Area/Moment and Compartmental Modeling of Pharmacokinetics During Pregnancy: Applications to Maternal/Fetal Exposures to Corticosteroids in Sheep and Rats

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Purpose. The pharmacokinetics of corticosteroids in pregnancy were analyzed to assess maternal/fetal disposition and factors controlling fetal exposure. Area/Moment equations and compartmental models for estimating pharmacokinetic parameters from single dose data during pregnancy were developed.

Methods. Betamethasone in the maternal/fetal circulations of sheep was measured by HPLC after maternal intramuscular injection (n = 4) of 170 μg kg^{-1} of a depot formulation. Additional data for betamethasone in sheep and dexamethasone pharmacokinetics in rats were obtained from the literature. Area/Moment equations were derived using mass balance concepts, statistical moments, and Laplace theory. Area/Moment analysis, compartmental modeling, and allometric scaling to man for betamethasone were performed using WinNonlin and ADAPT II programs.

Results. Polyexponential maternal/fetal profiles for corticosteroids were observed. Clearance terms for corticosteroid transfer from fetus to mother were 4-fold higher than the clearance term for transfer in the opposite direction. A placental efflux process may restrict fetal access of corticosteroids which are known PGP substrates. The elimination clearance estimates indicate that fetal metabolism plays a minor role in corticosteroid elimination.

Conclusions. Generalized and specific models for maternal/fetal pharmacokinetics were developed. An efflux transport mechanism, such as the known placental expression of PGP, could explain the limited fetal exposure of corticosteroids.

KEY WORDS: corticosteroid; fetus; p-glycoprotein; pharmacokinetics; pregnancy.

INTRODUCTION

Most papers published on the pharmacokinetics of drugs in pregnancy report clearance and volume parameters calculated using either traditional mammillary compartmental or non-compartmental methods (1–3). Other pharmacokinetic papers provide descriptive parameters such as area under the curve, half-life, terminal rate constants, concentration maximum (Cmax), time to reach Cmax, and mean residence time (4–5). More thorough studies calculate fetal and maternal elimination clearances and placental clearances using steady-state equations after maternal and fetal drug administration (6–7). Maternal/fetal drug distribution also has been described in the literature by means of physiologically based pharmacokinetic modeling, which is a very thorough and laborious method of quantifying drug disposition (8–9).

Comprehensive compartmental pharmacokinetic analysis involving models with maternal/fetal distribution, exchange, and elimination of drugs for evaluating data generated after single dose administration of drugs during pregnancy are performed very rarely (10). Complete pharmacokinetic characterization by these models in pregnancy requires the simultaneous analysis of paired maternal/fetal data obtained after both maternal and fetal drug administration. One objective of this communication is to describe area/moment and compartmental methods for obtaining all the essential pharmacokinetic parameters in these models.

Comprehensive pharmacokinetic characterization of drugs in pregnancy requires large animal models such as pregnant sheep where dosing and sampling can occur from both the mother and fetus. This experimental design is absolutely necessary to capture the role of fetal distribution and elimination in the overall pharmacokinetics of the drug. Rodent models are also popular in evaluating drug PK in pregnancy. Rodents offer the distinct advantage that their fetuses lack drug metabolizing capacity during most of their gestational life (11–14). Rodent fetuses are also unique in that due to their small size the amount of drug in the fetal body and the concentration in the plasma can be easily determined. The inability of the rodent fetus to eliminate drug and the analytical ability to determine both amount and concentration of the drug in the fetus allows calculation of maternal/fetal disposition parameters without the need for fetal dosing. The utility of this approach will also be demonstrated in this report.

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ABBREVIATIONS: $\_\_^{m}$, $\_\_^{f}$, site of drug administration, maternal (m) or fetal (f); $\__{m}$, $\__{f}$, $\__{Tm}$, $\__{Tf}$, site where drug is present, maternal (m) or fetal (f) or maternal tissue (Tm) or fetal tissue (Tf); $Dose^{m}$, maternal dose; $Dose^{f}$, fetal dose; C, plasma or serum concentration; Cmax, concentration maximum; A, drug amount; A_f^m (3), amount of drug in fetal body at 3 hours after maternal drug administration; AUC, area under the curve; AUMC, area under the moment curve; __/F, apparent pharmacokinetic parameters; CL_m, maternal elimination clearance; CL_f, fetal elimination clearance; CL_{mf}, maternal to fetal drug transfer clearance; CL_{fm}, fetal to maternal drug transfer clearance; CL_{dm}, maternal tissue distribution clearance; CL_{df}, fetal tissue distribution clearance; V_m, maternal central compartment volume; V_{Tm}, maternal tissue compartment distribution volume; V_f, fetal central compartment volume; V_{Tf}, fetal tissue compartment distribution volume; s, Laplace operator; $\overline{C}$, concentration in the Laplace domain; V_{ss}, steady-state volume of distribution; $V_{ss,app}$, apparent steady-state volume of distribution; $Vm_{ss,app}$, maternal apparent steady-state volume of distribution; $Vf_{ss,app}$, fetal apparent steady-state volume of distribution; $Vm_{ss,true}$, true maternal steady-state volume of distribution; $Vf_{ss,true}$, true fetal steady-state volume of distribution; V_{total}, total distribution space of the drug; Ci, intercept coefficients obtained by fitting a polyexponential function; λi, slope parameters obtained by fitting a polyexponential function; ka, absorption rate constant after intramuscular drug administration; F, bioavailability after intramuscular administration; BW, body weight; $\__{Human}$, allometrically scaled human pharmacokinetic parameters; $\__{Sheep}$, sheep pharmacokinetic parameters; BET, betamethasone; DEX, dexamethasone; GA, gestational age.

The synthetic corticosteroids, betamethasone and dexamethasone, will be used as model drugs for demonstrating the usefulness of these newly developed mathematical approaches for pharmacokinetic analysis in pregnancy. Corticosteroids are administered maternally to induce fetal lung maturation in women at risk of preterm delivery. This exogenous administration of steroids has been shown to reduce the incidence of neonatal respiratory distress syndrome and is considered medical intervention that improves health care and produces considerable cost saving (15). Data available for these steroids in the literature from pregnant rat and sheep will be analyzed using theoretical concepts presented in this work, with allometric extrapolation to man.

Corticosteroid pharmacokinetic profiles after maternal drug administration in humans (16,17), sheep (5,18), and rats (19) show total fetal to maternal concentration ratios of less than 1 ($\leq$0.45). Minor differences in the maternal and fetal plasma protein binding of these steroids in human (16,20,21), sheep (22) and rat (19) plasma do not explain the maternal/fetal concentration gradient. This indicates that some disposition process restricts the fetal access of exogenous corticosteroids in these species. Metabolic clearance of corticosteroids from the fetal/placental unit is the most common explanation for the low fetal exposure of corticosteroids after maternal drug administration (16,21). Since the rat fetus lacks metabolic capacity and still displays a maternal/fetal gradient for dexamethasone, it is unlikely that fetal metabolism occurs. *In vitro* metabolism studies of synthetic corticosteroids with human and rat placenta have shown that fluorinated corticosteroids such as dexamethasone and betamethasone exhibit minimal placental metabolism (19,23). Greater metabolism of fluorinated corticosteroids has been reported using the perfused human placenta preparation (24). However, the results from the perfused placenta experiments are difficult to rely upon because these experiments were performed with low albumin concentrations in the perfusion medium, which does not resemble the *in vivo* situation. Thus the disposition processes controlling fetal corticosteroid exposure are poorly understood. Comprehensive meta-analysis of the pharmacokinetic behavior of corticosteroids in pregnancy from several studies and species will be presented in this paper with focus on modeling methods and the processes that lead to restricted fetal access of corticosteroids.

THEORY

The derivation of the area/moment equations is based on the model presented in Fig. 1, which consists of maternal and fetal central compartments and one distribution compartment for both the mother and fetus. It can be shown that these equations hold for any linear system with n distribution compartments linked directly to the central compartments when elimination occurs only from the central maternal and fetal compartments. Since these equations are valid for models with this basic structure, these equations are general for an important pharmacokinetic system. These equations have been derived using mass balance concepts, Laplace theory, and principles of statistical moments (25–26).

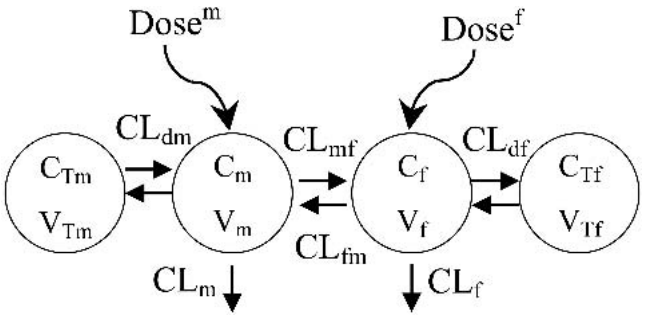

Fig. 1. The maternal/fetal pharmacokinetic model for deriving the area/moment equations.

