, diphenhydramine; [$^2H_{10}$]DPHM, deuterium-labeled diphenhydramine; CL_{mm}, maternal total body clearance; CL_{ff}, fetal total body clearance; CL_{mf}, maternal to fetal placental clearance; CL_{fm}, fetal to maternal placental clearance; CL_{mo}, maternal nonplacental clearance; CL_{fo}, fetal nonplacental clearance; C_m, maternal plasma steady-state DPHM concentration after maternal administration; C_f, fetal plasma steady-state DPHM concentration after maternal administration; C_m', maternal plasma steady-state DPHM (or [$^2H_{10}$]-DPHM) concentration after fetal administration; C_f', fetal plasma steady-state DPHM (or [$^2H_{10}$]DPHM) concentration after fetal administration; k_o, maternal drug infusion rate; k_o', fetal drug infusion rate; M-UF, maternal steady-state plasma unbound fraction of the drug; F-UF, fetal steady-state plasma unbound fraction of the drug; r, Pearson correlation coefficient.

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10.1021/js990244l CCC: $18.00 Published on Web 10/26/1999

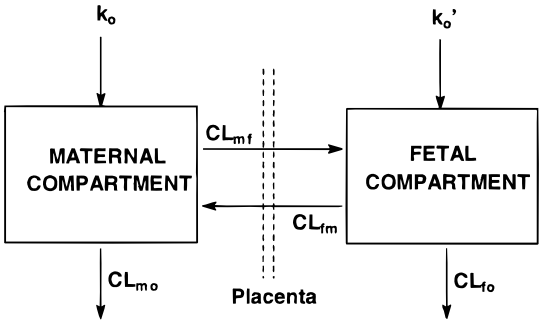

Figure 1—A representation of various placental and nonplacental drug clearances in the two-compartment pharmacokinetic model of the maternal-fetal unit. CL_{mo}: maternal nonplacental clearance; CL_{fo}: fetal nonplacental clearance; CL_{mf}: placental clearance from the mother to the fetus; CL_{fm}: placental clearance from the fetus to the mother.

clearance,[4] *in utero* fetal hepatic drug uptake and its relation to fetal drug clearance,[5] and *in utero* functional capacity of fetal drug metabolism pathways compared to the mother.[6] As part of these studies, we have determined DPHM placental and nonplacental clearances in 18 pregnant sheep during the final two weeks of their gestation. In the current study, we have retrospectively examined the inter-relationships between maternal and fetal clearances (placental and nonplacental) and plasma concentrations of DPHM among the data obtained from the above three studies in order to identify the most important factor(s) determining these concentrations after maternal as well as fetal drug administration.

Experimental Section

Animals and Surgical Preparation—A total of 18 pregnant sheep were employed in these studies. All studies were 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 employed have already been described in previous publications.[4−6] Briefly, 18 pregnant Dorset Suffolk cross-bred ewes, with a maternal body weight of 76.9 ± 12.6 kg (mean ± SD), were surgically prepared between 115 and 129 days gestation (term ~145 days). Surgery was performed aseptically under halothane (1−2%) and nitrous oxide (60%) anesthesia (balance O_2), following induction with intravenous (iv) sodium pentothal (1 g) and intubation of the ewe. Polivinyl or silicone rubber catheters (Dow Corning, Midland, MI) were implanted in both fetal femoral arteries and lateral tarsal veins and a maternal femoral artery and vein. Catheters were also implanted in the fetal carotid artery ($n = 4$), common umbilical vein ($n = 2$), fetal trachea ($n = 18$), fetal urinary bladder (via a suprapubic incision, $n = 5$), and the amniotic cavity ($n = 18$) for purposes unrelated to this manuscript. In some animals, electrodes (Cooper Corporation, Chatsworth, CA) were implanted biparietally on the dura to record the fetal electrocorticogram (ECoG). In four of the animals, a transit-time 4SB blood flow transducer (Transonic Systems, Inc., Ithaca, NY) was placed around the common umbilical artery to measure umbilical blood flow. The catheters, electrodes, and flow transducer cables were tunneled subcutaneously and exteriorized via a small incision on the flank of the ewe and were stored in a denim pouch when not in use. All catheters were flushed daily with approximately 2 mL of sterile 0.9% sodium chloride containing 12 units of heparin/mL to maintain catheter patency. Intramuscular injections of ampicillin 500 mg were given to the ewe on the day of surgery and for 3 days postoperatively. Ampicillin (500 mg) was also given via the amniotic cavity immediately following surgery and daily thereafter. Following surgery, animals were kept in holding pens with other sheep and were given free access to food and water. The sheep were allowed to recover for 4−8 days prior to experimentation.

Protocol—All experiments were conducted between 124 and 140 days gestation. A total of 31 experiments were conducted in 18 pregnant sheep. Each animal received one of the following:

(1) A 90 min separate maternal and fetal steady-state DPHM (DPHM hydrochloride, Sigma Chemical Co., St. Louis, MO) infusion with an appropriate washout period between ($n = 8$, experiments from ref 4).

(2) A 6 h separate maternal and fetal steady-state DPHM infusion with an appropriate washout period between ($n = 3$, experiments from ref 6).

(3) A 6 h separate maternal and fetal steady-state [$^2H_{10}$]DPHM (a deuterium labeled analogue of DPHM synthesized in our laboratory; Tonn et al., 1993) infusion with an appropriate washout period between ($n = 2$, experiments from ref 6).

