DISPOSITION OF VALPROIC ACID IN MATERNAL, FETAL, AND NEWBORN SHEEP: PLACENTAL TRANSFER, PLASMA PROTEIN BINDING, AND CLEARANCE

SANJEEV KUMAR, HARVEY WONG, SAM AU YEUNG, K. WAYNE RIGGS, FRANK S. ABBOTT, AND DAN W. RURAK

Division of Pharmaceutics and Biopharmaceutics, Faculty of Pharmaceutical Sciences (S.K., H.W., S.A.Y., K.W.R., F.S.A.) and British Columbia Research Institute of Children’s and Women’s Health, Department of Obstetrics and Gynecology, Faculty of Medicine (D.W.R.), The University of British Columbia, Vancouver, British Columbia, Canada

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ABSTRACT:

Separate 24-h maternal and fetal infusions of valproic acid (VPA) were administered to five pregnant sheep at 125 to 138 days gestation (term ~145 days) to determine maternal-fetal disposition. The pharmacokinetics of VPA were also investigated in five newborn 1-day-old lambs after a 6-h drug infusion. Plasma, urine, and amniotic and fetal tracheal fluid samples were analyzed for VPA using gas chromatography-mass spectrometry. During maternal drug infusion, the average steady-state fetal/maternal unbound VPA plasma concentration ratio was 0.81 ± 0.09. Unbound maternal-to-fetal VPA placental clearance (69.0 ± 20.2 ml/min/kg) was similar to that in the other direction (61.9 ± 24.2 ml/min/kg); this indicates passive placental diffusion and intermediate placental permeability of VPA in sheep. Newborn unbound VPA clearance (0.66 ± 0.28 ml/min/kg) was much lower than in the mother (5.4 ± 2.7 ml/min/kg) or the fetus (62.1 ± 22.4 ml/min/kg), and exhibited pronounced Michaelis-Menten characteristics. The elimination half-life of the drug was much longer in the newborn (18.6 ± 2.6 h) relative to the mother (5.6 ± 1.4 h) and the fetus (4.6 ± 1.9 h). Thus, VPA elimination in newborn lambs is much slower as compared with adult sheep, a situation similar to that in humans. Plasma protein binding of VPA was saturable, with similar VPA binding capacities and affinities in maternal and fetal plasma. VPA was extensively displaced from binding sites in the newborn lamb during the first 1 to 2 days of life, possibly because of increased plasma free fatty acid concentrations at birth. Thereafter, newborn plasma appeared to have a similar VPA binding capacity but lower affinity compared with the mother and the fetus.

Valproic acid (2-propylpentanoic acid, VPA) is a low-molecular-mass (144.2 Da) antiepileptic drug with a unique branched-chain fatty acid structure (Davis et al., 1994). There are approximately 12,000 births per year to epileptic women in the U.S. alone, and ~95% of these women are on antiepileptic therapy during pregnancy (Vorhees et al., 1988). VPA is one of the four major drugs (phenytoin, phenobarbital, carbamazepine, and VPA) used to treat epilepsy in the pregnant population (Lindhout and Omtzigt, 1994; Malone and D’Alton, 1997). The use of all these drugs is associated with an increased risk of numerous major birth defects, including cardiovascular and neural tube defects, orofacial clefts, genitourinary defects, and dysmorphic syndromes (Lindhout and Omtzigt, 1994; Malone and D’Alton, 1997). In addition to the teratogenic effects associated with the use of VPA and other antiepileptic drugs, prenatal exposure to these compounds may also result in alterations in cognitive function and behavior during postnatal life (Trimble, 1990; Koch et al., 1996).

VPA undergoes extensive placental transfer in animals as well as humans (Dickinson et al., 1979, 1980; Ishizaki et al., 1981; Nau et al., 1981, 1984; Nau and Krauer, 1986; Kondo et al., 1987). In humans, cord-to-maternal blood VPA concentration ratios at birth range from 0.5 to 4.6 (Dickinson et al., 1979; Ishizaki et al., 1981; Nau et al., 1981, 1982, 1984; Nau and Krauer, 1986; Kondo et al., 1987). Although cord-to-maternal blood concentration ratios of drug concentrations are highly dependent on the timing of drug administration and sampling (Rurak et al., 1991), the above data indicate a high degree of fetal VPA exposure after maternal administration in humans. We have also previously examined the placental transfer of VPA in chronically catheterized pregnant sheep during late gestation after i.v. bolus administration (Gordon et al., 1995). After maternal i.v. bolus administration, VPA appeared rapidly in plasma (within 2 min), and the
average fetal drug exposure index based on fetal-to-maternal arterial plasma area under the curve of arterial plasma concentration-time profile (AUC) ratio was 0.41.

In humans, the plasma protein binding of VPA gradually decreases in the mother over the course of gestation, whereas that in the fetus gradually increases, such that at birth, fetal VPA plasma protein binding exceeds that in the mother (Nau and Krauer, 1986). Also, there is an additional reduction in maternal plasma protein binding of the drug at birth due to elevated plasma free fatty acids (Nau et al., 1984; Nau and Krauer, 1986). These phenomena result in fetal accumulation of the drug and a greater than unity cord-to-maternal blood VPA ratio at birth (Nau et al., 1984).

The reported elimination half-life of VPA in less than 1-month-old human newborns ranges from 15.1 to 80 h, which is ~2- to 8-fold longer than the values in adults (Dickinson et al., 1979; Ishizaki et al., 1981; Nau et al., 1981, 1984; Irvine-Meek et al., 1982; Kondo et al., 1987; Gal et al., 1988). Similar findings have been reported for newborns of other species such as rats and guinea pigs (Yu et al., 1987; Haberer and Pollack, 1994). This indicates a much reduced elimination capacity for the drug during the immediate newborn period in all species studied.

In spite of the considerable body of data described above, there have been very few studies to systematically examine the fetal and newborn disposition and metabolism of VPA in animals or humans, and the exact reasons behind the impaired VPA elimination in human or animal newborns are not known. Thus we undertook a series of studies in chronically catheterized pregnant sheep and newborn lambs to examine the steady-state placental transfer, comparative pharmacokinetics and plasma protein binding (this article), as well as renal excretion and metabolism (accompanying article, Kumar et al., 2000b) of VPA in the mother, fetus, and newborn. The specific issues that we have addressed in the current manuscript are the mechanism of VPA placental transfer (active or passive), the extent of fetal drug exposure after maternal dosing, and the role of different factors determining fetal VPA exposure, such as placental permeability, drug lipophilicity, and fetal drug clearance capacity. In addition, we have quantitatively evaluated and compared the VPA elimination capacity in maternal, fetal, and newborn sheep so as to assess the development of VPA elimination routes during late gestation and early newborn period in this species.

**Experimental Procedures**

**Animals and Surgical Preparation.** 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.

**Pregnant sheep.** Five pregnant Dorset Suffolk cross-bred ewes, with a maternal body weight of 77.5 ± 10.6 kg (mean ± S.D.), were surgically prepared between 121 and 125 days gestation (term ~145 days) using procedures described previously (Kumar et al., 1997). Briefly, surgery was conducted under halothane (1–2%)-nitrous oxide (70%) anesthesia after induction with i.v. pentothal (1 g) and used sterile techniques throughout. After a midline abdominal incision to the ewe, access to the fetal head and hindquarters was gained via two separate uterine incisions in the areas devoid of major blood vessels and placental cotyledons. Polyvinyl catheters (Dow Corning, Midland, MI) were implanted in both fetal femoral arteries and lateral tarsal veins, a fetal carotid artery, fetal trachea, amniotic cavity, and in four animals, in the fetal urinary bladder (via a suprapubic incision). Amniotic fluid lost during surgery was replaced with warm sterile irrigation saline, and the uterine and abdominal incisions were closed in layers. Catheters were also implanted in a maternal femoral artery and vein. After a recovery period of at least 3 days, the sheep were moved to a monitoring pen adjacent to and in full view of the holding pen for experimentation purposes.

**Newborn lambs.** For newborn lamb studies, five additional pregnant sheep were prepared surgically as above at 121 to 132 days gestation. Lambs were allowed to undergo spontaneous delivery at term (139–143 days) along with their intact catheters. Experiments on the lambs were started the day after birth. On the day of experiment, the lambs were placed in small pens in full view of the mother, and were fed mother’s colostrum at intervals. After completion of the drug infusion period (6-h), the lambs were returned to their mother. Cumulative newborn lamb urine samples were collected in the following manner. A sterile bag was attached to the bladder catheter, then bag was placed in a small plastic housing that was bandaged to the abdomen of the animal. This permitted free movement of the lambs within the pen and also allowed them to nurse on their mother ad libitum.