Mass balance consideration for the model presented in Fig. 1 will be used for deriving the four fundamental clearances. For this model the dose administered into any compartment can leave the system only through clearance processes designed CL_m (maternal elimination) and CL_f (fetal elimination). Furthermore, the amount of drug administered to the mother or fetus must also equal the total loss of drug from the dosed compartment less the amount of drug that returns back from the corresponding maternal or fetal compartment. The following four equations can be written for maternal ($Dose^m$) and fetal ($Dose^f$) doses, by utilizing these restrictions and realizing that clearance times area under the curve (AUC) represents the amount of drug handled by a particular process from time zero to infinity:

$$Dose^m = CL_m \cdot AUC_m^m + CL_f \cdot AUC_f^m \quad (1)$$

$$Dose^f = CL_m \cdot AUC_m^f + CL_f \cdot AUC_f^f \quad (2)$$

$$Dose^m = (CL_m + CL_{mf}) \cdot AUC_m^m - CL_{fm} \cdot AUC_f^m \quad (3)$$

$$Dose^f = (CL_f + CL_{fm}) \cdot AUC_f^f - CL_{mf} \cdot AUC_m^f \quad (4)$$

where m and f refer to the mother and fetus, with the superscript denoting the site of drug administration and the subscript representing the site where the drug is present, and CL_{mf} and CL_{fm} represent maternal to fetal and fetal to maternal transfer clearances.

Solving Eqs. 1 and 2 jointly yields the maternal and fetal clearance terms:

$$Cl_m = \frac{Dose^m \cdot AUC_f^f - Dose^f \cdot AUC_f^m}{AUC_m^m \cdot AUC_f^f - AUC_f^m \cdot AUC_m^f} \quad (5)$$

$$Cl_f = \frac{Dose^f \cdot AUC_m^m - Dose^m \cdot AUC_m^f}{AUC_m^m \cdot AUC_f^f - AUC_f^m \cdot AUC_m^f} \quad (6)$$

Substituting Eqs. 5 and 6 in Eqs. 3 and 4 gives solutions for CL_{mf} and CL_{fm}:

$$Cl_{mf} = \frac{Dose^f \cdot AUC_f^m}{AUC_m^m \cdot AUC_f^f - AUC_f^m \cdot AUC_m^f} \quad (7)$$

$$Cl_{fm} = \frac{Dose^m \cdot AUC_m^f}{AUC_m^m \cdot AUC_f^f - AUC_f^m \cdot AUC_m^f} \quad (8)$$

These clearance equations are identical to the formulas used for calculating these parameters from steady-state data (7) except that infusion rate would replace dose and steady-state concentration would replace AUC.

The differential equations and initial conditions after ma-

ternal drug administration for the model presented in Fig. 1 are as follows:

$$V_m \frac{dC_m^m}{dt} = -(CL_m + CL_{mf} + CL_{dm}) \cdot C_m^m + CL_{dm} \cdot C_{Tm}^m + CL_{fm} \cdot C_f^m, \quad C_m^m(0) = Dose^m/V_m \tag{9a, b}$$

$$V_{Tm} \frac{dC_{Tm}^m}{dt} = CL_{dm} \cdot C_m^m - CL_{dm} \cdot C_{Tm}^m, \quad C_{Tm}^m(0) = 0 \tag{10a, b}$$

$$V_f \frac{dC_f^m}{dt} = -(CL_f + CL_{fm} + CL_{df}) \cdot C_f^m + CL_{df} \cdot C_{Tf}^m + CL_{mf} \cdot C_m^m, \quad C_f^m(0) = 0 \tag{11a, b}$$

$$V_{Tf} \frac{dC_{Tf}^m}{dt} = CL_{df} \cdot C_f^m - CL_{df} \cdot C_{Tf}^m, \qquad C_{Tf}^m(0) = 0 \tag{12a, b}$$

where subscripts Tm and Tf refer to maternal and fetal tissue compartments, V stands for volume of distribution, C refers to concentrations including the initial concentration (C(0)), and CL_{dm} and CL_{df} refer to maternal and fetal tissue distribution clearances.

The Laplace transforms for Eqs. 9 to 12 are:

$$(s + CL_m/V_m + CL_{mf}/V_m + CL_{dm}/V_m) \cdot \overline{C}_m^m - (CL_{dm}/V_m) \cdot \overline{C}_{Tm}^m - (CL_{fm}/V_m) \cdot \overline{C}_f^m = Dose^m/V_m \tag{13}$$

$$\overline{C}_{Tm}^m = \frac{(CL_{dm}/V_{Tm}) \cdot \overline{C}_m^m}{(s + CL_{dm}/V_{Tm})} \tag{14}$$

$$(s + CL_f/V_f + CL_{fm}/V_f + CL_{df}/V_f) \cdot \overline{C}_f^m - (CL_{df}/V_f) \cdot \overline{C}_{Tf}^m = (CL_{mf}/V_f) \cdot \overline{C}_m^m \tag{15}$$

$$\overline{C}_{Tf}^m = \frac{(CL_{df}/V_{Tf}) \cdot \overline{C}_f^m}{(s + CL_{df}/V_{Tf})} \tag{16}$$

Substituting Eq. 16 in Eq. 15 and solving for $\overline{C}_f^m$ gives:

$$\overline{C}_f^m = \frac{(CL_{mf}/V_f) \cdot \overline{C}_m^m}{s + \frac{CL_f}{V_f} + \frac{CL_{fm}}{V_f} + \frac{CL_{df}}{V_f} - \frac{(CL_{df}/V_f)(CL_{df}/V_{Tf})}{(s + CL_{df}/V_{Tf})}} \tag{17}$$

Substituting Eqs. 17 and 14 in Eq. 13 and simplifying gives the Laplace transform of the maternal plasma concentration after maternal dosing:

$$\overline{C}_m^m = \frac{Dose^m/V_m}{\left[s + \frac{CL_m}{V_m} + \frac{CL_{mf}}{V_m} + \frac{CL_{dm}}{V_m} - \frac{(CL_{dm}/V_m)(CL_{dm}/V_{Tm})}{(s + CL_{dm}/V_{Tm})} - \frac{(CL_{fm}/V_m)(CL_{mf}/V_f)}{s + \frac{CL_f}{V_f} + \frac{CL_{fm}}{V_f} + \frac{CL_{df}}{V_f} - \frac{(CL_{df}/V_f)(CL_{df}/V_{Tf})}{(s + CL_{df}/V_{Tf})}} \right]} \tag{18}$$

Similar derivations for dosing and measurement of drug in the fetus yield:

$$\overline{C}_f^f = \frac{Dose^f/V_f}{\left[s + \frac{CL_f}{V_f} + \frac{CL_{fm}}{V_f} + \frac{CL_{df}}{V_f} - \frac{(CL_{df}/V_f)(CL_{df}/V_{Tf})}{(s + CL_{df}/V_{Tf}} - \frac{(CL_{mf}/V_f)(CL_{fm}/V_m)}{s + \frac{CL_m}{V_m} + \frac{CL_{mf}}{V_m} + \frac{CL_{dm}}{V_m} - \frac{(CL_{dm}/V_m)(CL_{dm}/V_{Tm})}{(s + CL_{dm}/V_{Tm})}} \right]} \tag{19}$$

Equations 18 and 19 can be used for deriving AUC and area under the moment curve (AUMC) relationships using the special properties of Laplace transforms, that is,

$$AUC = \lim_{s \to 0} \overline{C} \text{ and } AUMC = -1 \cdot \lim_{s \to 0} (d\overline{C}/ds):$$

$$AUC_m^m = \frac{Dose^m}{CL_m + CL_{mf} - \frac{CL_{fm} \cdot CL_{mf}}{CL_f + CL_{fm}}} \tag{20}$$

$$AUMC_m^m = \frac{Dose^m \left[V_m + V_{Tm} + \frac{CL_{fm} \cdot CL_{mf}}{(CL_f + CL_{fm})^2} \cdot (V_f + V_{Tf}) \right]}{\left(CL_m + CL_{mf} - \frac{CL_{fm} \cdot CL_{mf}}{CL_f + CL_{fm}} \right)^2} \tag{21}$$

$$AUC_f^f = \frac{Dose^f}{CL_f + CL_{fm} - \frac{CL_{mf} \cdot CL_{fm}}{CL_m + CL_{mf}}} \tag{22}$$

$$AUMC_f^f = \frac{Dose^f \left[V_f + V_{Tf} + \frac{CL_{fm} \cdot CL_{mf}}{(CL_m + CL_{mf})^2} \cdot (V_m + V_{Tm}) \right]}{\left(CL_f + CL_{fm} - \frac{CL_{fm} \cdot CL_{mf}}{CL_m + CL_{mf}} \right)^2} \tag{23}$$