(4) A 6 h simultaneous steady-state infusion of DPHM to the mother and [$^2H_{10}$]DPHM to the fetus ($n = 5$, experiments from ref 5).

The doses were prepared in sterile water for injection and were sterilized by filtering through a 0.22 μm nylon syringe filter (MSI, Westboro, MA) into a capped empty sterile injection vial.

Drug (DPHM or [$^2H_{10}$]DPHM) was administered to the mother in each experiment as a 20 mg iv loading dose over 1.0 min, followed immediately by an infusion at 670 μg/min via the maternal femoral vein. In fetal experiments, a 5.0 mg iv loading dose of DPHM or [$^2H_{10}$]DPHM was given via the fetal lateral tarsal vein over 1.0 min, followed by an infusion of the same compound at 170 μg/min. Simultaneous serial blood samples were collected from the fetal (1.5 mL) and maternal (3.0 mL) femoral arterial catheters. Fetal femoral arterial samples (0.5 mL) were also collected at the same time intervals for blood gas analysis and measurement of glucose and lactate concentrations. All fetal blood removed for sampling was replaced at intervals during the experiment by an equal volume of maternal blood obtained prior to the start of the experiment. Amniotic and tracheal fluid (2.0 mL) and fetal (5.0 mL) and maternal urine (10.0 mL) samples were also collected in some animals to examine the excretion of DPHM into these fluids; these data have been reported previously.[4−6]

Maternal and fetal blood samples collected for drug analysis were placed into heparinized Vacutainer tubes (Becton-Dickinson, Rutherford, NJ), gently mixed, and then centrifuged at 2000*g* for 10 min. The plasma supernatant was removed and placed into clean borosilicate test tubes with poly(tetrafluoroethylene) (PTFE)-lined caps. Amniotic fluid and urine samples were also placed into clean borosilicate test tubes. All samples were stored frozen at −20 °C until the time of analysis.

Physiological Recording and Monitoring Procedures—From at least 24 h prior to and at least 24 h after each infusion period, amniotic pressure, fetal tracheal and femoral arterial pressures, and fetal heart rate were continuously monitored. In some animals with implanted cortical electrodes and fetal bladder catheters, fetal electrocortical activity and urine flow rate were also measured. Some of these data have been reported separately.[7]

Fetal blood pH, P_{O_2}, and P_{CO_2} were measured using an IL 1306 pH/blood gas analyzer (Allied Instrumentation Laboratory, Milan, Italy). Blood O_2 saturation and hemoglobin concentration were determined using a Hemoximeter (Radiometer, Copenhagen, Denmark). Blood glucose and lactate concentrations were determined with a 2300 STAT plus glucose/lactate analyzer (Y.S.I. Inc. Yellow Springs, OH). All of these fetal blood gases and metabolite concentrations have been reported in our earlier publications and were within the normal range observed in our and other laboratories at this stage of gestation in fetal sheep.[4−6]

Protein Binding of DPHM and [$^2H_{10}$]DPHM in Fetal and Maternal Plasma—The plasma protein binding/unbound fraction of DPHM (or [$^2H_{10}$]DPHM) was measured ex vivo in pooled fetal and maternal steady-state plasma samples using an equilibrium dialysis procedure as described by Yoo et al. (1993).[4] Maternal plasma protein binding was measured in plasma samples obtained during maternal drug infusion, whereas fetal plasma protein binding was measured in plasma samples obtained during fetal drug infusion.

Drug Analysis—The concentrations of DPHM in all biological fluids collected were measured using either a gas chromatographic nitrogen phosphorus detection method[8] (studies in ref 4) or by a GC-MS assay capable of measuring both DPHM and [$^2H_{10}$]DPHM simultaneously[9] (studies in refs 5 and 6). Both these assays have been shown to be comparable to each other with a similar limit of quantitation (2.0 ng/mL).[9]

Pharmacokinetic Analysis—The maternal and fetal steady-state arterial plasma DPHM and [$^2H_{10}$]DPHM concentration data were treated according to a two-compartment open model in order

Table 1—Steady-State Maternal and Fetal Plasma Unbound Fractions and Total Plasma Concentrations of DPHM in 18 Pregnant Sheep

	steady-state unbound fraction		total steady-state DPHM plasma concentration (ng/mL)[a]			
			maternal infusion		fetal infusion	
ewe	maternal plasma	fetal plasma	C_m	C_f	C_m'	C_f'
121	0.048	0.165	360.3	35.5	53.9	658.0
125	0.110	0.173	215.8	29.6	26.5	697.9
130	0.072	0.301	185.4	20.8	25.3	274.9
133	0.087	0.222	207.8	56.0	26.4	367.0
138	0.168	0.326	197.8	49.6	39.5	509.8
202	0.157	0.364	152.9	18.3	22.4	323.4
204	0.193	0.402	236.1	29.1	36.5	192.1
480	0.293	0.263	140.3	51.2	17.9	557.9
2101	0.066	0.296	225.4	18.2	31.9	227.6
122z	0.082	0.428	266.0	27.8	41.5	137.2
2177	0.069	0.255	236.7	32.6	43.9	187.8
2181	0.091	0.191	244.1	114.2	39.6	383.6
2241	0.050	0.299	331.6	35.5	66.3	283.7
4230	0.145	0.326	224.6	124.1	37.4	250.7
4227	0.180	0.242	251.5	60.7	40.2	374.7
2174	0.032	0.262	268.1	30.5	27.8	176.1
1225A	0.106	0.527	179.1	3.5	33.9	132.5
303Y	0.211	0.377	181.1	38.9	24.5	225.6
mean	0.120	0.301	228.0	43.1	35.3	331.1
SD	0.069	0.094	56.1	31.2	11.9	172.4

