DISPOSITION OF VALPROIC ACID IN MATERNAL, FETAL, AND NEWBORN SHEEP II: METABOLISM AND RENAL ELIMINATION

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

Abstract:

Metabolism and renal excretion of valproic acid (VPA) were examined in maternal, fetal, and newborn sheep to identify the underlying reasons for the previously observed reduced VPA clearance in newborn lambs. Plasma and urine from VPA infusion studies in maternal, fetal, and newborn sheep were analyzed for VPA and its metabolites [VPA-glucuronide; β-oxidation products: (E)-2-ene, (E)-3-ene, and 3-keto VPA; hydroxylated metabolites: 3-hydroxy, 4-hydroxy, and 5-hydroxy VPA (5-OH VPA); and 4-ene VPA, 4-keto VPA, 2-propylglutaric acid, and 2-propylsuccinic acid] using gas chromatography-mass spectrometry. All measured metabolites were detectable in maternal and fetal plasma, with 3-keto- and 5-OH VPA being at higher concentrations in the fetus. Plasma concentrations of (E)-2-ene, (E)-3-ene, 3-keto, and 5-OH VPA were higher in the newborn compared with the mother, whereas those of the other metabolites were similar. A smaller percentage of the dose was excreted as VPA-glucuronide, compared with the mother (77.0 ± 7.8%). Similarly, a lower fraction of the dose was excreted unchanged in newborn urine (11.0 ± 5.8%) relative to the urine of the mother (19.3 ± 5.8%); however, significantly larger percentages were excreted as (E)-2-ene (0.11 ± 0.04 versus 0.02 ± 0.01%), 3-keto (11.6 ± 3.5 versus 1.6 ± 0.8%), 4-hydroxy (6.1 ± 3.2 versus 2.3 ± 1.3%), and 5-OH VPA (2.2 ± 0.6 versus 0.8 ± 0.6%). The major reason for the reduced VPA elimination in newborn lambs appears to be impaired renal excretion and glucuronidation capacity. As a result, a larger fraction of the dose is channeled to β-oxidation and hydroxylation pathways. The β-oxidation activities are high at birth; this may explain the high plasma concentrations of (E)-2-ene and 3-keto VPA observed in newborn lambs and human newborns exposed to VPA.

The antiepileptic, valproic acid (VPA), is a simple branched short-chain fatty acid that exhibits an extremely complex metabolic fate (Fig. 1). There are approximately 50 known VPA metabolites, of which at least 16 are observed consistently in humans (Baillie and Sheffels, 1995). The major routes of VPA metabolism can be divided into three categories: glucuronidation, mitochondrial β-oxidation, and microsomal oxidative metabolism including desaturation and ω- and ω-1 oxidations (Fig. 1). Glucuronidation is the major route of VPA metabolism and results in the formation of 1-ω-acyl-β-D-ester-linked glucuronide (Dickinson et al., 1979). In different studies, 10 to 70% of the dose has been recovered in urine as VPA-glucuronide in humans (Gugler et al., 1977; Dickinson et al., 1989; Levy et al., 1990). A significant fraction of the VPA dose is also metabolized via the β-oxidation pathway in humans. The VPA metabolites formed via the mitochondrial β-oxidation pathway include 2-ω-propyl-2-pentenoic acid (2-ene VPA) (predominantly as the E-isomer), 2-ω-propyl-3-pentenoic acid (3-ene VPA) (predominantly the E-isomer), 2-ω-propyl-3-hydroxypentanoic acid (3-OH VPA), and 2-ω-propyl-3-oxopentanoic acid (3-keto VPA) (Bjorge and Baillie, 1991; Li et al., 1991). The 3-OH VPA metabolite may also be formed via cytochrome P450-mediated microsomal hydroxylation in addition to the β-oxidation pathway (Rettnermeier et al., 1987). The 3-keto VPA metabolite is a prominent urinary metabolic product of VPA in humans, and may account for 10 to 60% of the total administered dose (Dickinson et al., 1989; Levy et al., 1990; Sugimoto et al., 1996). Small amounts (≤1–3% of dose) of other β-oxidation metabolites, (E)-2-ene and 3-OH VPA, are also detectable in human urine. The (E)-3-ene VPA metabolite arises from the isomerization of (E)-2-ene VPA, and may be further metabolized to (E,E)-2,3′-diene VPA via β-oxidation (Fig. 1) (Bjorge and Baillie, 1991). The products of microsomal ω- and ω-1 hydroxylation of VPA are 2-ω-propyl-5-hydroxypentanoic acid (5-OH