**Pregnant Sheep Experiments.** All experiments on pregnant sheep were completed between 125 and 138 days gestation (term ~145 days). Two sets of experiments were carried out on all five pregnant sheep in a randomized manner and with an appropriate washout period in between.

**Maternal administration.** A bolus loading dose of VPA (sodium valproate; Sigma Chemical Co., St. Louis, MO) equivalent to 20.1 mg VPA/kg maternal body weight was administered to the ewe via the maternal femoral venous catheter over 1 min; this was followed immediately by a 24-h continuous infusion of the drug at 138.3 μg/min/kg via the same route.

**Fetal administration.** The fetal experimental protocol was similar to that for the maternal experiments described above, except that doses were administered via the fetal lateral tarsal vein and were reduced to one-fourth the maternal doses (i.e., 5.0 mg/kg bolus and 34.6 μg/min/kg infusion rate based on maternal body weight).

**Newborn Lamb Experiments.** As mentioned above, the newborn lamb experiments were begun the day after birth. Drug administration involved a 10 mg/kg bolus administered over 1 min via the lateral tarsal vein, followed immediately by a continuous 6-h infusion at 138.3 μg/min/kg via the same route. VPA was infused to newborn lambs only for a period of 6 h for two reasons. Firstly, the drug caused marked sedation in lambs so much so that it interfered with their normal feeding behavior. Secondly, long-term infusion necessitated separation of the lambs from their mothers for at least the infusion duration, and hence restriction of their free movement, nursing, and maternal-newborn bonding.

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

In all pregnant sheep experiments, serial blood samples were collected from the fetal (2 ml) and maternal (3.0 ml) femoral arterial catheters at 5 min, and 0.5, 1, 3, 6, 9, 12, 20, 22, and 24 h during the infusion, and at 0.5, 1, 3, 6, 9, 12, 24, 36, 48, 60, and 72 h postinfusion. 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 during the experiment was replaced, at intervals, by an equal volume of blood obtained from the mother before the start of the experiment or from another ewe (after the first day). Samples of maternal and fetal urine, amniotic fluid, and fetal tracheal fluid were also collected at predetermined intervals.

During the newborn lamb experiments, serial femoral arterial blood samples (2-ml) were collected at 5 min, and 0.5, 1, 2, 3, 4, 5, and 6 h during the infusion, and at 0.5, 1, 2, 4, 6, 18, 30, 42, 54, 66, 78, and 90 h postinfusion. Cumulative urine samples were also collected for 96 h.

All maternal, fetal, and newborn blood samples were placed into heparinized Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) and centrifuged at 2000g for 10 min. The plasma supernatant was removed and placed into clean borosilicate test tubes with polytetrafluoroethylene-lined caps. Samples were stored frozen at ~20°C until the time of analysis.

**Determination of VPA Plasma Protein Binding.** The unbound plasma concentrations of VPA were measured ex vivo in all fetal, maternal, and newborn plasma samples by an ultrafiltration procedure at 1000g for 30 min using Centrifree micropartition devices (Amicon, Inc., Danver, MA). Briefly, 0.75 ml of plasma was placed into Centrifree micropartition devices, followed by centrifugation at 1000g for 30 min and measurement of ultrafilterate VPA concentrations as described below. Plasma samples for the determination of unbound VPA concentrations were stored in separate aliquots so as to avoid repetitive thawing that could result in lipolysis and release of free fatty acids,
Drug and Metabolite Assay. The concentrations of VPA and its metabolites in all biological fluids and plasma ultrafiltrate were measured using a previously developed gas chromatographic-mass spectrometric analytical method (Yu et al., 1995). Validation studies demonstrate that the variability and bias of this assay for all compounds does not exceed 15% (Yu et al., 1995). In this article, only the VPA concentrations will be reported. The metabolite data are presented in the companion article (Kumar et al., 2000b). The lower limit of quantitation of this assay for VPA was 25 ng/ml using 0.1 ml of plasma.

Physiological Recording and Monitoring Procedures. Fetal blood pH, PO₂, and PACO₂ were measured using an IL 1306 pH/blood gas analyzer (Allied Instrumentation Laboratory, Milan, Italy). Blood O₂ 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).

Pharmacokinetic Analyses. For the analysis of protein binding data in maternal, fetal, and newborn plasma, plasma protein-bound concentrations of the drug were first calculated from the difference between corresponding experimentally measured total and unbound concentrations. Plasma protein binding parameters were calculated separately for maternal, fetal, and newborn plasma using the respective pooled bound and unbound plasma concentration data from all animals. Rosenthal plots (bound/unbound concentration versus bound concentration) were first constructed to identify multiplicity of the binding sites. The bound versus unbound concentration data were then fitted to the appropriate binding model using the nonlinear least-squares regression method (D’Argenio and Schumitzky, 1997). The best binding model was identified based on the reduction in sum of squared residuals and Akaike’s Information Criterion (AIC).

Net steady-state maternal and fetal clearances of the unbound drug (CLₘₑₙₚₙ and CLₙₑₙₚₙ, respectively) were calculated by dividing the respective VPA infusion rate by the corresponding steady-state arterial plasma unbound VPA concentration (Gibaldi and Perrier, 1982). The maternal and fetal net clearances of the total drug (CLₘₑₙₚₙ and CLₙₑₙₚₙ, respectively) were calculated in an analogous fashion, except that total instead of unbound maternal and fetal arterial plasma concentrations were used.

Placental and nonplacental clearances of unbound VPA in the ewe and the fetus were calculated from the maternal and fetal steady-state arterial plasma VPA concentrations according to the two-compartment model described earlier (Szeto et al., 1982b). This model is based on separate steady-state maternal and fetal drug administration and assumes bidirectional placental transfer and drug elimination from both maternal and fetal compartments. Thus, the model is defined by four clearance parameters, including maternal-to-fetal placental clearance (CLₘₖₜ), fetal-to-maternal placental clearance (CLₖₘₜ), maternal nonplacental clearance (CLₘₙₚ), and fetal nonplacental clearance (CLₙₙₚ), all clearances of the total drug. Maternal and fetal total clearances of the total drug (CLₘₚ and CLₙₚ, respectively) equal the sum of their respective placental and nonplacental clearances. All these clearance parameters were calculated from the following equations (eqs. 1–6) as described previously (Szeto et al., 1982b).

\[
\begin{align*}
CL_{mm} &= \frac{k_\text{m}}{C_m - C'_{mm} \cdot (C_m'/C_m)}
\end{align*}
\]

\[
\begin{align*}
CL_{mf} &= \frac{k_\text{m}'}{C'_m - C_{mm} \cdot (C_m'/C_m)}
\end{align*}
\]

\[
\begin{align*}
CL_{nf} &= CL_{mf} \cdot (C_m'/C_m)
\end{align*}
\]

\[
\begin{align*}
CL_{nm} &= CL_{mm} - (C_m'/C'_m)
\end{align*}
\]

\[
\begin{align*}
CL_{nf} &= CL_{nm} - CL_{mf}
\end{align*}
\]

\[
\begin{align*}
CL_{nf} &= CL_{mf} - CL_{nm}
\end{align*}
\]

The symbols \(k_\text{m}\) and \(k_\text{m}'\) denote the drug infusion rates to the mother and the fetus, respectively. \(C_m\) and \(C'_m\) are the steady-state plasma drug concentrations in the mother and the fetus after maternal VPA administration, respectively. and \(C_{mm}'\) and \(C'_m\) are the steady-state maternal and fetal plasma drug concentrations, respectively, after fetal VPA administration.

The net maternal and fetal clearances described above sum the overall rate of drug elimination from the maternal-fetal unit via maternal and fetal nonplacental routes after maternal or fetal drug administration, respectively. These clearances are related to the corresponding two-compartment estimates of total maternal and fetal clearance by the following relationships (Szeto et al., 1982b; Wang et al., 1986):

\[
\begin{align*}
CL_{mm} &= CL_{mm}^\text{total} + CL_{mf} \cdot \frac{C'_m}{C_m}
\end{align*}
\]

\[
\begin{align*}
CL_{nf} &= CL_{nf}^\text{total} - \frac{C_m'}{C_m}
\end{align*}
\]

and,

\[
\begin{align*}
CL_{mf} &= CL_{mf} + CL_{mm} \cdot \frac{C_m'}{C'_m}
\end{align*}
\]

\[
\begin{align*}
CL_{nf} &= CL_{nf} - CL_{mf} \cdot \frac{C'_m}{C'_m}
\end{align*}
\]

The presence of saturable plasma protein binding presented a complexity in the estimation of placental and nonplacental clearances for VPA using the above method. The two-compartment model assumes an altered linear pharmacokinetics between the maternal and fetal drug administration experiments. However, this assumption was violated for VPA because of significantly different steady-state maternal plasma unbound VPA fractions during maternal and fetal dosing (0.35 ± 0.13 versus 0.18 ± 0.08, respectively; Table 1); this would result in proportional alterations in total (not unbound) maternal VPA clearance during the two experimental periods for this low-clearance drug (Wilkinson and Shand, 1975). Thus, steady-state unbound plasma VPA concentrations were used for the estimation of maternal and fetal placental and nonplacental clearances. The “effective” placental and nonplacental clearances of total VPA in the mother and the fetus were calculated as the product of respective unbound clearance parameter and the steady-state maternal (in case of maternal clearances) or fetal (in case of fetal clearances) plasma unbound fraction.