[a] C_m and C_f: steady-state maternal and fetal total femoral arterial plasma DPHM concentrations, respectively, after maternal administration; C_m' and C_f', steady-state maternal and fetal total femoral arterial plasma DPHM concentrations, respectively, after fetal administration.

to estimate the placental and nonplacental clearance parameters of DPHM (or [$^2H_{10}$]DPHM when present) in the ewe and fetus (Figure 1). This model assumes steady-state plasma concentrations and drug elimination from both the maternal and fetal compartments. The equations to estimate placental and nonplacental clearance parameters have been previously described.[3] Pharmacokinetic modeling of the data, wherever necessary, was carried out using the nonlinear least-squares fitting program ADAPT II.[10]

Statistical Analysis—All values are reported as mean ± SD. All linear correlational analyses were performed by computing Pearson correlation coefficient (r). The significance level was $p < 0.05$ in all cases. 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.[11]

Results

The average maternal body weight was 76.9 ± 12.6 kg, and the estimated fetal body weights on the day of maternal and fetal DPHM (or [$^2H_{10}$]DPHM) infusion were 2.61 ± 0.61 and 2.56 ± 0.54 kg, respectively.

The mean gestational age on the day of maternal and fetal steady-state DPHM infusion experiments was 130.9 ± 4.1 (range 124−140) and 130.4 ± 3.7 (range 125−136) days, respectively, and these were not statistically different (paired t-test, $p > 0.05$). Average washout period between maternal and fetal DPHM infusion experiments was 2.4 ± 2.2 d. Table 1 presents maternal and fetal steady-state plasma unbound fractions and steady-state total plasma concentrations of DPHM in 18 pregnant sheep. Maternal and fetal clearances (total body, placental and nonplacental clearances) of the drug in these 18 sheep calculated using the two-compartment pharmacokinetic model are presented in Table 2. The average maternal plasma unbound fraction (M-UF) was significantly lower compared to the average fetal plasma unbound fraction (F-UF, unpaired t-test, $p < 0.0001$). Maternal and fetal steady-state unbound plasma drug concentrations were calculated by multiplying the appropriate total plasma concentration

Table 2—Steady-State Maternal and Fetal DPHM Clearances in 18 Pregnant Sheep during Late Gestation

	clearance[a] (mL/min/kg)					
ewe	CL_{mm}[b]	CL_{mo}[c]	CL_{mf}[c]	CL_{ff}[c]	CL_{fo}[c]	CL_{fm}[c]
121	28.2	27.8	7.4	71.7	29.4	42.3
125	43.7	43.2	18.2	126.2	65.1	61.1
130	37.9	37.2	24.8	235.4	108.8	126.6
133	37.3	35.8	53.6	203.9	101.8	102.1
138	56.3	54.9	31.8	120.2	25.6	94.6
202	49.6	48.9	40.4	356.9	150.8	206.1
204	46.3	44.6	43.0	328.6	128.4	200.2
480	56.8	55.5	78.3	201.8	100.3	101.5
2101	36.8	36.1	25.2	312.6	138.2	174.3
122z	41.5	39.4	48.1	459.9	177.1	282.8
2177	39.0	37.3	54.6	396.3	106.6	289.7
2181	43.8	40.5	97.8	209.0	75.4	133.6
2241	30.7	29.7	26.1	243.4	51.6	191.8
4230	43.7	38.2	125.1	267.4	92.1	175.3
4227	31.9	30.6	31.2	145.5	53.9	91.5
2174	24.6	23.5	44.9	317.2	187.5	129.7
1225A	45.5	45.2	7.6	452.6	115.1	337.5
303Y	57.6	55.1	52.6	285.4	133.0	152.4
mean	41.7	40.2	45.0	263.0	102.3	160.7
SD	9.6	9.4	30.3	111.8	46.4	80.7

[a] CL_{mm}, Maternal total clearance; CL_{mf}, maternal placental clearance; CL_{mo}, maternal nonplacental clearance; CL_{ff}, fetal total clearance; CL_{fm}, fetal placental clearance; CL_{fo}, fetal nonplacental clearance. [b] Per kg maternal weight. [c] Per kg estimated fetal weight at the time of fetal experiment.

with the corresponding plasma unbound fraction. The average maternal and fetal steady-state unbound plasma drug concentrations after maternal administration (C^u_m and C^u_f, respectively) were 25.1 ± 11.4 (range 8.6−45.6) and 12.0 ± 8.6 (range 1.9−40.4) ng/mL, respectively; these same unbound concentrations after fetal drug infusion (C^u_m' and C^u_f', respectively) were 3.9 ± 1.8 (range 0.9−7.2) and 89.3 ± 32.0 (range 46.1−166.2) ng/mL, respectively.

All clearances, except CL_{mm} and CL_{mo}, are normalized to the estimated fetal body weight on the day of experiment; CL_{mm} and CL_{mo} are normalized to maternal body weight. Since CL_{mo} and CL_{mf} are normalized differently, their sum does not equal CL_{mm} in Table 2. All fetal clearances were significantly higher compared to the corresponding maternal clearance parameters (unpaired t-test, $p < 0.0001$ in all cases), as reported previously.[4−6] However, the contribution of CL_{fo} to CL_{ff} (39.5 ± 10.7%) was significantly lower compared to that of CL_{mo} to CL_{mm} (96.3 ± 2.8%) (unpaired t-test, $p < 0.0001$).