The most widely used equation for calculating the steady-state volume of distribution (V_{ss}) is given by $Dose \cdot AUMC/AUC^2$. However, it has been pointed out (27) that this equation for V_{ss} is valid only if the system exhibits a mammillary pharmacokinetic structure with clearance only from the central compartment. The models being described here for pregnancy have elimination occurring from both the maternal and fetal compartments. The term $Dose \cdot AUMC/AUC^2$ can be described here as apparent V_{ss} ($V_{ss,app}$) terms:

$$Vm_{ss,app} = \frac{Dose^m \cdot AUMC_m^m}{AUC_m^{m\,2}} = V_m + V_{Tm} + \frac{CL_{fm} \cdot CL_{mf}}{(CL_f + CL_{fm})^2} \cdot (V_f + V_{Tf}) \tag{24}$$

$$Vf_{ss,app} = \frac{Dose^f \cdot AUMC_f^f}{AUC_f^{f\,2}} = V_f + V_{Tf} + \frac{CL_{fm} \cdot CL_{mf}}{(CL_m + CL_{mf})^2} \cdot (V_m + V_{Tm}) \tag{25}$$

In a mammillary pharmacokinetic system V_{ss} is the sum of the volumes of all equilibrating compartments in that system. Thus, by analogy in a pharmacokinetic model of pregnancy the true maternal V_{ss} ($Vm_{ss,true}$) is represented by the sum of the volumes of all the maternal central and peripheral compartments and the true fetal V_{ss} ($Vf_{ss,true}$) is represented

by the sum of the volumes of all the fetal central and peripheral compartments. Using these terms, Eqs. 24 and 25 can be simply written as:

$$\frac{\text{Dose}^m \cdot \text{AUMC}_m^m}{\text{AUC}_m^{m^2}} = \text{Vm}_{ss,true} + \frac{\text{CL}_{fm} \cdot \text{CL}_{mf}}{(\text{CL}_f + \text{CL}_{fm})^2} \cdot \text{Vf}_{ss,true} \tag{26}$$

$$\frac{\text{Dose}^f \cdot \text{AUMC}_f^f}{\text{AUC}_f^{f^2}} = \text{Vf}_{ss,true} + \frac{\text{CL}_{fm} \cdot \text{CL}_{mf}}{(\text{CL}_m + \text{CL}_{mf})^2} \cdot \text{Vm}_{ss,true} \tag{27}$$

Equations 26 and 27 indicate that if the traditional mammillary model equations are used for calculating V_{ss} in pregnancy, then the estimate of V_{ss} will be erroneous because: i) The maternal V_{ss} will be contaminated by fetal volume terms and *vice versa*, ii) Maternal/fetal transfer clearances will contribute toward both maternal and fetal V_{ss}, iii) Fetal elimination clearance will influence maternal V_{ss} and *vice versa*.

Substituting Eqs. 5 through 8 in Eqs. 26 and 27 and simplifying gives equations for the true maternal and fetal V_{ss}.

$$\text{Vm}_{ss,true} = \text{Dose}^m \left\{ \frac{\text{AUMC}_m^m \cdot \text{AUC}_f^{f^2} - \text{AUMC}_f^f \cdot \text{AUC}_f^m \cdot \text{AUC}_m^f}{\text{AUC}_f^{f^2} \cdot \text{AUC}_m^{m^2} - \text{AUC}_m^{f^2} \cdot \text{AUC}_f^{m^2}} \right\} \tag{28}$$

$$\text{Vf}_{ss,true} = \text{Dose}^f \left\{ \frac{\text{AUMC}_f^f \cdot \text{AUC}_m^{m^2} - \text{AUMC}_m^m \cdot \text{AUC}_f^m \cdot \text{AUC}_m^f}{\text{AUC}_f^{f^2} \cdot \text{AUC}_m^{m^2} - \text{AUC}_m^{f^2} \cdot \text{AUC}_f^{m^2}} \right\} \tag{29}$$

Total distribution space of the drug will be given by:

$$\text{V}_{total} = \text{Vm}_{ss,true} + \text{Vf}_{ss,true} \tag{30}$$

The central and tissue compartment volume of distribution terms are given by Eqs. 31 to 34 for mother and fetus. It should be noted that when more than one peripheral compartment exists on either the maternal or fetal side, then the terms V_{Tm} and V_{Tf} represent the sum of all corresponding peripheral compartmental volumes.

$$\text{V}_m = \text{Dose}^m / \text{C}_m^m(0) \tag{31}$$

$$\text{V}_f = \text{Dose}^f / \text{C}_f^f(0) \tag{32}$$

$$\text{V}_{Tm} = \text{Vm}_{ss,true} - \text{V}_m \tag{33}$$

$$\text{V}_{Tf} = \text{Vf}_{ss,true} - \text{V}_f \tag{34}$$

Distribution clearance terms can be calculated as (28):

$$\text{CL}_{dm} = \text{Dose}^m \frac{\sum_{i=1}^{n} \text{Ci}_m^m \cdot \lambda \text{i}_m^m}{\left(\sum_{i=1}^{n} \text{Ci}_m^m \right)^2} - \text{CL}_m - \text{CL}_{mf} \tag{35}$$

$$\text{CL}_{df} = \text{Dose}^f \frac{\sum_{i=1}^{n} \text{Ci}_f^f \cdot \lambda \text{i}_f^f}{\left(\sum_{i=1}^{n} \text{Ci}_f^f \right)^2} - \text{CL}_f - \text{CL}_{fm} \tag{36}$$

where Ci represents intercept coefficients and λi represents slope parameters that can be obtained by fitting polyexponential functions to maternal/fetal pharmacokinetic profiles. If more than one peripheral compartment exists on either the maternal or fetal side, then the terms CL_{dm} and CL_{df} represent the sum of all corresponding maternal and fetal peripheral distribution clearances respectively.

METHODS

Data Sources

The first set of data for betamethasone in pregnant sheep is part of a pharmacokinetic study (M. Schwab, T. Coksaygan, M. Samtani, W. J. Jusko, and P. W. Nathanielsz. Pharmacokenitics and cardiovascular effects of betamethasone in pregnant sheep after different doses; is the clinical dose too high? Manuscript submitted for publication (2004)). Data were obtained after administering a betamethasone prodrug depot formulation (Celestan Depot, Essex, Munich) intramuscularly to four animals. These animals (gestational age: 117 ± 1 days) received 170 μg kg^{-1} of the formulation, consisting of 50% betamethasone as betamethasone phosphate and 50% betamethasone acetate. Assuming a weight of 70 kg for a pregnant woman, the 170 μg kg^{-1} dose corresponds to the 12-mg dose recommended by the National Institutes of Health for administration to pregnant women in premature labor (15). Maternal and fetal betamethasone plasma samples were collected at 15 and 30 min and 1, 2, 3, 4, 6, 8, 12, and 24 h after intramuscular injection. Samples were frozen at −20°C until analyzed. Samples were analyzed using a well-established normal phase HPLC assay for betamethasone (29). Samples fell below the lower limit of quantification of 13 nM after eight hours in the maternal samples and only eight fetal samples showed presence of betamethasone.

Additional betamethasone data for the prodrug depot formulation in pregnant sheep and humans were extracted from the literature (5,30,31). Dexamethasone pharmacokinetic data after an intravenous injection of dexamethasone phosphate in pregnant rats were also published (19). Literature data were recaptured by computer digitalization (Sigma Scan, Jandel Scientific, Corte Madera, CA, USA) or by using the reported data (19). The characteristics of the data collected from all studies are listed in Table I.

Area/Moment Pharmacokinetic Analysis for Betamethasone in Pregnant Sheep

Area/Moment equations presented in the previous section indicated that four data sets are needed after both maternal and fetal drug administration for deciphering the pharmacokinetic parameters of the system. The pharmacokinetic profiles presented by Moss *et al.* (5) represent one such data set for betamethasone that is amenable to area/moment analysis under two assumptions. One is that the intramuscular administration of betamethasone acts like an instantaneous drug input, as the equations developed above are for IV bolus administration. This assumption is reasonable considering the fact that betamethasone plasma concentrations peak within 15-30 min after intramuscular administration. Second, the maternal profile after fetal administration in this data set is incomplete due to assay difficulties and an assumption is needed regarding the terminal slope of this curve to calculate

Table I. Characteristics of the Corticosteroid Data Collected from the Literature

Species	BW[a]	Fetal BW[a]	GA[b]	Steroid	Dose	Data collected	Ref.
Sheep	53.8 kg	2.1 kg	117 day	BET[c]	$Dose^m$ = 0.17 mg/kg	C_m^m and C_f^m	N/A[d]
Sheep	55 kg	1.4 kg	103 day	BET[c]	$Dose^m$ = 0.5 mg/kg	C_m^m and C_f^m	5
					$Dose^f$ = 0.5 mg/kg	C_f^f and C_m^f	
Sheep	58 kg	2.5 kg	127 day	BET[c]	$Dose^m$ = 0.5 mg/kg	C_f^m	30
					$Dose^f$ = 0.5 mg/kg	C_f^f	
					$Dose^m$ = 0.2 mg/kg	C_f^m	
					$Dose^f$ = 0.2 mg/kg	C_f^f	
Human	64 kg	2.2 kg[e]	33 week	BET[c]	$Dose^m$ = 12 mg	C_m^m	31
Rat	328 gm	42 gm[f]	20 day	DEX[g]	$Dose^m$ = 1.9 mg/kg	C_m^m, C_f^m, A_f^m (3)[h]	19

[a] BW: body weight.
[b] GA: gestational age.
[c] BET: betamethasone.
[d] Submitted for publication.
[e] Ref. 34.
[f] Ref. 33: Total rat fetal BW = Average litter size (11.4) · Average BW of each fetus (3.7 g).
[g] DEX: dexamethasone.
[h] A_f^m (3): Amount of drug in fetal body at 3 hours.