All other pharmacokinetic parameters were calculated by the equations described below (Gibaldi and Perrier, 1982). Maternal and fetal parameters were calculated using the data from maternal and fetal infusion experiments, respectively. Mean residence time (MRT) of VPA (total or unbound):

\[
\begin{align*}
\text{MRT}_{\text{total or unbound drug}} &= \frac{\text{AUMC}^{\text{total or unbound drug}}}{2(k_\tau + \gamma D_{\text{bolus}})}
\end{align*}
\]

where \(k_\tau\), \(\gamma\), and \(D_{\text{bolus}}\) are the infusion rate, infusion duration, and initial bolus dose of VPA, respectively. Plasma AUMC₀₋∞ and AUMC₀₋∞ values of the unbound and total drug were calculated by the linear trapezoidal rule.

Total body clearance (CLₜₜₜ) of VPA (total or unbound):

\[
\begin{align*}
\text{CL}_{\text{total or unbound}} &= \frac{\text{Total i.v. dose}}{\text{AUC}^{\text{total or unbound}}}
\end{align*}
\]

Area-weighted unbound fraction of the drug (\(f_p\)):

\[
\begin{align*}
\text{AUC}^{\text{total or unbound drug}} = \frac{\text{AUC}^{\text{total or unbound drug}}}{\text{AUC}^{\text{total drug}}}
\end{align*}
\]

Steady-state volume of distribution of the unbound drug (\(V_{d_{uu}}\)):

\[
\begin{align*}
V_{d_{uu}} = (CL_{\text{total or unbound drug}} \times \text{MRT}_{\text{unbound drug}})
\end{align*}
\]

Steady-state volume of distribution of the total drug (\(V_{d_{t}}\)):

\[
\begin{align*}
V_{d_{t}} = (CL_{\text{total or unbound drug}} \times \text{MRT}_{\text{total drug}})
\end{align*}
\]
TABLE 1
Mean steady-state maternal and fetal plasma concentrations of total and unbound VPA, maternal-fetal plasma VPA concentration ratios, and steady-state plasma unbound fractions in five pregnant ewes

<table>
<thead>
<tr>
<th>Maternal Administration</th>
<th>E4241</th>
<th>E5108</th>
<th>E105x</th>
<th>E4133</th>
<th>E1226</th>
<th>Mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal body weight (kg)</td>
<td>83.0</td>
<td>67.1</td>
<td>87.5</td>
<td>85.0</td>
<td>65.0</td>
<td>77.5 ± 10.6</td>
</tr>
<tr>
<td>Estimated fetal body weight (kg)</td>
<td>3.01</td>
<td>3.14</td>
<td>3.06</td>
<td>2.64</td>
<td>2.79</td>
<td>2.93 ± 0.21</td>
</tr>
<tr>
<td>Total maternal plasma concentration (C_m, μg/ml)</td>
<td>101.0</td>
<td>55.2</td>
<td>208.3</td>
<td>79.9</td>
<td>88.3</td>
<td>106.5 ± 59.3</td>
</tr>
<tr>
<td>Total fetal plasma concentration (C_f, μg/ml)</td>
<td>79.5</td>
<td>36.2</td>
<td>173.0</td>
<td>49.0</td>
<td>55.3</td>
<td>78.6 ± 55.06</td>
</tr>
<tr>
<td>Unbound maternal plasma concentration (C_m, μg/ml)</td>
<td>39.5</td>
<td>20.0</td>
<td>113.1</td>
<td>16.8</td>
<td>23.3</td>
<td>41.7 ± 40.5</td>
</tr>
<tr>
<td>Unbound fetal plasma concentration (C_f, μg/ml)</td>
<td>29.9</td>
<td>13.6</td>
<td>103.2</td>
<td>13.4</td>
<td>18.5</td>
<td>35.7 ± 38.3</td>
</tr>
<tr>
<td>C_f/C_m ratio</td>
<td>0.79</td>
<td>0.68</td>
<td>0.83</td>
<td>0.61</td>
<td>0.63</td>
<td>0.70 ± 0.106</td>
</tr>
<tr>
<td>C_unbound/f_total ratio</td>
<td>0.85</td>
<td>0.68</td>
<td>0.91</td>
<td>0.80</td>
<td>0.79</td>
<td>0.81 ± 0.096</td>
</tr>
<tr>
<td>Steady-state maternal plasma unbound fraction</td>
<td>0.35</td>
<td>0.36</td>
<td>0.54</td>
<td>0.21</td>
<td>0.26</td>
<td>0.35 ± 0.13</td>
</tr>
<tr>
<td>Steady-state fetal plasma unbound fraction</td>
<td>0.38</td>
<td>0.38</td>
<td>0.60</td>
<td>0.27</td>
<td>0.34</td>
<td>0.39 ± 0.12</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fetal administration</th>
<th>E4241</th>
<th>E5108</th>
<th>E105x</th>
<th>E4133</th>
<th>E1226</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated fetal body weight (kg)</td>
<td>2.62</td>
<td>3.51</td>
<td>2.74</td>
<td>2.99</td>
<td>3.07</td>
</tr>
<tr>
<td>Total maternal plasma concentration (C_m, μg/ml)</td>
<td>33.0</td>
<td>20.3</td>
<td>68.6</td>
<td>28.7</td>
<td>28.7</td>
</tr>
<tr>
<td>Total fetal plasma concentration (C_f, μg/ml)</td>
<td>51.7</td>
<td>37.6</td>
<td>102.1</td>
<td>58.5</td>
<td>43.7</td>
</tr>
<tr>
<td>Unbound maternal plasma concentration (C_m, μg/ml)</td>
<td>6.5</td>
<td>3.8</td>
<td>20.3</td>
<td>3.7</td>
<td>2.9</td>
</tr>
<tr>
<td>Unbound fetal plasma concentration (C_f, μg/ml)</td>
<td>17.1</td>
<td>7.7</td>
<td>40.1</td>
<td>19.3</td>
<td>9.4</td>
</tr>
<tr>
<td>C_f/C_m ratio</td>
<td>0.64</td>
<td>0.54</td>
<td>0.67</td>
<td>0.49</td>
<td>0.66</td>
</tr>
<tr>
<td>C_unbound/C_total ratio</td>
<td>0.38</td>
<td>0.50</td>
<td>0.51</td>
<td>0.19</td>
<td>0.30</td>
</tr>
<tr>
<td>Steady-state maternal plasma unbound fraction</td>
<td>0.20</td>
<td>0.19</td>
<td>0.30</td>
<td>0.13</td>
<td>0.10</td>
</tr>
<tr>
<td>Steady-state fetal plasma unbound fraction</td>
<td>0.33</td>
<td>0.20</td>
<td>0.39</td>
<td>0.33</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Steady-state volume of distribution of the total drug corrected for the effects of saturable plasma protein binding (Vd_u): Vd_u = f_p*Vd_p

For drugs with saturable plasma protein binding, Vd_p is constant only for a particular f and can be used to relate steady-state plasma concentrations to the amount of the drug in the brain if the steady-state unbound fraction of the drug is equal to f (McNamara et al., 1983). Also for such drugs, the Vd_m of the total drug obtained using eq. 13 overestimates the “true” Vd, instead, Vd_u provides a more reliable estimate of true Vd. McNamara et al., 1983). As with Vd_p above, this Vd_u parameter is also constant only for a particular f or a steady-state fetal unbound fraction equivalent to f.

Maternal and fetal terminal elimination half-life (t_1/2) of the total and unbound drug was obtained from a two-compartment model fitting of the data using the nonlinear least-squares regression software WinNonlin (Scientific Consulting, Inc., Apex, NC). Maternal t_1/2 values were calculated from the data obtained during maternal VPA infusion, whereas fetal t_1/2 values were calculated from those obtained during fetal infusion. The terminal plasma t_1/2 of the total and unbound drug in the newborn lamb was approximated using an one-compartment model. These model fittings were carried out using a weighting factor of 1/predicted (y^2).