Inter-Relationships between Maternal and Fetal Plasma DPHM Concentrations, Unbound Fractions and the Two-Compartment Model Clearance Estimates—DPHM concentration in maternal plasma after maternal drug infusion (C_m) exhibited a highly significant negative linear relationship with M-UF of the drug (Figure 2A). In contrast, fetal plasma concentration after maternal infusion (C_f) was not related significantly to either maternal ($r = 0.1751$, $p = 0.5$) or fetal ($r = -0.3676$, $p > 0.1$) plasma unbound fraction (data not shown). Analogous to the maternal situation, DPHM concentration in the fetal plasma after fetal drug infusion (C_f') was inversely related to F-UF (Figure 2B). Also, maternal plasma DPHM concentration after fetal drug infusion (C_m') was not related to F-UF ($r = -0.0966$, $p > 0.5$; data not shown), whereas its negative relationship with M-UF was near statistical significance ($r = 0.4612$, $p = 0.05$, data not shown). The data in Figure 3 demonstrate the relationships between maternal and fetal total body clearances (CL_{mm} and CL_{ff}, respectively) and respective steady-state plasma unbound fractions of DPHM. CL_{ff} appears to be linearly related to F-UF whereas the relationship of CL_{mm} with M-UF is closer

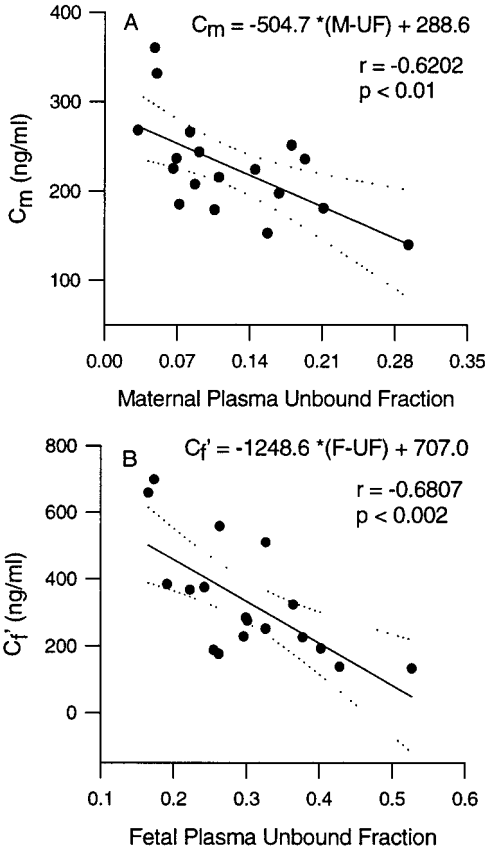

Figure 2—Relationships between (A) maternal unbound fraction and steady-state plasma concentration of the drug after maternal drug administration, and (B) fetal unbound fraction and steady-state plasma concentration of the drug after fetal drug administration. Scatter points are the experimental data in different sheep. The regression line (solid) and the 95% confidence interval (dotted) are also shown.

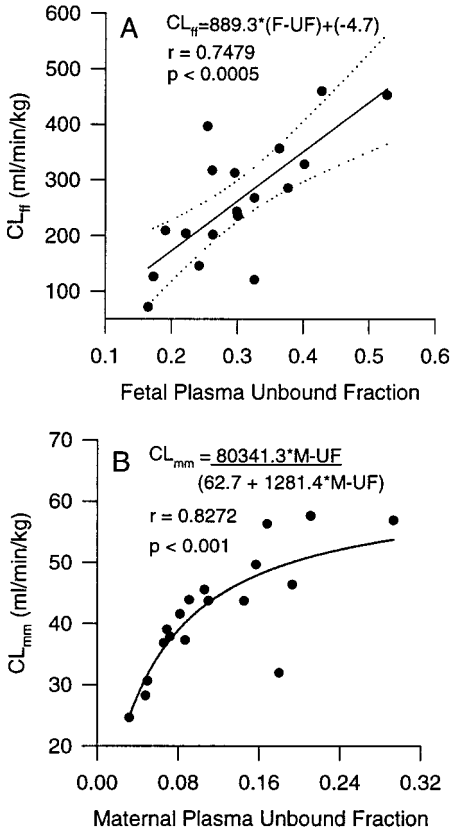

Figure 3—Relationships between fetal and maternal DPHM clearances and the corresponding plasma unbound fractions of the drug. (A) CL_{ff} vs F-UF; (B) CL_{mm} vs M-UF. The CL_{mm} vs M-UF relationship was analyzed according to the well-stirred model of organ clearance. M-UF: steady-state maternal plasma unbound fraction; F-UF: steady-state fetal plasma unbound fraction; CL_{mm}: maternal total body clearance; CL_{ff}: fetal total body clearance.

to a hyperbola. Hence, the data in Figure 3B were fitted with a well-stirred model of organ drug elimination.[12]