AUC and AUMC. We will show that the pharmacokinetic nature of these pregnancy models is such that all terminal maternal/fetal profiles are expected to be parallel to one another. This property of the system allows the terminal slope of the missing maternal profile to be fixed based on the terminal decline of maternal concentrations after maternal drug administration, as was measured by Moss *et al.* over 24 h.

For area/moment analysis all AUC and AUMC values were calculated using the WinNonlin pharmacokinetic software package (Pharsight Corporation, Mountain View, CA, USA) by the linear trapezoidal rule with extrapolation to infinity. For calculation of the maternal distribution clearances, a biexponential function was fitted to C_m^m vs. time data using $1/(Y_{predicted})^2$ as the weighting scheme in WinNonlin.

Compartmental Pharmacokinetic Model for Betamethasone in Pregnant Sheep

All the depot formulation betamethasone sheep pharmacokinetic data were fitted simultaneously using the model shown in Fig. 2 and the equations given below. The equations and initial conditions for maternal drug administration are:

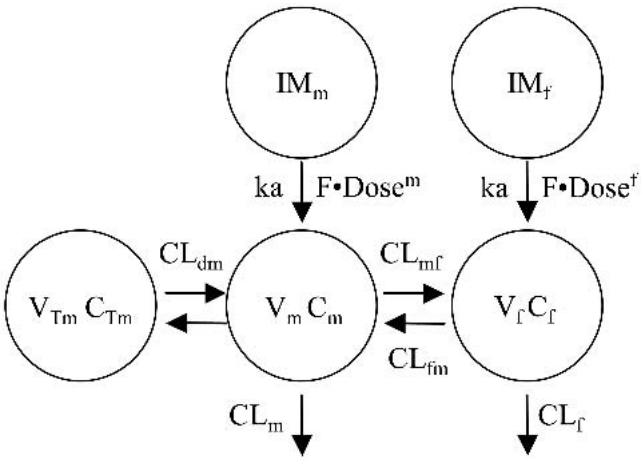

Fig. 2. Pharmacokinetic model for describing the maternal/fetal disposition of betamethasone in pregnant sheep.

$$\frac{dA_{IM}^m}{dt} = -ka \cdot A_{IM}^m, \qquad A_{IM}^m(0) = Dose^m \tag{37a,b}$$

$$\frac{dA_m^m}{dt} = ka \cdot A_{IM}^m - \left(\frac{CL_m/F}{V_m/F} + \frac{CL_{mf}/F}{V_m/F} + \frac{CL_{dm}/F}{V_m/F}\right) \cdot A_m^m + \frac{CL_{dm}/F}{V_{Tm}/F} \cdot A_{Tm}^m + \frac{CL_{fm}/F}{V_f/F} \cdot A_f^{m,}, \qquad A_m^m(0) = 0 \tag{38a,b}$$

$$\frac{dA_{Tm}^m}{dt} = \frac{CL_{dm}/F}{V_m/F} \cdot A_m^m - \frac{CL_{dm}/F}{V_{Tm}/F} \cdot A_{Tm}^m, \qquad A_{Tm}^m(0) = 0 \tag{39a,b}$$

$$\frac{dA_f^m}{dt} = -\left(\frac{CL_f/F}{V_f/F} + \frac{CL_{fm}/F}{V_f/F}\right) \cdot A_f^m + \frac{CL_{mf}/F}{V_m/F} \cdot A_m^m, \qquad A_f^m(0) = 0 \tag{40a,b}$$

$$C_m^m = \frac{A_m^m}{V_m/F} \quad \text{and} \quad C_f^m = \frac{A_f^m}{V_f/F} \tag{41,42}$$

where the subscript IM refers to the intramuscular drug administration compartment, A refers to amounts including the initial amount, A(0), and the terms expressed as a function of the intramuscular bioavailability (F) refer to apparent pharmacokinetic parameters. A similar set of six equations was used for fetal drug administration.

The modeling assumed that the absorption rate constant (ka) and bioavailability (F) are similar for betamethasone after maternal and fetal drug administration. All pharmacokinetic processes were assumed to be linear. The inclusion of a peripheral maternal compartment was based on the results of the area/moment analysis and the goodness-of-fit criteria. The estimates from the area/moment analysis were used as initial starting values for the fitting procedure.

Compartmental Pharmacokinetic Model for Dexamethasone in Pregnant Rat

Dexamethasone is primarily eliminated by cytochrome P450 metabolism (32). The inability of the rat fetus to me-

tabolize drugs allowed simplification of the pharmacokinetic model that was used for describing the disposition of dexamethasone in pregnant rats. A simple two-compartment maternal/fetal exchange system with elimination occurring only from the maternal compartment was applied (Fig. 3). The maternal/fetal serum concentrations were fitted using traditional differential equations and their initial conditions:

$$V_m \frac{dC_m^m}{dt} = -(CL_m + CL_{mf}) \cdot C_m^m + CL_{fm} \cdot C_f^m, \quad (43a,b)$$

$$C_m^m(0) = Dose^m/Vm$$

$$V_f \frac{dC_f^m}{dt} = -CL_{fm} \cdot C_f^m + CL_{mf} \cdot C_m^m, C_f^m(0) = 0 \quad (44a,b)$$

Additionally, the amount of dexamethasone per gram of fetal tissue was fitted simultaneously with the expression $C_f^m \cdot V_f/BW_f$ where BW_f is the total rat fetal body weight of 42 g obtained from the literature (33). This expression reflects that volume of distribution is a proportionality factor relating the concentration of drug in serum to the total amount of drug in the compartment or body.

The initial estimates for fitting the rat data were obtained using:

$$CL_m = Dose^m/AUC_m^m \quad (45)$$

$$V_m = Dose^m/C_m^m(0) \quad (46)$$

$$V_f = \frac{\text{Amount in fetal body at three hr}}{\text{Concentration in fetal serum at 3 hr}} \quad (47)$$

For traditional multicompartment models, the derivation of distribution clearance is based on the initial rate of decline of the central compartment plasma concentration profile (28). The initial rate of decline in plasma concentrations relates to drug distribution into the entire peripheral distribution space. In models of pregnancy the distribution clearance (Cl_{mf}) for drug transfer only into the fetal distribution space needs to be estimated. Unlike traditional models, where concentrations in peripheral distribution spaces are not known, the fetal plasma data in pregnancy allows computation of Cl_{mf} using the initial rate of increase in fetal concentrations. Thus by using the early slope of the fetal up-curve, an initial estimate for the distribution clearance specific to this compartment can be obtained. The initial estimate for CL_{mf} is obtained by first fitting the fetal profile after maternal drug administration to the following polyexponential function:

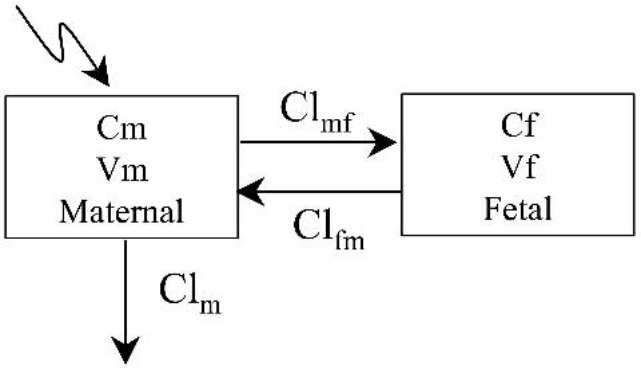

Fig. 3. Simple two compartment maternal-fetal exchange model with maternal drug elimination for capturing the pharmacokinetics of dexamethasone in pregnant rat.

$$C_f^m = C_1 e^{-\lambda_1 \cdot t} - C_1 e^{-\lambda_2 \cdot t} \quad (48)$$

The differential equation for C_f^m as time approaches zero is:

$$\text{as } t \to 0, V_f \frac{dC_f^m}{dt} \cong CL_{mf} \cdot C_m^m(0) \quad (49)$$

The parameter estimates from fitting Eq. 48 are used for calculating the initial rate of change of C_f^m:

$$\text{as } t \to 0, \frac{dC_f^m}{dt} \cong C_1 \cdot (\lambda_2 - \lambda_1) \quad (50)$$

Substituting Eq. 50 in 49 and simplifying gives the initial estimate for CL_{mf}:

$$Cl_{mf} = V_f \cdot C_1 \cdot (\lambda_2 - \lambda_1)/C_m^m(0) \quad (51)$$

Finally, CL_{fm} can be obtained by using moment concepts and recognizing that AUC_m^m/AUC_f^m is equal to the ratio of the two transfer clearances (CL_{fm}/CL_{mf}). Thus:

$$CL_{fm} = \frac{AUC_m^m}{AUC_f^m} \cdot CL_{mf} \quad (52)$$

All polyexponential fittings and calculations of AUC and initial concentrations for obtaining initial estimates for dexamethasone pharmacokinetic analysis were performed using WinNonlin.