Statistical Analysis. All data are reported as mean ± S.D. unless otherwise indicated. The achievement of steady state for total and unbound VPA concentrations in maternal and fetal plasma was established according to two criteria: 1) the slope of the plasma concentration versus time curve should not be significantly different from zero, and 2) the coefficient of variation of the measured concentrations should be <10%. Maternal and fetal steady-state plasma concentrations measured during the same infusion experiment were compared using the paired t test. All maternal versus fetal pharmacokinetic parameters including net clearances, two-compartment clearances (placental, nonplacental, and total), overall clearances, volumes of distribution, half-lives, and MRT values were also compared using the paired t test. Amniotic and fetal tracheal fluid VPA concentrations were also compared with fetal plasma unbound concentrations from the same experiment using the paired t test. Newborn pharmacokinetic parameters were compared with those in the ewe and fetus using the unpaired t test. The significance level was P < .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 (Koong et al., 1975).

Results

The average maternal body weight was 77.5 ± 10.6 kg and estimated fetal body weights on the day of maternal and fetal VPA infusion were 2.93 ± 0.21 and 2.98 ± 0.34 kg, respectively. The mean gestational age on the day of maternal and fetal experiments was 129.8 ± 4.3 and 130.2 ± 2.2 days, respectively, and these were not statistically different (paired t test, P > .05). During maternal experiments, the control period fetal femoral arterial blood pH, Po2, Pco2, O2-saturation, and hemoglobin, glucose, and lactate concentrations were 7.344 ± 0.047, 20.8 ± 5.9 mm Hg, 50.4 ± 1.8 mm Hg, 41.6 ± 15.4%, 12.6 ± 3.2 g/dl, 0.54 ± 0.08 mM, and 0.91 ± 0.40 mM, respectively. Likewise during fetal administration, the control values for these variables were 7.313 ± 0.067, 20.0 ± 4.1 mm Hg, 49.9 ± 2.6 mm Hg, 38.0 ± 13.3%, 12.8 ± 3.6 g/dl, 0.95 ± 0.48 mM, and 1.27 ± 0.80 mM, respectively. Apart from a small increase in fetal blood lactate concentrations during both maternal (1.40 ± 0.54 mM) and fetal (1.40 ± 0.25 mM) infusions, there were no systematic changes in any of these variables during the experimental period. Because appropriate control saline infusion experiments were not carried out, a more detailed analysis of these data was not possible.

Placental Transfer and Maternal and Fetal Plasma Protein Binding of VPA. Figure 1A shows representative total as well as unbound maternal and fetal arterial blood plasma concentration-time profiles of VPA during and after a 24-h maternal VPA infusion. Figure 1B shows similar profiles during and after a 24-h fetal VPA infusion. Total as well as unbound VPA concentrations in maternal and fetal plasma were at steady state during the 6- to 24-h infusion period according to the criteria described in the data analysis section. Placental transfer of VPA was rapid, with significant concentrations detected on the other side of placenta at the first sampling time (5 min) after drug dosing (Fig. 1).

Table 1 presents the steady-state maternal and fetal femoral arterial unbound as well as total plasma concentrations and unbound fractions of VPA during separate maternal and fetal 24-h infusion experiments. During maternal drug infusion, the fetal steady-state unbound and
total plasma concentrations ($C^m_1$ and $C^f_1$) were significantly lower compared with the corresponding maternal values ($C^m_1$ and $C^m_2$). Thus, the indices of fetal drug exposure based on both unbound and total steady-state fetal/maternal plasma concentration ratios ($0.81 \pm 0.09$ and $0.70 \pm 0.10$) were significantly less than unity. Similarly, during fetal drug infusion, the steady-state maternal unbound as well as total plasma concentrations ($C^m_1$ and $C^m_2$) were significantly lower than the corresponding fetal values ($C^f_1$ and $C^f_2$). Also, the $C^m_1$/$C^f_1$ ($0.60 \pm 0.08$) and $C^m_2$/$C^f_2$ ($0.38 \pm 0.13$) ratios were significantly lower than unity.

Plasma protein binding of VPA was saturable (or nonlinear) in both the mother and fetus, and thus varied inversely with total VPA concentration. The range of plasma-unbound VPA fractions in maternal and fetal plasma during maternal drug administration were 0.09 to 0.45 and 0.12 to 0.44, respectively. Similarly, these ranges during fetal drug infusion were 0.10 to 0.21 and 0.12 to 0.53, respectively. VPA plasma unbound fraction in both maternal and fetal plasma at steady state was constant during individual administration experiments because maternal and fetal plasma total as well as unbound plasma concentrations were relatively constant (Fig. 1). Table 1 also presents steady-state maternal and fetal plasma unbound fractions during both maternal and fetal drug infusions. The average steady-state maternal plasma unbound fraction of VPA during fetal drug infusion was significantly lower compared with that during maternal drug infusion ($0.18 \pm 0.08$ versus $0.35 \pm 0.13$). However, the average steady-state fetal plasma unbound fractions during maternal and fetal drug infusions were not significantly different ($0.39 \pm 0.12$ versus $0.29 \pm 0.08$).

Because of insufficient data points for individual animals to characterize plasma protein binding at a wide enough range of concentrations, binding parameters were estimated separately for the mother and the fetus using pooled maternal and fetal data from all experiments. Figure 2 shows VPA binding characteristics in maternal and fetal plasma using pooled bound and unbound VPA concentration data from all experiments. Rosenthal plots in both maternal and fetal plasma (Fig. 2, A and C, respectively) show a biphasic curvilinear relationship. The initial steep declining portion of the Rosenthal plots suggests the presence of a high-affinity but low-capacity (saturable) binding site, whereas the relatively flat portion of the curves suggests a linear (nonsaturable) or another saturable binding site with a low affinity but high binding capacity. Statistically better fits (lower AIC and sum of squares, smaller c.v. values for fitted parameters) were obtained when the bound versus unbound concentration data were fit to a two-site binding model with one saturable and one nonsaturable binding site (eq. 15) as compared with a one-site binding model.

$$C_u = \frac{B_{\text{max}1} \cdot C_u}{K_{d1}} + \frac{B_{\text{max}2} \cdot C_u}{K_{d2}}$$

where, $C_b$ and $C_u$ are the corresponding bound and unbound plasma concentrations, $B_{\text{max}1}$ and $B_{\text{max}2}$ are the maximal VPA binding capacities of the first and second binding site, respectively. $K_{d1}$ and $K_{d2}$ are the equilibrium dissociation constants of VPA at the first and second binding site, respectively.

Figure 2, B and D, shows the scatter plots of the pooled bound versus unbound VPA concentration data in maternal and fetal plasma, respectively, from all the experiments. The model-predicted lines based on eq. 15 are also depicted, and indicate excellent fit of the data to the above model. From Fig. 2, B and D, it appears that the data from one animal (E105x), which had higher VPA plasma concentrations compared with the other four ewes, could unduly influence the final maternal and fetal VPA binding parameter estimates. Hence, maternal and fetal VPA plasma protein binding parameters were also estimated after excluding the data from this animal. Table 2 presents the estimates of binding parameters of VPA in maternal and fetal plasma obtained from the entire pooled data as well as those obtained after excluding the data from E105x. The estimates of VPA binding parameters for maternal plasma were similar using the entire data or after excluding E105x from analysis. However, data from E105x do appear to significantly influence the binding parameters for fetal plasma (Table 2). Using the entire data, the maximal VPA binding capacity of maternal and fetal plasma at the high-affinity saturable binding site ($B_{\text{max}1}$) is similar (62.8 versus 65.0 µg/ml); however, after excluding the data from E105x, the capacity of fetal plasma at this site appears somewhat lower (46.4 µg/ml). Similarly, the affinity of this binding site toward VPA appears substantially lower in fetal plasma relative to that in maternal plasma using the entire pooled data ($K_{d1}$ of 7.6 versus 13.0 µg/ml in maternal and fetal plasma, respectively). However, there is no difference in the VPA binding affinity of maternal and fetal plasma after the data from E105x are excluded ($K_{d1}$ of 6.8 versus 7.2 µg/ml, respectively). Thus, it appears that in E105x, fetal plasma had a substantially higher capacity and lower affinity for VPA binding as compared with the remaining four ewes. Although the exact reasons underlying this remain unclear, it may result from the...
presence of higher plasma protein concentrations in combination with significant concentrations of certain endogenous substances in fetal plasma that can compete with VPA for binding sites (e.g., bilirubin, free fatty acids).