To determine the influence of various two-compartment clearance terms on maternal and fetal plasma DPHM concentrations after maternal or fetal drug administration, different concentration vs clearance relationships were analyzed according to the simple steady-state clearance model of the form: $CL = I_o/C_{ss}$. The majority of the interanimal variability in C_m was reflected in the estimated value of total CL_{mo} (not weight-normalized, because the total clearance is the actual determinant of plasma concentrations) as demonstrated by an excellent fit of the concentration vs clearance data to this model (Figure 4A). However, C_m was not significantly related to the other three clearance parameters of the two-compartment model (CL_{mf}, CL_{fo}, and CL_{fm}). Similarly, the majority of interanimal variability in C_f was reflected in the final estimates of CL_{mf} (Figure 4B). In contrast, when the drug was administered to the fetus, the interanimal variability in C_f' was due to relatively equal contributions from the magnitude of CL_{fo} and CL_{fm} (Figures 4C,D). Also, in contrast to the situation with C_f above (Figure 4B), the C_m' concentration was not related to the magnitude of CL_{fm} (Figure 4E). Instead the variation in C_m' among different animals was best explained by the differences in their maternal nonplacental clearance (Figure 4F).

Relationships between the Indices of Fetal Drug Exposure/Placental Transfer and Plasma Protein Binding—The average C_f/C_m ratio based on total plasma drug concentrations was 0.20 ± 0.14. The same ratio calculated using unbound drug concentrations was significantly higher (0.50 ± 0.30, paired *t*-test, $p < 0.0001$). Plasma total drug C_f/C_m ratio was not significantly correlated with maternal or fetal nonplacental clearance or F-UF; its positive relationship with M-UF was only near statistical significance ($r = 0.4029$, $p < 0.1$, data not shown). The mean C_m'/C_f' ratios during fetal drug administration based on total and unbound plasma drug concentrations were 0.14 ± 0.08 and 0.05 ± 0.02, respectively, the latter being significantly lower (paired *t*-test, $p < 0.0001$). In contrast to the C_f/C_m ratio above, the C_m'/C_f' ratio was positively correlated with total (not weight-normalized) CL_{fo} and CL_{fm} (Figures 5A,B), whereas its inverse relationship with total CL_{mo} was only near statistical significance ($r = -0.4138$, $p < 0.1$, data not shown). The C_m'/C_f' ratio also exhibited a highly significant positive relationship with F-UF (Figure 5C) but not with M-UF (data not shown).

Discussion

A number of variables such as the lipophilicity and plasma protein binding of the drug, placental blood flows (uterine and umbilical), the efficiency of maternal and fetal drug elimination/metabolism, and the gestational age of the fetus have been postulated to affect the degree of placental drug transfer and fetal drug exposure.[1,2,13] The influence of many of these variables on the kinetics of placental drug transfer and fetal drug exposure has not been extensively studied under controlled experimental conditions either in vitro or in vivo. Our objective in this study was to examine the role of different factors that determine plasma concentrations of DPHM in the mother and the fetus after maternal as well as fetal drug admin-