Allometric Scaling of Betamethasone Pharmacokinetics

Pharmacokinetic parameters for betamethasone in sheep were scaled to humans using:

$$CL/F_{Human} = \left(\frac{BW_{Human}}{BW_{Sheep}}\right)^{0.75} \cdot CL/F_{Sheep} \quad (53)$$

$$V/F_{Human} = \frac{BW_{Human}}{BW_{Sheep}} \cdot V/F_{Sheep} \quad (54)$$

$$ka_{Human} = \left(\frac{BW_{Human}}{BW_{Sheep}}\right)^{0.25} \cdot ka_{Sheep} \quad (55)$$

where BW are body weights. Parameters CL_m/F, V_m/F, CL_{mf}/F, ka, CL_{dm}/F, and V_{tm}/F were scaled using maternal human and sheep weights of 61.8 and 53.6 kg, while CL_f/F, V_f/F, and CL_{fm}/F were scaled using fetal human and sheep weights of 2.2 and 2 kg. The sheep BW is an average value from three published studies (Table I). The human maternal BW is that of a 33-week pregnant woman whose betamethasone pharmacokinetic profile is reported in the literature (31). The human fetal BW for a 33-week conceptus is based on literature norms (34). The scaled human parameters were used for conducting simulations of human pharmacokinetic profiles and expected fetal/maternal concentration ratios using the model presented in Fig. 2 and Eqs. 37 to 42. The simulation results were compared with the human maternal profile for betamethasone in pregnancy (31) and the reported fetal to maternal concentration ratio of betamethasone (17).

Compartmental Pharmacokinetic Analysis

All compartmental pharmacokinetic modeling was performed using the maximum likelihood estimator within the ADAPT II computer program (35). The variance model was:

$$\text{Variance} = \text{Coefficient} \cdot Y(t)^{\text{Power}} \quad (56)$$

where Coefficient and Power are variance parameters that were fitted, and Y(t) represents the model output function. The goodness-of-fit was assessed using Akaike Information Criterion (AIC), Schwartz Criterion (SC), correlation coefficients (R^2), examination of residuals, and visual inspection. Simulation of the human pharmacokinetic profiles and expected fetal/maternal concentration ratios using the allometrically scaled parameters was performed using ADAPT II.

RESULTS

Betamethasone Profiles in Pregnant Sheep

The typical characteristics of betamethasone pharmacokinetics are shown in Figs. 4 to 6. Betamethasone has restricted access to the fetal compartment. Upon administration of betamethasone to the mother the fetal concentrations reach only about 30% of the maternal concentrations. Upon administration of the drug to the fetus, the concentrations of betamethasone initially decline very rapidly, before becoming parallel to all the other profiles, which decline with a terminal half-life of 10.3 h. The concentrations of the drug that reach the maternal circulation after fetal drug administration are so low that they pose an analytical limitation and there are few data points for the C_m^f profile in the literature. This is because dosing is done on a per kilogram basis and the fetal weight is less than 5% of the total body weight. Thus the total dose administered to the fetus is considerably smaller than the amount that would be administered to the mother. After administration of the small dose to the fetus, the minuscule amount of drug that diffuses across the placenta into the mother is diluted in the large maternal distribution volume, which acts as a sink, greatly reducing maternal drug exposure. Despite the small fetal dose of betamethasone, the fetal AUC of betamethasone is greater after fetal dosing than the fetal AUC observed after maternal drug administration. Thus Moss *et al.* (5) have proposed that fetal drug administration may offer a novel route of betamethasone administration, which allows substantial dose reduction, greater fetal drug exposure, and greatly reduced maternal exposure to the drug. However, there are practical difficulties administering drug via this route.

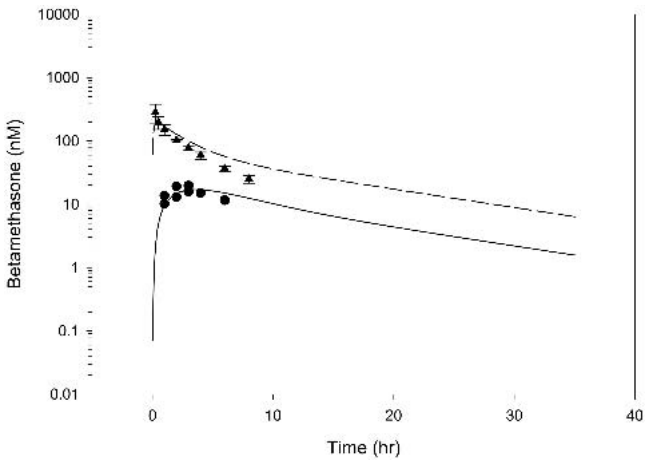

Fig. 4. Maternal and fetal plasma concentrations of betamethasone in pregnant sheep. The pharmacokinetic model shown in Fig. 2 was fitted to the data from Schwab *et al.* (submitted for publication). Triangles with standard deviation bars represent C_m^m data and circles represent C_f^m data for the eight fetal samples where betamethasone could be detected. Lines represent model fittings.

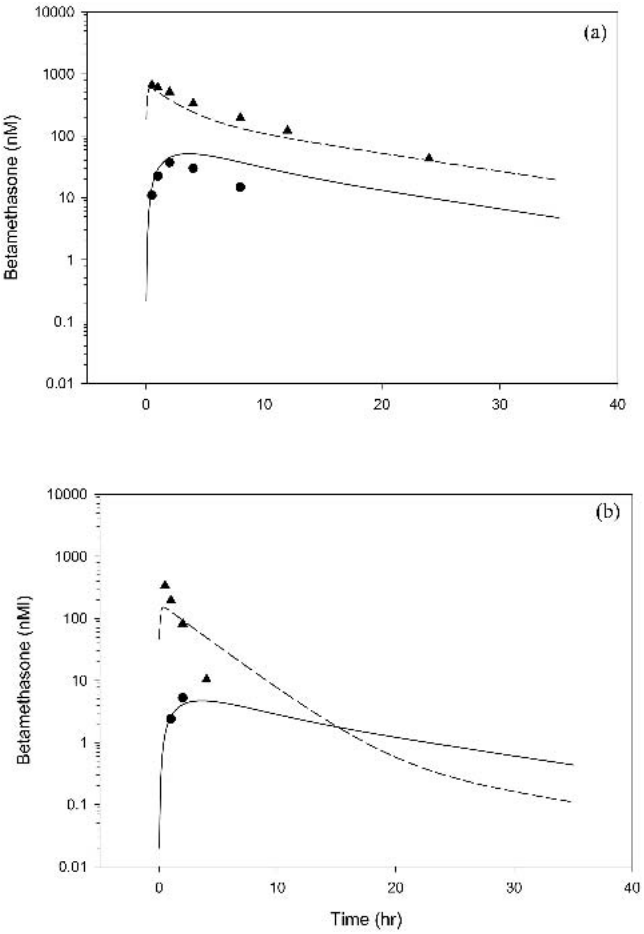

Fig. 5. Maternal and fetal plasma concentrations of betamethasone in pregnant sheep. The pharmacokinetic model shown in Fig. 2 was fitted to the data from Moss *et al.* (5). (a) Symbols and lines are defined as in Fig. 4. (b) Triangles and circles represent C_f^f and C_m^f data with lines representing model fittings.