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857
VPA) and 2-n-propyl-4-hydroxypentanoic acid (4-OH VPA), respectively (Rettemmeier et al., 1987). Additional oxidation of 5-OH VPA leads to 2-propylglutaric acid (2-PGA), whereas that of 4-OH VPA results in the formation of 2-n-propyl-4-oxopentanoic acid (4-keto VPA) and 2-propylsuccinic acid (2-PSA) (Granneman et al., 1984). In addition to 2-ene and 3-ene VPA, another monounsaturated metabolite of VPA is 2-n-propyl-4-pentenoic acid (4-ene VPA). In contrast to 2-ene and 3-ene VPA, 4-ene VPA is formed via a distinct microsomal cytochrome P-450-mediated desaturation reaction (Rettie et al., 1987). The study of 4-ene VPA formation and its subsequent fate has received considerable attention because of its possible involvement in VPA-induced idiosyncratic hepatotoxicity. The 4-ene VPA metabolite can be subsequently metabolized via mitochondrial \(\beta\)-oxidation to form the diunsaturated metabolite (E)-2,4-diene VPA (Kassahun and Baillie, 1993). One hypothesis of VPA-induced hepatotoxicity is that subsequent oxidative metabolism of 4-ene VPA, possibly via (E)-2,4-diene VPA formation, leads to the generation of chemically reactive and potentially toxic intermediates that are capable of reacting with and depleting mitochondrial glutathione stores (Kassahun and Abbott, 1993; Kassahun and Baillie, 1993; Tang and Abbott, 1996). This may eventually result in mitochondrial alterations and inhibition of \(\beta\)-oxidation, two characteristic features of VPA-induced hepatotoxicity (Kesterson et al., 1984). In our companion article on VPA pharmacokinetics in pregnant sheep and newborn lambs (Kumar et al., 2000), striking differences in the pharmacokinetics of VPA in newborn lambs as compared with maternal sheep have been outlined. These include a reduced clearance, a longer elimination half-life, and a pronounced Michaelis-Menten type nonlinear pharmacokinetic profile. Interestingly, these differences also appear to exist in human newborns exposed to VPA either via direct neonatal administration for control of seizures or via in utero placental transfer and persistence of the drug in the neonatal circulation after birth. Thus it was of interest to elucidate the underlying cause(s) of these differences. This includes possible differences in the functional capacity of various VPA metabolism pathways and the renal elimination of VPA in the newborn lamb compared with maternal sheep. In addition, the possible involvement of VPA metabolites in its idiosyncratic hepatotoxicity and pharmacological activity necessitates detailed study of fetal and newborn exposure to these compounds as compared with the adult. In this study, we have examined comparative maternal, fetal, and newborn metabolism and renal elimination of VPA in sheep.

**Materials and Methods**

The experimental details regarding the surgical procedures performed on animals are described in the companion manuscript (Kumar et al., 2000).
Drug Administration Protocols. The detailed protocols for drug administration are also described in Kumar et al. (2000). Briefly, the following experiments were conducted.

Pregnant Sheep Experiments. Two sets of experiments were carried out on 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 \( \mu \text{g/min/kg} \) via the same route.

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

Newborn Lamb Experiments. 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 based on maternal body weight (b).