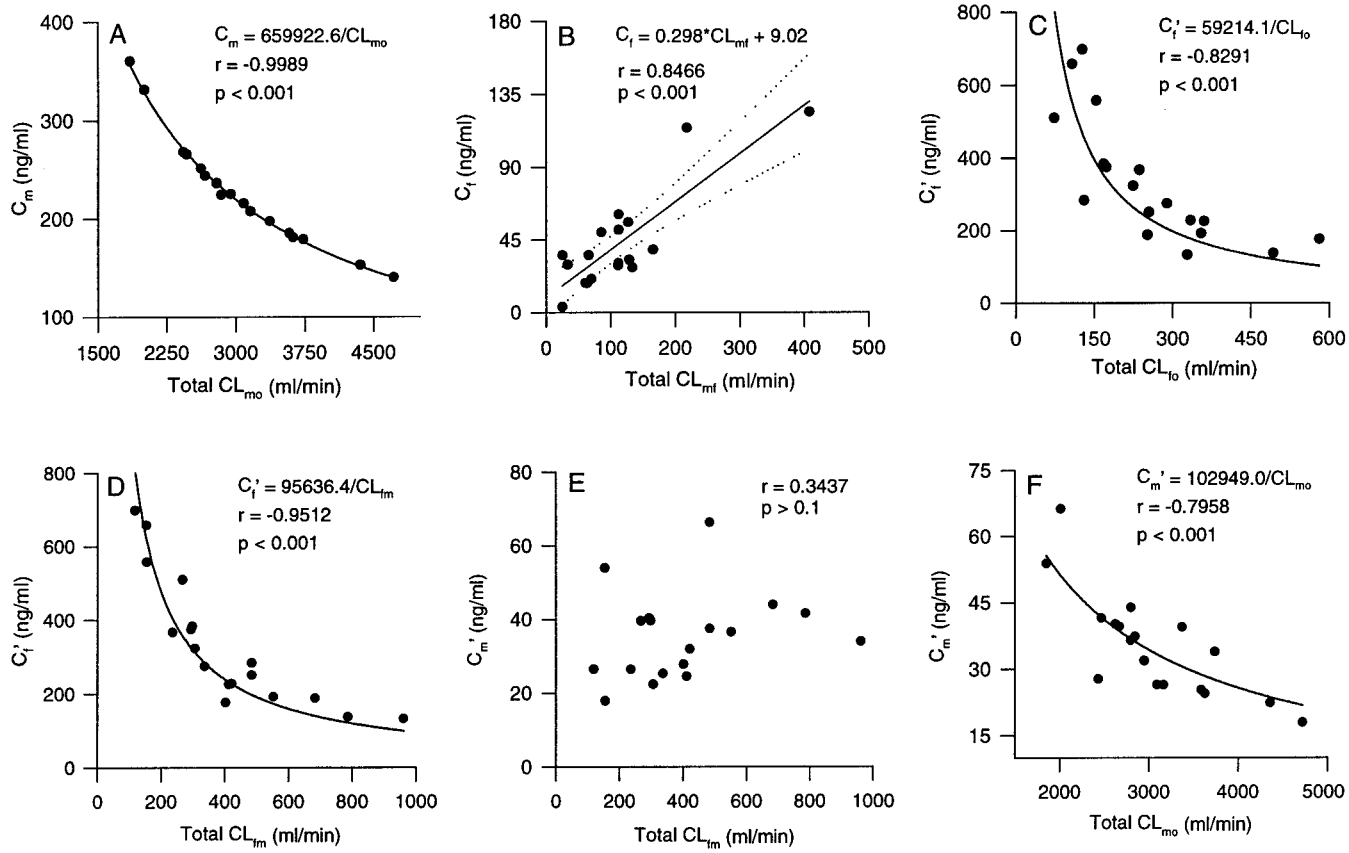

Figure 4—Influence of various clearance parameters of the two-compartment model on different maternal–fetal plasma concentrations. (A) CL_{mo} vs C_m; (B) CL_{mf} vs C_f; (C) CL_{fo} vs C_f'; (D) CL_{fm} vs C_f'; (E) CL_{fm} vs C_m'; and (F) CL_{mo} vs C_m'. All relationships except B and E were analyzed according to the steady-state clearance model, $CL = I_0/C_{ss}$; the solid lines represent the best-fit lines determined by this model. CL_{mo}: maternal nonplacental clearance; CL_{mf}: placental clearance from the mother to the fetus; CL_{fo}: fetal nonplacental clearance; CL_{fm}: placental clearance from the fetus to the mother; C_m': steady-state maternal plasma DPHM concentration after fetal infusion; C_f': steady-state fetal plasma DPHM concentration after fetal infusion.

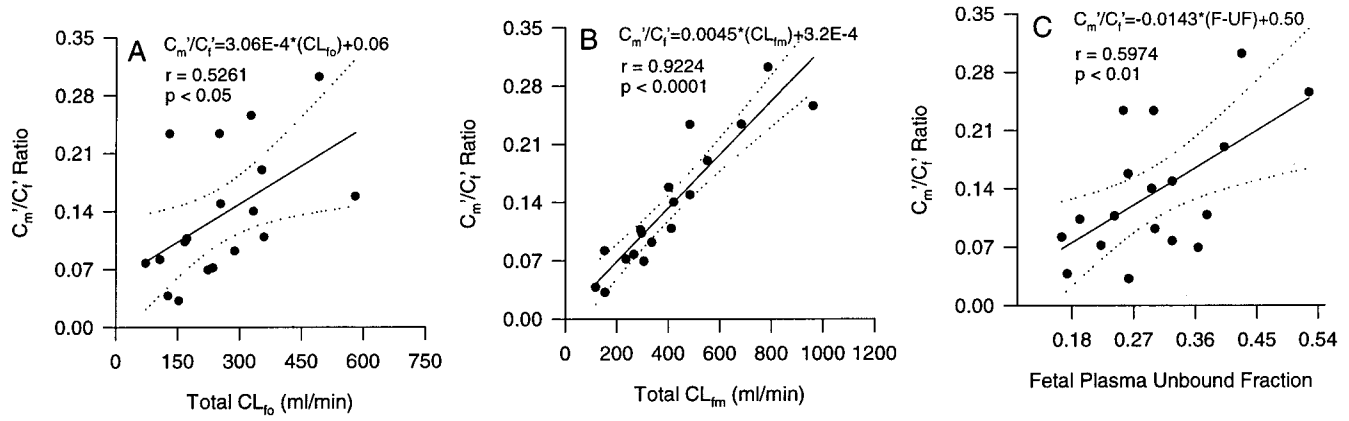

Figure 5—Relationships between the index of steady-state placental drug transfer after fetal administration (C_m'/C_f' ratio) and its determining factors. (A) C_f'/C_m' vs CL_{fo}; (B) C_f'/C_m' vs CL_{fm}; and (C) C_f'/C_m' vs F-UF. Scatter points are the experimental data in different sheep. The regression line (solid) and the 95% confidence interval (dotted) are also shown. CL_{fo}: fetal nonplacental clearance; CL_{fm}: placental clearance from the fetus to the mother; F-UF: steady-state fetal plasma unbound fraction; C_m': steady-state maternal plasma DPHM concentration after fetal infusion; C_f': steady-state fetal plasma DPHM concentration after fetal infusion.

istration in chronically instrumented pregnant sheep during late gestation. We have utilized DPHM as a model high-clearance drug that undergoes rapid and extensive placental transfer in pregnant sheep for assessing the importance of different variables The average CL_{mo} and CL_{fo} (40.2 and 102.3 mL/min/kg, respectively, Table 2) of DPHM are ~70% and 75% of the reported hepatic blood flow in pregnant sheep and late gestation fetal lambs, respectively (60 and 137 mL/min/kg, respectively).[19,20] Similarly, the average CL_{fm} of DPHM (160.7 mL/min/kg, Table 2) is ~80% of the umbilical blood flow estimates in sheep at this stage of gestation (200 mL/min/kg[21]), suggesting a high rate of DPHM placental transfer.