Placental and Nonplacental Clearances of VPA in the Mother and the Fetus. Table 3 presents the weight-normalized steady-state maternal and fetal clearances of VPA based on unbound as well as total drug concentrations. Maternal net, total, and nonplacental clearances are normalized to maternal body weight, whereas all other clearances are normalized to the estimated fetal body weight. Normalization of maternal as well as fetal placental clearance to estimated fetal body weight allows for an easier comparison of the bidirectional placental transfer efficiency. The net maternal and fetal clearances are lower compared with the corresponding two-compartment estimates of total maternal or fetal clearance because of the relationships described in eqs. 7a and 8a (Szeto et al., 1982b; Wang et al., 1986). Fetal net (CL_{u\text{f(net)}}) and total (CL_{u\text{ff}}) clearances of the unbound drug were significantly greater compared with the corresponding maternal clearances (CL_{u\text{m(net)}} and CL_{u\text{mm}} , respectively). However, there was no significant difference between maternal and fetal placental clearances of the unbound drug (CL_{u\text{mf}} and CL_{u\text{fm}} , respectively). Also, CL_{u\text{fo}} was significantly greater than CL_{u\text{mo}}.

The maternal clearances of the total VPA (CL_{u\text{m(net)}} , CL_{u\text{mm}} , CL_{u\text{mf}} , and CL_{u\text{mo}}) presented in Table 3 are calculated as the product of steady-state maternal plasma unbound fraction during maternal infusion and the corresponding maternal clearance of the unbound drug. Similarly, the fetal clearances of total VPA (CL_{u\text{f(net)}} , CL_{u\text{ff}} , CL_{u\text{fm}} , and CL_{u\text{fo}}) are the product of steady-state fetal plasma unbound fraction during fetal drug infusion and the corresponding fetal clearance of the unbound drug. As with the unbound clearances above, CL_{u\text{f(net)}} , CL_{u\text{ff}} , and CL_{u\text{fo}} values were significantly higher compared with CL_{u\text{m(net)}} , CL_{u\text{mm}} , and CL_{u\text{mo}} values, respectively; however, CL_{u\text{mf}} and CL_{u\text{fm}} values were not significantly different.

### TABLE 2

Estimated plasma protein binding parameters of VPA in maternal, fetal, and newborn sheep obtained by fitting the pooled data to a one- or two-site binding model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Maternal</th>
<th>Fetal</th>
<th>Newborn</th>
</tr>
</thead>
<tbody>
<tr>
<td>B_{max1} (µg/ml)</td>
<td>62.8 ± 5.4</td>
<td>65.0 ± 8.4</td>
<td>71.8 ± 15.7</td>
</tr>
<tr>
<td>K_{d1} (µg/ml)</td>
<td>7.6 ± 1.3</td>
<td>13.0 ± 2.9</td>
<td>13.3 ± 6.6</td>
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<tr>
<td>B_{max2}/K_{d2}</td>
<td>0.34 ± 0.06</td>
<td>0.17 ± 0.07</td>
<td>(0.38 ± 0.22)</td>
</tr>
</tbody>
</table>

* Fit of the data to a two-site binding model
* Values in parentheses are the parameters estimated after excluding data from E105s.
* Fit of the data to a one-site binding model.
* B_{max1}, maximal VPA binding capacity of the plasma at the high-affinity saturable binding site; K_{d1}, dissociation constant of VPA binding at the high-affinity saturable binding site; B_{max2}/K_{d2}, maximal binding capacity/dissociation constant ratio at the low-affinity nonsaturable binding site.

Fig. 2. Maternal and fetal plasma protein binding characteristics of VPA; pooled data from all maternal and fetal VPA infusion experiments. C_{u\text{a}} and C_{u\text{c}} are the maternal or fetal plasma concentrations of the bound and unbound VPA. A and C, the Rosenthal plots of the data in maternal and fetal plasma, respectively, demonstrating a biphasic relationship. B and D, the relationship between C_{u\text{a}} versus C_{u\text{c}} in maternal and fetal plasma, respectively; actual data (scatter points) and model-predicted line obtained from the fit of the data to a two-site binding model are depicted. Different scatter plot symbols denote data from different animals.
Mean steady-state maternal and fetal placent al and nonplacental clearances of unbound and total VPA in five pregnant ewes during late gestation

Maternal net, total, and nonplacental clearances are expressed as milliliters per minute per kilogram of maternal body weight; all other clearances are expressed as milliliters per minute per kilogram of estimated fetal body weight.

<table>
<thead>
<tr>
<th>Unbound Clearances</th>
<th>( CL_{\text{unb}}^{\text{net}} )</th>
<th>( CL_{\text{unb}}^{\text{mat}} )</th>
<th>( CL_{\text{unb}}^{\text{fet}} )</th>
<th>( CL_{\text{unb}}^{\text{tot}} )</th>
<th>( CL_{\text{ub}}^{\text{tot}} )</th>
<th>( CL_{\text{ub}}^{\text{fet}} )</th>
<th>( CL_{\text{ub}}^{\text{mat}} )</th>
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<tr>
<td>E4241</td>
<td>3.9</td>
<td>5.8</td>
<td>3.3</td>
<td>80.2</td>
<td>64.1</td>
<td>94.6</td>
<td>24.5</td>
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<tr>
<td>E5108</td>
<td>6.9</td>
<td>10.5</td>
<td>5.8</td>
<td>88.7</td>
<td>86.3</td>
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<tr>
<td>E105x</td>
<td>1.2</td>
<td>2.3</td>
<td>0.8</td>
<td>46.6</td>
<td>27.5</td>
<td>51.1</td>
<td>14.4</td>
</tr>
<tr>
<td>E4133</td>
<td>8.2</td>
<td>9.7</td>
<td>8.0</td>
<td>47.8</td>
<td>50.9</td>
<td>60.0</td>
<td>7.5</td>
</tr>
<tr>
<td>E1226</td>
<td>5.9</td>
<td>7.8</td>
<td>4.0</td>
<td>81.9</td>
<td>78.1</td>
<td>103.1</td>
<td>52.5</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>5.2 ± 2.7</td>
<td>7.2 ± 3.3</td>
<td>4.4 ± 2.7</td>
<td>69.0 ± 20.2</td>
<td>61.4 ± 23.2</td>
<td>87.8 ± 32.4</td>
<td>25.9 ± 17.3</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Total Clearances</th>
<th>( CL_{\text{ub}}^{\text{tot}} )</th>
<th>( CL_{\text{ub}}^{\text{mat}} )</th>
<th>( CL_{\text{ub}}^{\text{fet}} )</th>
<th>( CL_{\text{ub}}^{\text{tot}} )</th>
<th>( CL_{\text{ub}}^{\text{fet}} )</th>
<th>( CL_{\text{ub}}^{\text{mat}} )</th>
</tr>
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<tr>
<td>E4241</td>
<td>2.0</td>
<td>1.1</td>
<td>28.0</td>
<td>21.2</td>
<td>31.3</td>
<td>8.1</td>
</tr>
<tr>
<td>E5108</td>
<td>1.3</td>
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<td>32.1</td>
<td>17.6</td>
<td>26.6</td>
<td>6.3</td>
</tr>
<tr>
<td>E105x</td>
<td>0.7</td>
<td>0.4</td>
<td>25.3</td>
<td>10.8</td>
<td>20.1</td>
<td>5.7</td>
</tr>
<tr>
<td>E4133</td>
<td>2.7</td>
<td>1.7</td>
<td>10.1</td>
<td>16.8</td>
<td>19.8</td>
<td>2.5</td>
</tr>
<tr>
<td>E1226</td>
<td>1.6</td>
<td>2.1</td>
<td>21.6</td>
<td>16.8</td>
<td>22.2</td>
<td>11.3</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>1.6 ± 0.7</td>
<td>2.2 ± 0.9</td>
<td>1.3 ± 0.6</td>
<td>23.4 ± 8.4</td>
<td>16.7 ± 3.7</td>
<td>24.0 ± 4.9</td>
</tr>
</tbody>
</table>

\(^{a}\) Significantly higher compared to the corresponding maternal clearance value \((P < .005)\).

\(^{b}\) Significantly higher compared to the corresponding maternal clearance value \((P < .05)\).

The average contribution of maternal nonplacental clearance to net maternal drug elimination \((\%CL_{\text{ub}}^{\text{mat}}/CL_{\text{ub}}^{\text{net}})\) based either on total or unbound concentrations was 79.6 ± 13.2%, with placental elimination accounting for the remainder \((20.4 ± 13.2\%)\). The contribution of fetal nonplacental clearance to net fetal drug elimination \((\%CL_{\text{ub}}^{\text{fet}}/CL_{\text{ub}}^{\text{net}})\) was significantly lower compared with that in the mother \((41.7 ± 19.6\%; P < .005)\). Thus, there is a significantly greater contribution of fetal placental clearance to net fetal drug elimination as compared with the mother \((58.3 ± 19.6\%)\).