Area/Moment Analysis of Betamethasone in Pregnant Sheep

The results from this analysis are presented in Table II. The area/moment estimates of $Vm_{ss,true}$ (116 L) was found to be larger than the maternal central compartment volume (92 L). This suggests that betamethasone has a peripheral distribution space in the mother, which has a volume of 24 liters. On the other hand, the fetal central (2.9 L) and true steady-state volumes (2.5 L) were very similar. This suggests that after fetal betamethasone dosing, the distribution within the fetus behaves like an instantaneous process. Finally, the esti-

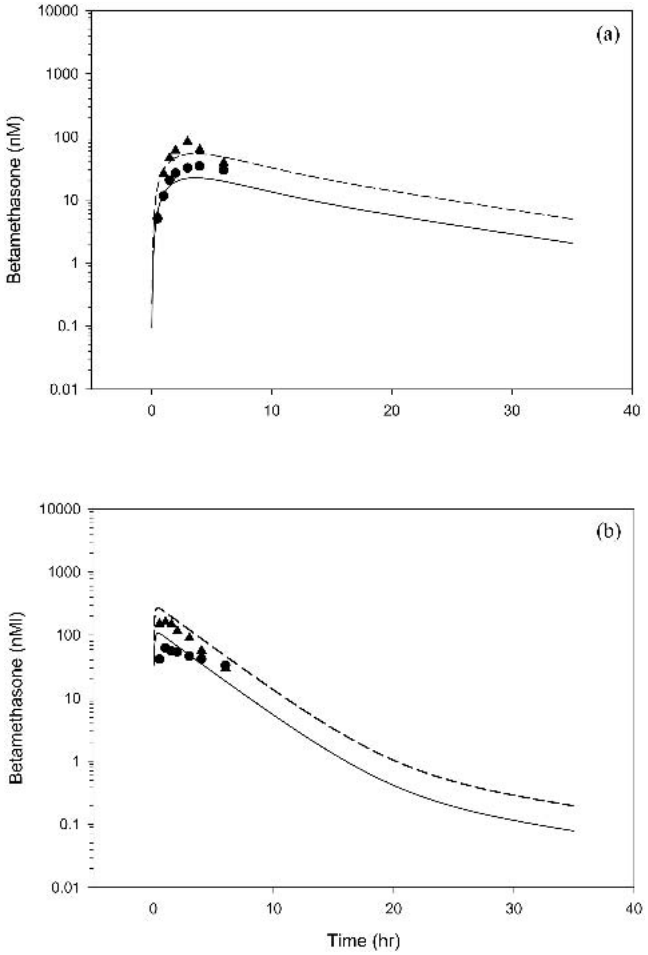

Fig. 6. Maternal and fetal plasma concentrations of betamethasone in pregnant sheep. The pharmacokinetic model shown in Fig. 2 was fitted to the data from Berry *et al.* (30). (a) Triangles and circles represent C_f^m data at 0.5 mg/kg and 0.2 mg/kg with lines representing model fittings. (b) Triangles and circles represent C_f^f data at 0.5 mg/kg and 0.2 mg/kg with lines representing model fittings.

mates of CL_{fm} and CL_m were much higher than the corresponding values of CL_{mf} and CL_f.

Compartmental Analysis of Betamethasone in Pregnant Sheep

The parameter estimates from the compartmental analysis are presented in Table II. In agreement with the area/moment results, the CL_{fm} and CL_m values were estimated to be higher than the estimate of CL_{mf} and CL_f. All parameters were obtained with a reasonable degree of precision. The parameter with the highest coefficient of variation (99.95%) was ka. This is not surprising because the absorption up-curve for betamethasone is missing due to its fast absorption from the IM site. The performance of the compartmental pharmacokinetic model is shown by the fitted curves in Figs. 4 through 6.

The signature pattern of the pharmacokinetic profiles in pregnancy exhibits four main characteristics that are expected theoretically and largely evident in Figs. 4 through 6: i) Drug appears in the fetal circulation after maternal drug administration; ii) Drug appears in the maternal circulation after fetal drug administration; iii) The profiles usually exhibit polyexponential characteristics regardless of maternal/fetal distribution spaces. This is exemplified by the C_f^f profiles for betamethasone; iv) Terminal slopes of the C_m^m, C_f^m, C_m^f, and C_f^f profiles show parallelism.

Table II. Pharmacokinetic Parameters for Betamethasone in Sheep Pregnancy

Parameter	Area/moment estimates[a]	Compartmental estimates[b]	Compartmental estimates CV%
CL_m/F (L/h)	12.66	16.27	15.62
CL_f/F (L/h)	1.41	0.93	68.23
V_m/F (L)	92.15	99.73	20.10
V_f/F (L)	2.92	9.88	12.78
CL_{mf}/F (L/h)	0.15	0.63	15.76
CL_{fm}/F (L/h)	1.42	2.28	28.12
ka (1/h)	N/A	9.06	99.95
CL_{dm}/F (L/h)	6.17	15.18	70.31
V_{tm}/F (L)	24.41	88.65	59.90

[a] Analysis of data in Fig. 5.
[b] Analysis of data in Figs. 4–6.

Compartmental Analysis of Dexamethasone in Pregnant Rats

As shown in Fig. 7, fetal dexamethasone concentrations rise rapidly after maternal dexamethasone administration and maternal/fetal concentration and fetal amount profiles decline with a parallel terminal slope. These characteristics are typical of an equilibrating two-compartment model, which adds credibility to the model structure presented in Fig. 3. The distribution of dexamethasone is very rapid and hence the maternal profile does not display a distinct early distribution phase. Rapid distribution of dexamethasone is indicated by its appearance in the fetal circulation at the first sampling time point of 15 min. The fetal dexamethasone concentration

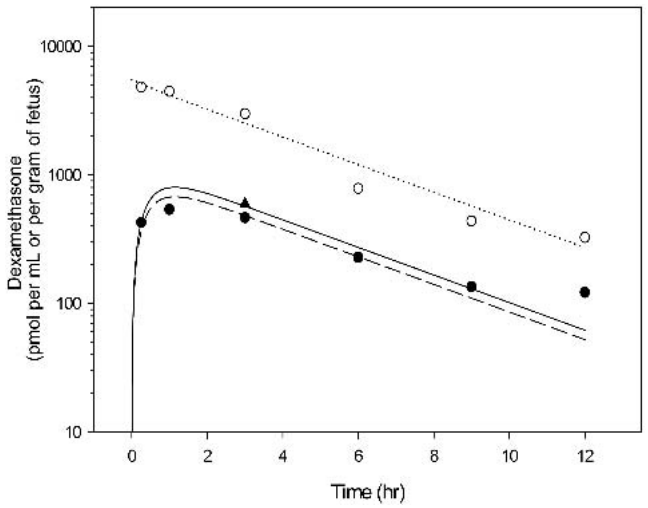

Fig. 7. Maternal and fetal plasma concentrations of dexamethasone in pregnant rats. The pharmacokinetic model in Fig. 3 was fitted to the data from Varma and Yue (19). Open circles represent C_m^m data and filled circles represent C_f^m data. The filled triangle represents amount of dexamethasone per gram of fetal tissue at 3 h. Lines represent model fitting.

and amount per ng of tissue are almost identical indicating that the drug distribution space is very close to the fetal body weight. Similar to the sheep betamethasone results, dexamethasone also exhibited restricted fetal access. Upon maternal drug administration the level of dexamethasone in the fetal circulation reached only about 20% of the corresponding maternal concentrations.

The inclusion of the amount of drug in the fetus is essential for obtaining reliable estimates for CL_{mf}, CL_{fm}, and V_f, as listed in Table III. Exclusion of the amount data gives high CV% for these parameters and the three parameters were found to be correlated with one another. Thus the information regarding the amount of drug in the fetal body is essential for distinguishing the role of fetal drug distribution from placental transfer processes. Eqs. 48–51 and the fetal upstroke data were used only to generate initial estimates of CL_{mf} for compartmental modeling. CL_{mf} also controls the AUC (Eq. 52) and the terminal slope. In compartmental modeling all the features of the data are used and hence the program converges with reasonable estimates, which is evident from the low CV% of CL_{mf} in Table III.

The maternal and fetal volumes reported in Table III have values very similar to the maternal and fetal body weights. This finding adds credibility to these estimates because it has been shown in the literature that the volume of distribution of dexamethasone in normal and adrenalectomized (ADX) rats is close to 1 L/kg (36,37). The maternal elimination clearance (60.8 ml/h) normalized to the total body weight of the pregnant rat (0.328 kg) equals 185 ml/h/kg, which is very close to the dexamethasone clearance of 157–195 ml h^{-1} kg^{-1} in normal and ADX rats (36,37). Thus it appears that the pharmacokinetics of dexamethasone are unaffected by pregnancy. Finally, similar to the sheep results, the estimate of CL_{fm} was substantially higher than CL_{mf}, indicating more rapid drug movement from fetus to mother. This consistently higher value of CL_{fm} vs. CL_{mf} may indicate that a placental efflux process mediates the transfer of corticosteroids from the fetal side back into the maternal circulation.

It should be pointed out that the pharmacokinetic parameters for dexamethasone in rats have been reported as true clearance and volume parameters. This is possible because the phosphate ester dexamethasone prodrug used in the rat studies was dosed intravenously and it generates the active steroid completely and rapidly (16). In contrast the pharmacokinetic parameters for betamethasone in sheep are reported as apparent clearance and volume parameters. The distribution and clearance parameters for betamethasone are confounded by the extent of bioavailability of betamethasone from the intramuscular site of injection and by the extent of drug released by the depot prodrug formulation. As discussed later the release of the active drug from the depot component of the betamethasone formulation is so slow that it does not give measurable levels of betamethasone. This poor release of betamethasone from the depot component of the formulation therefore markedly influences the CL/F and V/F estimates.