Inter-Relationships between Maternal and Fetal Plasma DPHM Concentrations, Unbound Fractions and the Two-Compartment Model Clearance Estimates—Although the two-compartment model is the simplest pharmacokinetic representation of the maternal–fetal unit, the exact relationships between the four clearance parameters of this model (CL_{mo}, CL_{mf}, CL_{fo}, and CL_{fm}) and various maternal–fetal plasma drug concentrations (C_m, C_f, C_f', C_m') are not directly obvious. Also, the influence of

maternal and fetal plasma drug protein binding in determining these concentrations is generally speculative and has rarely been determined experimentally. The data in Figure 2 indicate that maternal and fetal plasma protein binding is an important determinant of C_m and C_f', respectively, possibly because of its profound effects on respective clearance values (Figure 3). In contrast, C_f was not related to either M-UF or F-UF. When the drug is administered to the mother, it transfers across the placenta and enters the fetal circulation via the umbilical vein. A portion of the umbilical venous blood flow (~30−70%) passes through the fetal liver before reaching the fetal circulation. We have demonstrated earlier that after maternal dosing, a significant fraction of DPHM (~45%) transferred across the placenta and into the umbilical vein is metabolized by the fetal liver and does not reach the fetal circulation.[5] This phenomenon leads to a variable reduction in the "true" C_f and may underlie the lack of any relationship observed above.[5] Thus, for many drugs the major determinant of C_f after maternal drug administration may, in fact, be the extent of this fetal hepatic first-pass uptake/metabolism of the drug rather than maternal or fetal plasma protein binding or systemic clearance. This phenomenon also results in only a limited fetal exposure to DPHM after maternal administration (~20% compared to the mother) despite its high "near flow-limited" placental permeability. Analogous to C_f, the C_m' concentration bore no relationship with F-UF on the other side of the placenta. Instead its negative correlation with M-UF was near statistical significance, indicating that the latter could be a determinant of this concentration via its effects on maternal clearance of the drug (see below).

The estimated value of I_o from the fitting of plasma concentration vs clearance data to the $CL = I_o/C_{ss}$ relationship represents the rate of drug elimination via that clearance route. The data in Figure 4A indicate that CL_{mo} is the major determinant of C_m. This should generally be true for most high clearance drugs because the absolute magnitude of CL_{mo} will be much higher compared to any other clearance parameter (for DPHM, $CL_{mo} = 3058.5 \pm 745.5$ mL/min; $CL_{mf} = 114.5 \pm 88.6$ mL/min; $CL_{fo} = 257.5 \pm 135.0$ mL/min; $CL_{fm} = 408.5 \pm 225.1$ mL/min). The estimated value of the I_o coefficient (659.9 μg/min) relative to the total maternal drug infusion rate (670 μg/min) indicates that ~98% of the drug infused to the mother is eliminated via maternal nonplacental routes. Despite a variable underestimation of C_f, a large amount of variability in the measured C_f was carried over to the CL_{mf} parameter, indicating that this clearance parameter is also almost equally underestimated (Figure 4B). After fetal drug infusion, both CL_{fo} and CL_{fm} appear to be important determinants of C_f' (in contrast to the mother where only CL_{mo} is important, see above). The I_o coefficients of C_f' vs CL_{fo} and C_f' vs CL_{fm} relationships were 59.2 and 95.6 μg/min, respectively, which when added together approach the total fetal drug infusion rate of 170 μg/min. In contrast to the C_f vs CL_{mf} relationship (Figure 4B), the variability in C_m' was not related to the magnitude of estimated CL_{fm} (Figure 4E). This is understandable because, as discussed above, the major determinant of maternal plasma concentrations is expected to be CL_{mo}. Based on this, CL_{mo} does in fact appear to explain the variation in C_m' among different animals (Figure 4F).

Relationships between the Indices of Fetal Drug Exposure/Placental Transfer and Plasma Protein Binding—After maternal drug administration, the steady-state fetal-to-maternal arterial plasma concentration ratio (C_f/C_m) is commonly used as an index of the efficiency of placental drug transfer and fetal exposure to the drug.[1] Different total vs unbound C_f/C_m ratios indicate that the magnitude of total drug C_f/C_m ratio is partly determined by the differences in maternal and fetal plasma protein binding. It has been postulated that fetal plasma protein binding and total fetal clearance are important factors determining the steady-state C_f/C_m ratio and $C_f/C_m = CL_{mf}/[CL_{fm} + CL_{fo}]$.[1−3] However, in our experiments, the total drug C_f/C_m ratio neither exhibited any significant relationship with CL_{fm}, CL_{fo} or CL_{ff} ($CL_{fm} + CL_{fo}$), nor with F-UF. The positive relationship of C_f/C_m with M-UF approached statistical significance despite the errors in C_f, and this was mainly because of a highly significant negative correlation between C_m and M-UF (Figure 2A). The lack of expected relationships among the above variables may also be related to the errors in the measurement of "true C_f" due to fetal first-pass hepatic uptake of the drug present in the umbilical venous blood.[5]