Amniotic and Fetal Tracheal Fluid Disposition of VPA. In all animals, VPA was detectable in amniotic and fetal tracheal fluids at the earliest sampling time, i.e., 5 min after the start of maternal or fetal VPA infusion. As with maternal and fetal plasma, the amniotic and tracheal fluid VPA concentrations were also at an apparent steady-state during the 6- to 24-h period of infusion (Fig. 3). In general, tracheal fluid concentrations exhibited somewhat larger fluctuations compared with amniotic fluid. The steady-state VPA concentrations in amniotic fluid during maternal and fetal infusion were 17.3 ± 13.8 and 11.0 ± 4.7 \(\mu g/ml\), respectively, whereas those in tracheal fluid were 2.2 ± 1.4 and 1.0 ± 0.5 \(\mu g/ml\), respectively. The tracheal fluid VPA concentrations were lower compared with the fetal plasma unbound concentrations during both fetal (statistically significant) and maternal (not significant) drug administration. Similarly, the amniotic fluid VPA concentrations were lower than the fetal plasma unbound VPA concentrations in all but one animal; however, the difference between means was not statistically significant during maternal or fetal drug administration. During the postinfusion period, the concentrations in these fluids appeared to decline in parallel with maternal and fetal plasma concentrations (Figs. 1 and 3).

Overall Maternal and Fetal Pharmacokinetics of VPA. Table 4 presents the overall pharmacokinetic parameters of VPA in the mother and the fetus. The mean maternal and fetal total body clearances of the unbound as well as the total drug presented in Table 4 are similar to the corresponding net steady-state clearances presented in Table 3. Like the steady-state net fetal clearance (Table 3), the fetal \( CL_{\text{ub}} \) and total body unbound clearance \((CL_{\text{unb}}^{\text{tot}})\) values were significantly higher than the corresponding maternal values (Table 4). Fetal terminal elimination half-life of the unbound drug \((t_{1/2\text{ub}})\) was significantly shorter than the corresponding maternal \(t_{1/2\text{ub}}\) (Normal 3.1 ± 1.3 versus 5.5 ± 1.9 h). However, there was no significant difference between maternal and fetal \( t_{1/2\text{ub}} \) values based on total plasma drug concentrations \((t_{1/2\text{ub}})\) (5.6 ± 1.4 versus 4.6 ± 1.9 h). Maternal \( t_{1/2\text{ub}} \) values based on maternal plasma unbound \((t_{1/2\text{ub}}^{\text{mat}})\) and total drug \((t_{1/2\text{ub}}^{\text{tot}})\) concentrations were not statistically different \((5.5 ± 1.9 \text{ versus } 5.6 ± 1.4 \text{ h})\). In contrast, fetal \( t_{1/2\text{ub}}^{\text{fet}} \) was significantly shorter than fetal \( t_{1/2\text{ub}}^{\text{mat}} \) \((3.1 ± 1.3 \text{ versus } 4.6 ± 1.9 \text{ h})\). There was no significant difference between MRT of the drug in maternal and fetal circulation based either on total or unbound drug concentrations. However, in both the mother and the fetus, the MRT of the unbound drug was significantly shorter compared with that for the total drug. All fetal steady-state volume of distribution parameters were significantly greater than the corresponding maternal values.

Pharmacokinetics of VPA in Newborn Lambs. Figure 4A shows representative plasma concentration versus time profiles of VPA in E4241, showing apparent steady-state concentrations in these fluids during the 6- to 24-h infusion period and subsequent rapid decline in concentrations during the postinfusion phase.
same pharmacokinetic parameters for the unbound drug. Similar to (C) were also observed in all animals after fetal VPA infusion and unbound VPA in maternal and fetal plasma during the initial 6-h period of the 24-h maternal VPA infusion in E5108, showing lack of any drug accumulation. Profiles in NL0123z during the 6-h infusion period, showing continuous accumulation of the drug in newborn plasma; C, typical plasma concentration versus time profiles of total VPA in maternal and fetal plasma during the initial 6-h period of the 24-h maternal VPA infusion in E5108, showing lack of any drug accumulation. Profiles similar to (C) were also observed in all animals after fetal VPA infusion.

Table 4

<table>
<thead>
<tr>
<th>Maternal</th>
<th>Unbound VPA</th>
<th>Total VPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL&lt;sub&gt;u&lt;/sub&gt;</td>
<td>t&lt;sub&gt;1/2,b&lt;/sub&gt;</td>
<td>MRT&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>E4241</td>
<td>4.1</td>
<td>5.0</td>
</tr>
<tr>
<td>E5108</td>
<td>7.0</td>
<td>4.0</td>
</tr>
<tr>
<td>E105x</td>
<td>1.4</td>
<td>7.4</td>
</tr>
<tr>
<td>E4133</td>
<td>8.3</td>
<td>3.5</td>
</tr>
<tr>
<td>E1226</td>
<td>6.0</td>
<td>7.5</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>5.4 ± 2.7</td>
<td>5.5 ± 1.9</td>
</tr>
</tbody>
</table>

Fetal

| E4241 | 62.4 | 3.8 | 1.8 | 6.65 | 20.4 | 4.8 | 0.33 | 3.89 | 2.18 |
| E5108 | 86.1 | 2.4 | 1.0 | 4.92 | 17.5 | 4.3 | 0.20 | 2.07 | 1.90 |
| E105x | 30.3 | 5.0 | 4.2 | 7.72 | 10.1 | 7.4 | 0.33 | 3.84 | 2.57 |
| E4133 | 52.1 | 1.9 | 1.7 | 5.31 | 16.5 | 2.1 | 0.32 | 1.87 | 1.68 |
| E1226 | 79.8 | 2.3 | 1.5 | 6.97 | 16.9 | 4.5 | 0.21 | 1.86 | 1.48 |
| Mean ± S.D. | 62.1 ± 22.4<sup>a</sup> | 3.1 ± 1.3<sup>b,d</sup> | 2.0 ± 1.3<sup>c</sup> | 6.31 ± 1.17<sup>e</sup> | 16.3 ± 3.8<sup>d</sup> | 4.6 ± 1.9 | 3.0 ± 1.9 | 0.28 ± 0.07 | 2.70 ± 1.06<sup>e</sup> | 1.78 ± 0.61<sup>e</sup> |

<sup>a</sup> Vd<sub>u</sub>: corrected steady-state volume of distribution of the total drug in liters per kilogram; f<sub>p</sub>: area weighted unbound fraction of the drug. Superscript ‘u’ in the above symbols refers to the same pharmacokinetic parameters for the unbound drug.

<sup>b</sup> Significantly shorter than fetal t<sub>1/2,b</sub> (<i>P</i> < .05).

<sup>c</sup> Significantly shorter than maternal t<sub>1/2,b</sub> (<i>P</i> < .05).

<sup>d</sup> Significantly shorter than maternal t<sub>1/2,b</sub> (<i>P</i> < .005).

<sup>e</sup> Significantly shorter than the corresponding total VPA MRT (<i>P</i> < .05).

<sup>f</sup> Significantly larger than the corresponding maternal volume parameter (<i>P</i> < .0005).

Fig. 4. Plasma VPA concentrations during a 6 h i.v. infusion protocol in newborn lambs and the first 6 h of a 24-h maternal i.v. infusion protocol in pregnant sheep.

A, representative plasma concentration versus time profile of total and unbound VPA in the newborn lamb NL0123z; B, unbound and total VPA plasma concentrations in NL0123z during the 6-h infusion period, showing continuous accumulation of the drug in newborn plasma; C, typical plasma concentration versus time profiles of total and unbound VPA in maternal and fetal plasma during the initial 6-h period of the 24-h maternal VPA infusion in E5108, showing lack of any drug accumulation. Profiles similar to (C) were also observed in all animals after fetal VPA infusion.

maternal and fetal plasma during the initial 6 h of the 24-h infusion period after maternal or fetal dosing (Fig. 4C). Also, in contrast to the mother and the fetus (Fig. 1), a typical convexity was evident during the postinfusion portion of the unbound and total plasma concentration versus time pharmacokinetic profile in the newborn lamb (Fig. 4A). This convexity is characteristic of Michaelis-Menten nonlinear pharmacokinetic behavior (Gibaldi and Perrier, 1982). Pharmacokinetic parameters of unbound and total VPA in four newborn lambs are presented in Table 5 (plasma samples were not available from the fifth lamb due to catheter failure). Although VPA appears to exhibit pronounced nonlinear pharmacokinetics in lambs, valid comparisons of newborn pharmacokinetic parameters with those of the mother and the fetus can be made because of a similar range of plasma concentrations observed. The average CL<sub>u</sub> as well as CL<sub>t</sub>, values in newborn lambs was significantly lower than the corresponding values in the mother and the fetus. The terminal t<sub>1/2</sub> and MRT of the unbound as well as total VPA were significantly longer in newborn lambs compared with the corresponding values in the mother and the fetus. All steady-state volume of distribution parameters (Vd<sub>ss</sub>, Vd<sub>ss</sub>, Vd<sub>ss</sub><sup>u</sup>) in the newborn lamb were significantly lower compared with the fetus but were not statistically different from those of the mother.