Table III. Pharmacokinetic Parameters for Dexamethasone in Rat Pregnancy

Parameter	Estimate	CV%
V_m (ml)	236.20	6.71
V_f (ml)	49.93	29.80
CL_m (ml/h)	60.78	6.24
CL_{fm} (ml/h)	102.10	58.60
CL_{mf} (ml/h)	17.22	50.86

Scaling of Pregnant Sheep Pharmacokinetics to Humans

The difference in metabolic capability between rat and human fetuses prevents animal scale-up of dexamethasone pharmacokinetics to humans. However, the sheep parameters are amenable to allometric scaling. The results of the simulation obtained by using allometrically scaled human parameters and literature data for maternal betamethasone pharmacokinetics in humans (31) are presented in Fig. 8. It appears that the simulation captured similar concentrations and the terminal slope of the maternal profile, but missed the Cmax and the time to reach Cmax. The simulated profile reached a higher Cmax value earlier and declined in a biexponential fashion as compared to the observed maternal profile, which behaves like a Bateman function. The fetal to maternal concentration ratio rose slowly and reached a steady value of 0.26, which is very close to the reported ratio of 0.28 for betamethasone in humans (17).

DISCUSSION

Compartmental and Area/Moment Analysis in Pregnant Sheep

Area/Moment analysis is based on the estimation of area under the drug concentration vs. time plot and area under the curve produced by plotting the product of concentration and time vs. time. These areas can be easily computed by numerical integration using the trapezoidal rule. The use of area/moment analysis in pharmacokinetics is hardly new, but the application of these concepts to drug disposition in pregnancy is both novel and important. These methods do not require the assumption of a mammillary pharmacokinetic system, which is a common assumption for non-compartmental pharmacokinetic analysis. Area/Moment analysis is more general method that can be applied to any pharmacokinetic scheme

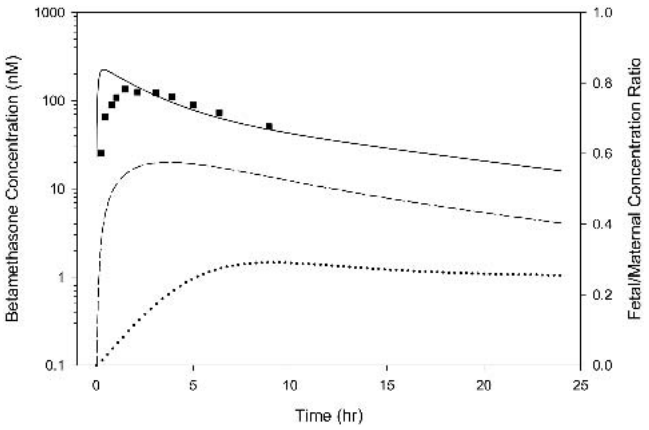

Fig. 8. Simulation of human betamethasone profiles using parameters obtained by allometrically scaling sheep pharmacokinetic parameters. Closed squares represent human maternal concentrations of betamethasone in pregnancy from Ref. 31 (dose = 12 mg). Solid, dashed and dotted lines represent the maternal profile, fetal profile and fetal/maternal concentration ratio for betamethasone.

including non-mammillary systems such as pregnancy where elimination of drugs can occur from both the mother and fetus. The methods described in this paper provide an easy computational approach for calculating the essential pharmacokinetic parameters describing maternal/fetal exchange, distribution, and elimination of drugs in pregnancy providing a minimal model for this system. The disadvantage of area/moment analysis is the inability to quantitate and predict the time-course of drug concentrations and view the appropriateness of the calculated parameters. Compartmental analysis does not suffer from such a disadvantage, but requires the assumption of a structural model and represents a higher-level computational method.

Differences were observed in the parameter estimates obtained for betamethasone by area/moment and compartmental methods. Reasons for differences are as follows: i) Certain assumptions were made in calculating the area/moment parameters, which include bolus input of drug and a fixed terminal slope for the C_f^m data. The compartmental analysis is not restricted by the assumption of a bolus input and the simultaneous analysis of all the data helps predict the missing portion of the C_f^m profile. Fitting the betamethasone data in pregnant sheep exemplifies these features of simultaneous modeling. ii) The compartmental estimates are obtained from simultaneous fitting of data from three sources, while the area/moment estimates are results for data from one study (5). Variability of the data from the three different sources could account for differences in parameter estimates. iii) Most importantly, the compartmental analysis makes use of data that were gathered at different dose levels and at different gestational ages. There is some evidence that fetal pharmacokinetic parameters can change as a function of gestational age (38,39) due to developmental changes occurring in the fetus. Thus the estimates obtained from compartmental analysis represent dose- and time-averaged values of pharmacokinetic parameters of betamethasone. The area/moment estimates, on the other hand, represent parameters for the 0.5 mg/kg dose at gestational age 103 days for pregnant sheep.

Despite the differences between the compartmental and area/moment estimates; the latter serves as good method for generating initial estimates for the compartmental modeling procedure. Such estimates also serve as an excellent diagnostic tool for deciding on the need for peripheral compartments on either the maternal and/or fetal sides. Thus, the area/moment analysis indicated that $Vm_{ss,true}$ is greater than V_m, suggesting that a peripheral compartment would be necessary for describing betamethasone pharmacokinetics. This diagnostic indication was confirmed by the goodness-of-fit criteria in compartmental modeling.

Compartmental Modeling of Dexamethasone Pharmacokinetics in Pregnant Rats

An interesting study (40) implicates an active transfer process from the fetal to maternal side in rats for dexamethasone. The drug was administered to both the pregnant rat and its fetuses to decipher the disposition parameters. This study had many drawbacks. i) The study made use of serial fetal sampling with three to four fetuses removed at each time point to collect fetal blood. The animals were subjected to repeated surgeries and anesthesia for sample collection, which could have affected dexamethasone pharmacokinetics. ii) Labeled and unlabeled drug were administered simultaneously to the mother and fetus to obtain the complete C_m^m, C_f^m, C_m^f, and C_f^f data set. However, fetal drug administration was by injection into the peritoneal cavities of all the fetuses and this route of drug administration was assumed to provide instantaneous and complete fetal drug input, without affecting fetal physiology and dexamethasone disposition. These assumptions may not be true. iii) Each of the four maternal and fetal profiles had four data points and this sparse data set caused constraints during curve fitting, which led to inaccurate parameter estimates. iv) There are also some issues with the use of radiolabeled drug for fetal drug administration and its interference with the assay for the unlabeled drug administered to the mother. To reduce the extent of this problem the authors administered a 10-fold lower dose to the fetus and assumed (based on data from non-pregnant animals) linear pharmacokinetics for all the placental, fetal and maternal pharmacokinetic parameters at the two different dose levels. v) Finally, the radioactivity measured in serum for maternal/fetal profiles after fetal drug administration was assumed to be solely from dexamethasone and not its metabolites. However, dexamethasone is extensively metabolized (32).

The dexamethasone pharmacokinetics described in this report simplifies the experimental and computational difficulties associated with studying pharmacokinetics in rat pregnancy. The data used for this analysis was from a study with a richer data set where animals were sacrificed at each time point for collection of maternal and fetal samples, without the need for repeated anesthesia and surgical manipulation of the animals. Maternal and fetal data after only maternal drug administration were required for estimating all the pharmacokinetic parameters. This was accomplished by considering the known absence of fetal drug metabolic capability and the knowledge of the amount and serum concentration of drug in the fetus. Parameter estimates were obtained with reasonable precision. Inference could be made about the role of placental efflux in restricting corticosteroid fetal access.

Scaling of Pregnant Sheep Pharmacokinetics to Humans

Considering the fact that the animal scaling was based on one species with little difference in weight, Fig. 8 indicates that the application of allometric principles to betamethasone pharmacokinetics in pregnancy gave reasonable results. The discrepancy between the simulated absorption phase and the observed betamethasone concentration at early time points can be explained by an analytical problem associated with measuring betamethasone in sheep plasma samples. The use of betamethasone prodrugs in pharmacokinetic studies poses the risk of overestimation of corticosteroid concentrations during the early portion of the pharmacokinetic profile due to *in vitro* hydrolysis of prodrugs after sample collection (41). We recently studied this problem and found that such artifacts in pharmacokinetic profiles could be prevented by sample treatment with enzyme inhibitors (41). Pharmacokinetic studies of betamethasone in sheep have usually ignored this problem because prevention requires sample stabilization with inhibitors such as sodium arsenate and the problem is limited to only the initial phase of the profile, which leads to over-estimation of AUC from time zero to sixty minutes by 40% and total AUC by only 7% (41). Human studies, on the other hand, have been performed very thoroughly where this

problem was circumvented by the use of sample stabilizing enzyme inhibitors (31). This difference in sample handling between sheep and human studies may explain the inability of the simulations to capture the initial phase of the maternal human data for betamethasone. Finally, the simulations did a good job at capturing the general exposure (AUC) and the fetal/maternal concentration ratio for betamethasone, which may indicate that disposition processes that control fetal exposure of betamethasone in sheep may also regulate fetal human exposure to betamethasone.