To overcome this problem, we evaluated the factors affecting the analogous index of placental transfer after fetal drug administration, i.e., the C_m'/C_f' ratio. On similar lines to the C_f/C_m ratio above, it can be hypothesized that CL_{fm}, CL_{mo}, and CL_{mf} will be the important factors determining the C_m'/C_f' ratio, i.e., $C_m'/C_f' = CL_{fm}/(CL_{mo} + CL_{mf})$. However, the C_m'/C_f' ratio did not show any relationship with CL_{mf} (again this could be due to errors in CL_{mf} estimates), and its inverse relationship with CL_{mo} (as well as CL_{mm}) was only near statistical significance. Thus, CL_{mo} (and CL_{mm}) does not appear to be an important determinant of the C_m'/C_f' ratio. Total CL_{fo}, total CL_{fm} and F-UF are the important variables determining C_f' (Figures 4C,D and 2B). An increase in any of these variables leads to a fall in C_f' (Figures 4C,D and 2B) and hence to a significant increase in the C_m'/C_f' ratio (Figures 5A−C), C_m' being unaffected by any of these factors. In contrast, the C_m'/C_f' ratio was not significantly related to M-UF. Thus, in this situation, CL_{fo}, CL_{fm}, and F-UF appear to be the most important factors determining the C_m'/C_f' ratio, mainly via their effects on C_f'.

The two-compartment model of the maternal−fetal unit involves both maternal and fetal drug elimination (Figure 1). This system never reaches a state of "true" equilibrium (no net transfer of drug across the placenta) after maternal or fetal drug administration. It, however, does reach a steady-state where the rate of placental drug transfer becomes equal to the rate of drug elimination from the other side. For example, after maternal dosing, fetal drug elimination creates and maintains a maternal-to-fetal gradient of (unbound) drug concentrations, thus leading to a continuous passage of the drug from the mother to the fetus at steady-state. Similarly, during fetal dosing, maternal drug elimination creates a fetal-to-maternal (unbound) drug concentration gradient and leads to continuous passage of the drug from the fetus to the mother. The driving force for placental transfer is this difference in unbound drug concentrations across the placenta. For compounds such as DPHM that are rapidly diffusible across the placenta and whose placental transfer is not limited by low placental permeability, the maternal and fetal unbound concentrations at the site of placental exchange should fully equilibrate with each other and the rate of placental drug transfer should be directly related to the magnitude of unbound concentration gradient and variables affecting this gradient. However, from a number of observations, it appears that at least for fetal-to-maternal placental drug transfer, the factors operating on the opposite side of the placenta have minimal, if any, effect on the kinetics of DPHM placental transport. These observations include (i) the C_m'/C_f' ratio is not affected by CL_{mo} and M-UF, and (ii) the C_m' concentration is not at all influenced by F-UF or CL_{fm}. This is in contrast to the above "unbound drug equilibrium" hypothesis of placental trans-

port where these variables are considered the predominant factors affecting passage of the drug across the placenta. Because of errors in the measurement of C_f, it is not entirely clear if the same phenomenon occurs in the maternal-to-fetal direction of DPHM placental transfer. The presence of this phenomenon in at least the fetal-to-maternal direction may indicate that the unbound concentrations of the drug on both sides of the placenta may not be in complete equilibrium with each other at the site of placental exchange, as is generally assumed. The steady-state concentrations of a number of highly diffusible markers, which are not plasma protein bound and have blood flow limited clearance (e.g., antipyrine, ethanol, D_2O), do not equilibrate completely between the maternal and fetal placental outflow vessels (uterine and umbilical veins, respectively) in the sheep and cow.[14−16] This has generally been attributed to the inefficiencies that exist within the placental vasculature such as partial shunting of the uterine and umbilical blood flows to nonexchange areas of the placenta and to nonplacental tissues, and unequal maternal−fetal perfusion in different regions of the placenta.[15−17] However, the fact that DPHM placental transport is tightly coupled to many variables operating only on one side of the placenta and to none on the other strongly suggests that the assumption of a complete equilibrium between the unbound drug concentrations on the two sides of placenta may not be entirely accurate. The possibility of this phenomenon can be realized by considering the anatomical structure of the epitheliochorial sheep placenta, which has a number of tissue layers separating maternal and fetal blood flows. Also, the available evidence on the geometrical arrangement of maternal and fetal placental blood flows at the placental exchange site suggests a relatively less efficient concurrent (sheep and cow) and pool flow (human) arrangement in many species.[15−17] These factors along with a rapid transit time of the blood through the placental circulation may lead to incomplete equilibration of the unbound drug concentrations in maternal and fetal blood at the placental exchange site even for compounds with very high placental permeability.[18] It remains to be determined if a similar phenomenon exists during drug passage through the hemochorial human placenta which has fewer anatomical tissue layers compared to sheep.

In summary, the major determinant of plasma DPHM concentrations in the mother after maternal as well as fetal administration is maternal plasma protein binding and maternal nonplacental clearance. In contrast, the major determinant of fetal plasma DPHM concentrations after maternal drug administration appears to be the extent of fetal first-pass hepatic drug uptake from the umbilical vein. After fetal drug administration, the fetal plasma concentrations are related to the extent of fetal plasma protein binding and fetal placental and nonplacental clearances. The index of fetal-to-maternal placental drug transfer after fetal administration (steady-state C_m'/C_f' ratio) is related to steady-state fetal plasma unbound fraction and fetal placental and nonplacental clearance. However, this index was not related to the magnitude of the factors operating on the maternal side of the placenta such as maternal plasma protein binding and maternal nonplacental clearance. This might indicate a lack of complete equilibration of the unbound drug concentrations on the two sides of the placenta at the exchange site.

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Acknowledgments

These studies were supported by funding from the Medical Research Council of Canada. S.K. was the supported by a University of British Columbia Graduate Fellowship. D.W.R. is the recipient of an investigatorship award from the British Columbia Children's Hospital Foundation.

JS990244L