Similar to the mother and the fetus, the plasma protein binding of VPA in newborn lambs was saturable over the concentration range encountered in these studies, with plasma unbound fractions ranging from 0.08 to 0.92. However, as opposed to the mother and the fetus, no distinct relationships were apparent when the entire pooled newborn bound and unbound VPA plasma concentration data were analyzed in a fashion similar to Fig. 2 (plot not shown). In contrast, there were two clear patterns of VPA binding in newborn plasma samples. These are depicted in Fig. 5, which shows the newborn VPA plasma protein binding characteristics in pooled data from the four lambs. There are profound differences in VPA binding characteristics of
newborn plasma sampled on day 1 (0–24 h; Fig. 5, A–B) and that sampled after day 1 (24–96 h; Fig. 5, C–D) of our experiments. On day 1 (Fig. 5A), the bound/unbound concentration ratio is positively related to the bound concentration as opposed to an inverse relationship between these two variables in the mother and the fetus (Fig. 2, A and C). Also, in contrast to the mother and the fetus, there is no obvious relationship between bound and unbound concentrations (Fig. 5B). However, in the newborn plasma samples obtained after day 1 (Fig. 5, C and D) the situation appears to be similar to that of the mother and the fetus (Fig. 2). As in the mother and the fetus, a biphasic relationship appears to exist in the Rosenthal plot of the data (Fig. 5C). However, enough data points were not available in each phase to adequately model the data according to a two-site binding model. Hence, these data were modeled according to a one-saturable-binding-site model and the estimates of binding parameters are presented in Table 2. Fit of the data to this one-site binding model resulted in a lower AIC as compared with the fit to a two-site binding model.

**Table 5**

Pharmacokinetic parameters of the unbound and total VPA in four newborn lambs

<table>
<thead>
<tr>
<th></th>
<th>Unbound VPA</th>
<th></th>
<th></th>
<th></th>
<th>Total VPA</th>
<th></th>
<th></th>
<th></th>
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<td></td>
<td>CL\textsubscript{ub}</td>
<td>t\textsubscript{1/2b}</td>
<td>MRT\textsuperscript{u}</td>
<td>VD\textsubscript{ub}</td>
<td>CL\textsubscript{tb}</td>
<td>t\textsubscript{1/2b}</td>
<td>MRT</td>
<td>f\textsubscript{u}</td>
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<tr>
<td>NL424\textsuperscript{b}</td>
<td>0.78</td>
<td>12.9</td>
<td>18.1</td>
<td>0.84</td>
<td>0.27</td>
<td>20.5</td>
<td>23.6</td>
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<td>NL4124</td>
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<td>9.7</td>
<td>12.8</td>
<td>0.77</td>
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<td>16.5</td>
<td>17.5</td>
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<tr>
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<td>0.16</td>
<td>20.6</td>
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<td>NL2241(1)</td>
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<td>13.4</td>
<td>17.4</td>
<td>0.53</td>
<td>0.25</td>
<td>16.8</td>
<td>21.7</td>
<td>0.49</td>
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<td>Mean ± S.D.</td>
<td>0.66 ± 0.28\textsuperscript{c}</td>
<td>12.1 ± 1.6\textsuperscript{d}</td>
<td>16.6 ± 2.5\textsuperscript{d}</td>
<td>0.63 ± 0.21\textsuperscript{d}</td>
<td>0.25 ± 0.07\textsuperscript{c}</td>
<td>18.6 ± 2.3\textsuperscript{d}</td>
<td>22.1 ± 3.4\textsuperscript{d}</td>
<td>0.40 ± 0.08</td>
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</table>

\textsuperscript{a} \text{Vd\textsubscript{ss}}: corrected steady-state volume of distribution of the total drug in liters per kilogram; \textit{f\textsubscript{u}}: area weighted unbound fraction of the drug. Superscript \textit{u} in the above symbols refers to the same pharmacokinetic parameters for the unbound drug.

\textsuperscript{b} This lamb was offspring of E4241.

\textsuperscript{c} Significantly lower than the corresponding maternal and fetal values in Table 4 (\(P < .05\)).

\textsuperscript{d} Significantly longer than the corresponding maternal and fetal values in Table 4 (\(P < .05\)).

\textsuperscript{e} Significantly lower than the corresponding fetal values in Table 4 (\(P < .005\)).

**Figure 5.** Plasma protein binding characteristics of VPA in newborn lambs during day 1 (A–B) and after day 1 (C–D) of experiments.

C\textsubscript{u} and C\textsubscript{b} are the plasma concentrations of the bound and unbound VPA. A and C, the Rosenthal plots. B and D, the relationships between C\textsubscript{b} and C\textsubscript{u}. The data in (D) were fitted to a one-site binding model and the model-predicted line is also shown. Different scatter plot symbols denote data from different animals. Striking differences in VPA binding characteristics between the two groups of data are evident.
Placental Transfer and Maternal and Fetal Plasma Protein Binding of VPA. The steady-state maternal and fetal total plasma concentrations of VPA during maternal as well as fetal drug administration were near the therapeutic range in humans (40–100 μg/ml, Davis et al., 1994). During maternal drug administration, the lower fetal steady-state plasma-unbound VPA concentrations compared with the mother indicate irreversible drug elimination by the fetus and/or the placenta (Szeto et al., 1982b; Wang et al., 1986). Fetal-to-maternal steady-state plasma concentration ratios (0.70 ± 0.10 and 0.81 ± 0.09 based on total and unbound drug, respectively) indicate high fetal VPA exposure after maternal administration. The average unbound VPA \( F_{f/0} \) ratio is higher than that of a number of other drugs studied in pregnant sheep at this stage of gestation. These include diphenhydramine (0.50, Kumar et al., 2000a), morphine (0.14, Szeto et al., 1982a), methadone (0.42, Szeto et al., 1982a), and metoclopramide (0.67, Riggs et al., 1990). This is in spite of the fact that VPA placental clearance (and permeability, see below) is lower compared with many drugs including diphenhydramine, methadone, and metoclopramide. However, the VPA \( F_{f/0} \) ratio is similar to that of acetaminophen (0.77, Wang et al., 1986). Because at steady state, \( F_{f/0} = \frac{C_{f/C_m}}{C_{f/C_m} + CL_f/(CL_f + CL_m)} \) (Szeto et al., 1982b), it is important to note that VPA and acetaminophen also have the lowest values for CL\(_{fo}\) among all these drugs. This emphasizes the importance of fetal drug elimination capacity in determining fetal drug exposure.

The estimates of plasma protein binding parameters after excluding the data from E105x, which had much higher VPA plasma concentrations as compared with the remaining four ewes, indicate a similar VPA binding affinity for maternal and fetal plasma. In contrast, the fetal plasma VPA binding capacity appears to be somewhat lower than that of the mother; this may be related to lower fetal plasma protein concentrations (Kwan et al., 1995). The steady-state unbound fraction in maternal plasma during maternal drug administration (0.35 ± 0.13; Table 1) appears to be somewhat higher than the range (0.05–0.15) observed in epileptic nonpregnant patients (Levy and Shen, 1995); however, it is in reasonable agreement with that measured in serum obtained from pregnant mothers at birth (0.27 ± 0.06) (Nau et al., 1984; Nau and Krauer, 1986). The pregnancy-related increase in VPA-unbound fraction in humans appears to be due to reductions in plasma albumin concentration and displacement by increased free fatty acid plasma concentrations during pregnancy (Nau et al., 1984; Riva et al., 1984; Nau and Krauer, 1986). We did not observe a higher fetal VPA plasma protein binding compared with the mother, as demonstrated in humans at birth; however, this may be related to the fact that our experiments were conducted 1 to 2 weeks before term.