Comparison of Pregnancy Models to Nonmammillary Reversible Metabolic Systems

The area/moment equations developed in this paper are very similar to equations developed for another nonmammillary system representing reversible metabolic interconversion of drugs (42). These equations for nonmammillary systems have been previously derived using complex matrix algebra (42). This report derives area/moment equations for nonmammillary systems, using traditional concepts in pharmacokinetics such as Laplace transforms and statistical moments. Furthermore, the equations for the two systems are similar but the meaning of the pharmacokinetic parameters in the two systems differ in the following ways: i) The volume terms in reversible metabolic systems can be segregated into two groups, one representing the parent drug and the other representing the metabolite. The two sets of volume terms are independent of one another. Similarly, in the models for pregnancy there are two sets of volume terms; those for the mother and the fetus. However, in pregnancy the two sets can be added together to obtain the total distribution space of the drug. ii) Exchange clearances represent metabolic interconversion of the parent and metabolite. In pregnancy models the exchange clearances reflect transfer of the drug across the placenta between mother and fetus. iii) Elimination clearances denote irreversible removal of the parent or metabolite. Elimination clearances in the pregnancy models represent irreversible removal of the drug either by the mother or the fetus. iv) Distribution clearance terms in reversible metabolic systems represent the velocity of distribution of either the parent drug or the metabolite. In pregnancy models, distribution clearances describe distribution of the drug in the mother or the fetus.

Protein Binding in Assessing Maternal/Fetal Pharmacokinetics

The compartmental and area/moment analysis above is based on an assumption that maternal and fetal binding of drug is identical, which is true for corticosteroids. If this assumption is not met for other drugs, then the above equations and methods should be applied to free drug concentrations to obtain estimates that are not influenced by differences in protein binding.

Role of PGP in Modulating Fetal Corticosteroid Exposure

It has been noted that levels of synthetic corticosteroids in the fetal circulation after maternal drug administration are much lower in humans, sheep, and rats. Furthermore, it can be seen in Figs. 4 through 6 that fetal concentrations after fetal betamethasone administration decline with a rapid rate initially as compared to the decline observed in the fetal circulation after maternal drug administration. This restricted fetal residence of corticosteroids has been attributed in the literature to fetal drug elimination. The latter cannot explain the polarized transfer (low maternal to fetal transfer vs. rapid fetal to maternal transfer) seen for betamethasone movement across the placental barrier. Furthermore, the inability of the rat fetus to metabolize drugs and the low value of CL_f/F obtained for sheep indicates that fetal elimination probably plays a minor role in limiting fetal corticosteroid exposure.

We have shown that regardless of the computation method (area/moment vs. compartmental) and regardless of the species (sheep or rat), the CL_{fm} value was always substantially higher than the CL_{mf}. Numerous reports have appeared showing that both endogenous and synthetic corticosteroids are substrates for the multidrug resistance (MDR) gene transporter, P-glycoprotein (43–53). Although most work with PGP mediated corticosteroid efflux has been performed with dexamethasone, recent studies have shown that transport efficiency of betamethasone by PGP is also relatively high (54). PGP functions as an efflux mechanism for extruding corticosteroids from the intracellular region to the extracellular space. An important and well-studied location for PGP expression in humans and rodents are placental trophoblast cells (55–57). PGP lines the brush border membrane facing the maternal circulation (58) and serves the function of protecting the developing conceptus by effluxing a broad range of substrates (59). Elegant experiments with MDR knockout mice have shown that PGP is capable of restricting the fetal transfer of drugs and toxic compounds and can protect the fetus from deleterious effects (60,61). Along with showing that dexamethasone is a good substrates for PGP (62), it has also been demonstrated that this transporter can have a significant impact on the *in vivo* distribution of dexamethasone (63). Thus, this novel placental efflux mechanism can explain the limited placental transfer of antenatal corticosteroids, the rapid decline in corticosteroid levels after fetal drug administration, and the high CL_{fm} vs. CL_{mf} values. Our analysis attributes the maternal to fetal concentration gradient of corticosteroids to the now known placental expression of efflux transporter PGP for which corticosteroids are good substrates.

Limitations of the Dataset and Meta-analysis

The compartmental modeling made use of a variety of model structures including peripheral compartments on the maternal and fetal side. The models selected are the most parsimonious models with the best goodness of fit criteria for the available data. However, each dataset used in this work had some limitations which imposed certain constraints in our modeling. The limitations are as follows: i) Derendorf *et al.* have measured betamethasone kinetics by RIA and found that low betamethasone levels (0.1–10 ng/ml) are maintained for 2 weeks after intra-articular injection of the depot formulation (64). Unfortunately all the literature information available for our meta-analysis provides data only for the first 24 h after drug administration in pregnancy. During the first 24 h betamethasone concentrations are high, which result from the fast releasing phosphate prodrug. The HPLC assay used in the present experiments is unable to monitor low concentration of betamethasone beyond 24 h, which may result from

the depot component of the formulation. Further, the depot formulation may not offer sustained-release properties either because there are species differences between human and sheep in the ability to activate betamethasone acetate and/or because the release rate of betamethasone is so slow that the amount released during the first 24 h contributes marginally. When comparing the maternal and fetal profiles of the depot formulation vs. those obtained by administering the phosphate prodrug alone, we found that the depot formulation released betamethasone only from its phosphate component over the first 8 h after administration (Schwab *et al.,* ob cit). It appears that the slow releasing component from the depot formulation offers very little therapeutic benefit in antenatal therapy for fetal maturation during preterm labor (31). This is because the low levels of betamethasone, if at all released from the depot formulation, will hardly reach the therapeutic target, which resides in the fetal lung, and will likely be inefficacious. ii) There are only two data points beyond ten hours in Figs. 4–6 for betamethasone pharmacokinetics in sheep. These two data points should be considered fairly reliable data because they were obtained from five animals, had extremely small error bars, and are in the high concentration range, which is well within the standard curve range of the assay used by Moss *et al.* (5). However, these two points dictate all the fitted terminal slopes in Figs. 4–6, a constraint imposed by the limited literature dataset available. iii) There is uncertainty regarding the early concentration maternal sheep data for betamethasone due to the problem of *ex vivo* hydrolysis of the soluble prodrug. We discussed this problem in our earlier publication (41). This analytical artifact causes profiles to assume biexponential character and estimates for CL_{dm}/F and V_{tm}/F are affected. iv) There are no data for betamethasone in the fetal circulation for the first thirty minutes after drug administration in any of the papers used for the meta-analysis. If there is a distribution phase at all for betamethasone in the fetal circulation, it was probably missed. v) Unlike the sheep studies, rats were dosed only with the phosphate ester prodrug of dexamethasone via the intravenous route. The phosphate ester corticosteroid prodrugs release the active steroid with a half-life of less than 10 min in humans (41). Biologic processes occur much faster in rats and we have unpublished results for dexamethasone pharmacokinetics in rats for this prodrug. In female rats the prodrug hydrolysis and dexamethasone distribution is complete within 15 min after injection (we controlled for *ex vivo* prodrug hydrolysis). The pregnant rat data in Fig. 7 starts at 15 min. By this time the rising and declining parts of the maternal curve are expected to be over. Because the data set is limited, we assumed the simplest and most parsimonious model, that is, instantaneous input with instantaneous distribution of dexamethasone in the mother rat. vi) Human data for betamethasone kinetics in pregnancy are sparse. It is unclear whether the allometric simulations captured the terminal slope of the maternal profile for betamethasone in humans because of this. However, the simulations do capture the maternal exposure (AUC for the data is 1129 nM h and AUC for the simulation is 1579 nM h) as well as the relative fetal exposure (fetal/maternal concentration ratio) to betamethasone indicating that the processes controlling betamethasone disposition may be similar in humans and sheep.

Corticosteroid use in antenatal therapy for fetal maturation during preterm labor has existed for over thirty years. The landmark clinical trial in 1972 by Liggins and Howie (65) was the first study that suggested that prematurely born infants had a decreased incidence of respiratory distress syndrome when the mothers were administered betamethasone prior to delivery. The original regimen of Liggins, which consisted of two doses of 6 mg betamethasone acetate suspended in a solution of 6 mg betamethasone phosphate, is still recommended as standard care in prenatal medicine (15). Despite their long history of use in pregnancy, there are several drawbacks in the studies reported on the pharmacokinetic properties of this important class of steroids. The present report comprehensively summarizes actual and theoretical corticosteroid pharmacokinetics in pregnancy, and highlights the need for future studies with optimal study designs for addressing the identified gaps in knowledge regarding corticosteroid pharmacokinetic properties. Despite all the above limitations, the application of the mathematical principles described in this paper to corticosteroid pharmacokinetics shows how these data analysis techniques can be used to generate hypotheses regarding mechanisms controlling the maternal/fetal disposition and transfer of drugs to the fetus in pregnancy.

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