Placental and Nonplacental Clearances of VPA in the Mother and the Fetus. The placental clearance of unbound VPA in the two directions is similar (69.0 versus 61.9 ml/min/kg; Table 3). With the exception of acetaminophen, this is in contrast to all other drugs studied in pregnant sheep where CL\(_{fm}\) is significantly higher than CL\(_{rot}\). Previously, we have demonstrated that a significant proportion of the drug transferred from the maternal circulation across the placenta may be taken up by the fetal liver before reaching the fetal circulation, thus leading to an underestimation of CL\(_{rot}\) relative to CL\(_{fm}\) (Kumar et al., 1997). Because VPA is a low clearance drug, a significant fetal hepatic first-pass uptake of the drug would not be expected and accurate estimates of CL\(_{rot}\) are likely obtained. The equal magnitude of placental clearance in both directions across the placenta also indicates a passive diffusion mechanism for placental transfer of VPA in sheep.

The CL\(_{fm}\) value of unbound VPA is lower compared with that of diphenhydramine (Kumar et al., 1997), methadone (Szeto et al., 1982a), metoclopramide (Riggs et al., 1990), and compounds with blood flow-limited placental diffusion (antipyrine and ethanol, ~200 ml/min/kg) (Wilkening et al., 1982); however, it is greater than that of morphine (Szeto et al., 1982a), labetalol (Yeleswaram et al., 1993), and acetaminophen (Wang et al., 1986). This indicates an intermediate placental permeability for VPA in sheep. Molecular size of VPA is smaller relative to all of the above compounds, except ethanol, and hence, its intermediate placental clearance is likely related to its polarity (octanol/water log \( P = 2.6 \)) and high degree of ionization at the physiological pH (\( pK_a = 4.8 \)).

The clearance data in Table 3 also indicate that fetal total and nonplacental clearances are larger compared with the corresponding maternal clearances, with CL\(_{fo}\) being remarkably high. One limitation of the two-compartment model is that the uptake/metabolism of the drug by the placenta, if present, is calculated as part of CL\(_{fo}\), or CL\(_{mom}\) estimates (Wang et al., 1986). VPA, due to its branched-chain fatty acid structure, enters fatty-acid \( \beta \)-oxidation pathways (Baillie and Sheffels, 1995). Various lipid metabolism pathways also exist in the placenta of sheep and many other species (Coleman, 1989). Moreover, \( \beta \)-acid compounds that are structurally similar to VPA (e.g., acetocetate and \( \beta \)-hydroxybutyrate), are metabolized by the sheep placenta at slow rates (~30 μmol/min for \( \beta \)-hydroxybutyrate and ~6.0 μmol/min for acetocetate) (Carver and Hay, 1995). Use of VPA by the placenta at a more conservative rate of 6.0 μmol/min during both maternal and fetal infusion experiments would introduce an error of ~15.5 ml/min/kg (~150%) in CL\(_{fo}\) and of ~0.27 ml/min/kg (~7%) in CL\(_{mom}\). Although we do not have direct evidence for placental metabolism of VPA, this may be a plausible explanation for the high CL\(_{fo}\) value observed in our studies. This is especially true in light of the fact that VPA clearance in newborn lambs immediately after birth is much lower compared with the estimated CL\(_{fo}\) (Table 4).

Amniotic and Fetal Tracheal Fluid Disposition of VPA. One feature of amniotic and tracheal fluid disposition of VPA is a lack of its significant accumulation in these fluids relative to maternal and fetal plasma. This is in contrast to a number of amine drugs such as metoclopramide (Riggs et al., 1987), diphenhydramine (Riggs et al., 1987), labetalol (Yeleswaram et al., 1993), and ritodrine (Wright et al., 1991), which accumulate in these fluids at concentrations ranging from ~4 to 15 times compared with fetal plasma, and subsequently decline at a slow rate. Excretion of the drug into fetal urine and its passage from the fetal systemic circulation across the fetal membranes play an important role in drug transfer into and out of amniotic and allantoic fluids (Szeto et al., 1979; Rurak et al., 1991). Low fetal urinary excretion of VPA (see Kumar et al., 2000b) appears at least partly responsible for the small amounts of VPA detected in amniotic fluid.

Overall Maternal and Fetal Pharmacokinetics of VPA. The adult human unbound and total VPA clearances (1.0–3.0 and 0.1–0.3 ml/min/kg, respectively) are somewhat lower compared with maternal sheep (Davis et al., 1994; Levy and Shen, 1995). Similarly, \( t_{1/2P} \) of unbound and total VPA in sheep is much shorter compared with that in nonpregnant and pregnant humans (9–18 h) (Nau et al., 1982; Davis et al., 1994; Levy and Shen, 1995). The \( V_d' \) of VPA in maternal sheep \((0.24 ± 0.10 liters/kg)\) is similar to that in humans \((0.13–0.20 liters/kg)\) (Davis et al., 1994; Levy and Shen, 1995). A generally large fetal \( V_d' \) value for VPA and many other drugs may in part be due to the fact that it includes a maternal volume component due to rapid distribution of a portion of the administered fetal dose to the ewe via placental transfer.
capacity compared with the mother and the fetus as reflected in a lower clearance and longer elimination half-life (Tables 4 and 5). Fetal-newborn VPA elimination differences are at least partly related to the loss of placentation route of drug elimination at birth. As discussed in Kumar et al. (2000b), glucuronidation and renal excretion of the unchanged drug are the two most important routes of VPA elimination in adult sheep. Both these routes are significantly underdeveloped in newborn lambs, thus leading to a slower clearance of VPA relative to the mother. The estimated VPA clearances in newborn lambs are very similar to the range observed in human neonates less than 1 month of age (total: 0.15–0.48 ml/min/kg; unbound: 0.7–4.2 ml/min/kg) (Irvine-Meek et al., 1982; Gal et al., 1988). Similar to our results in sheep, apparent elimination half-life of VPA in these human neonates (15.1–80 h) is much longer compared with that in human epileptic adults (9–18 h) (Ishizaki et al., 1981; Nau et al., 1981, 1984; Irvine-Meek et al., 1982; Gal et al., 1988; Davis et al., 1994; Levy and Shen, 1995).

The data presented in Fig. 5 elucidate a possible reason for the difference between the VPA binding properties of newborn versus maternal or fetal plasma. A positive relationship of bound/unbound concentration ratio with bound concentration in Fig. 5A indicates that with an increasing VPA plasma concentration, the bound concentration increases to a larger extent than the unbound concentration. This could occur if significant concentrations of a competitive VPA plasma protein binding displacer were present in the newborn lamb plasma. In this situation, an increase in VPA plasma concentration will lead to a competitive displacement of the displacer from the binding sites and to an increased binding of VPA. This would then result in an increase in the bound/unbound concentration ratio with increasing VPA concentration until all of the displacer has been displaced from the binding sites. The plasma unbound fractions of VPA correlate inversely with plasma concentrations of free fatty acids (Nau et al., 1984; Riva et al., 1984). Hence, increased plasma free fatty acids during pregnancy and the newborn period have been suggested as potential competitive displacers of VPA binding to plasma proteins (Nau et al., 1984; Riva et al., 1984). Plasma concentrations of free fatty acids rise dramatically soon after birth in sheep as well as in the human newborn (Noble, 1980; Nau et al., 1984) and this may be responsible for the anomalous VPA plasma protein binding characteristics in newborn lambs. Also, the plasma concentrations of free fatty acids begin to decline after the initial 2 to 3 days of life both in the lamb and the human newborn (Noble, 1980; Nau et al., 1984). In parallel with this, the VPA binding characteristics of newborn lamb plasma after day 1 of our experiments tend to approach those of maternal and fetal plasma (Fig. 5, C and D). Somewhat lower VPA binding affinity of newborn plasma may be related to the fact that the free fatty acid concentrations in newborn plasma may still be higher as compared with the mother and the fetus until a few days after birth. The increased plasma-unbound concentrations of VPA in the immediate newborn period due to displacement from plasma binding sites could have important pharmacological or toxicological implications, including increased penetration of the drug across the blood-brain barrier and an augmented possibility of acute as well as long-term central nervous system adverse effects.

In summary, VPA undergoes rapid placental transfer via passive diffusion and results in a high degree of fetal exposure in sheep. The drug exhibits an intermediate placental permeability, possibly due to its polarity and high degree of ionization at physiological pH. Although there is evidence of fetal VPA elimination, the remarkably high fetal nonplacental clearance estimates may indicate possible placentoblastic metabolism of the drug. Similar to the human newborns, VPA exhibits a slower Michaelis-Menten elimination and longer elimination half-life in newborn lambs as compared with adult sheep.

In addition, VPA appears to be extensively displaced from the binding sites in newborn lamb plasma during the initial 1 to 2 days of life, possibly as a result of elevated plasma free fatty acids at birth. The maximal VPA binding capacities of the maternal, fetal, and newborn plasma (after the initial 2 days of life) are similar, whereas the binding affinity of newborn plasma appears to be somewhat lower.

References