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. 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. Cumulative maternal urine was collected via a Foley bladder catheter inserted via the urethra of the ewe on the morning of the experiment, and attached to a sterile polyvinyl bag. Fetal urine flow rate was estimated using a computer-controlled roller pump assembly developed in our laboratory. Fetal bladder catheter was allowed to drain by gravity into a sterile reservoir (10-ml syringe barrel) to which a disposable DTX transducer was connected. When the pressure in the reservoir increased above a preset level (usually 3 mm Hg) as a result of urine accumulation, the computer activated a roller pump (DIAS, Ex154; DIAS Inc., Kalamazoo, MI), which pumped a calibrated volume of urine from the reservoir back to the amniotic cavity (via the amniotic catheter). The cumulative volume pumped per min, which equals fetal urine production rate per min, was stored on diskette. During the experimental period and at specified sampling time intervals, fetal urine samples (5 ml) were collected by attaching a sterile sample collection syringe to the amniotic catheter via a 3-way stop-cock.

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 \(-20^\circ \text{C}\) 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 method at 1000g for 30 min using Centrifree micropartition devices (Amicon, Inc., Danver, MA) (see Kumar et al., 2000).

Drug and Metabolite Analysis. Concentrations of VPA and its metabolites were measured using a previously developed sensitive and specific gas chromatography-mass spectrometry assay method (Yu et al., 1995). Earlier validation studies have demonstrated that the variability and bias of this assay for all compounds does not exceed 15% (Yu et al., 1995). The concentrations of VPA-glucuronide in maternal, fetal, and newborn urine were measured using a base hydrolysis procedure as follows. The samples were adjusted to pH 12.5, incubated at 60°C for 1 h, and the total VPA (unconjugated + conjugated) was quantified by the above gas chromatography-mass spectrometry analysis method. The difference of total and unconjugated (unhydrolyzed) VPA concentrations gave the concentration of VPA-glucuronide. This procedure was preferred over hydrolysis with \( \beta \)-glucuronidase because VPA-glucuronide has been shown to rearrange to at least six \( \beta \)-glucuronidase-resistant structural isomers via migration of the acyl moiety away from the C-1 position and subsequent ring opening, mutarotation, and lactone formation (Dickinson et al., 1984). These rearrangements are pH-, temperature-, and storage time-dependent (Dickinson et al., 1989). The hydrolysis with alkali, however, is capable of measuring total VPA-glucuronide in spite of these possible rearrangements (Dickinson et al., 1989).

Pharmacokinetic Analysis. Renal clearance values for the total and unbound VPA in the ewe and the newborn lamb were calculated by dividing the total amount of unconjugated VPA excreted in urine by the respective plasma area under the curve from time zero to infinity of the total or unbound drug. Fetal renal clearance of total and unbound drug was calculated by dividing the urinary excretion rate determined at each sampling point by the corresponding total or unbound fetal plasma drug concentration at that time. Urinary excretion rate of the drug at each sampling point was determined from the concentration of the drug in fetal urine and the average urine flow rate during the 0.5 h preceding the sampling time point. Fetal renal clearances calculated at each sampling point were averaged to obtain a mean value for this parameter.

Statistical Analysis. All data are reported as mean \( \pm \) S.D. The maternal and fetal maximal plasma concentrations (Cmax) of various VPA metabolites (during both maternal and fetal infusion) were compared against each other using a paired \( t \) test. Because maternal and fetal Cmax values of various VPA metabolites are not completely independent and are influenced by each other due to placental transfer of metabolites, they were compared with newborn plasma Cmax values independently, using unpaired \( t \) tests. The significance level was \( P < .05 \) in all cases.

Results

Plasma Concentrations of VPA Metabolites in Maternal, Fetal, and Newborn Sheep. A number of metabolites of VPA such as those formed via fatty acid \( \beta \)-oxidation [(E)-2-ene, (E)-3-ene, and 3-keto VPA], cytochrome P450-mediated desaturation (4-ene VPA), and hydroxylation and subsequent oxidation (3-OH, 4-OH, 5-OH, and 4-keto VPA, 2-PSA, 2-PGA) were measured in maternal and fetal plasma and after VPA administration (Table 1). Maternal and fetal plasma concentrations of 3-OH VPA and 2-PSA were generally below the lower limit of quantitation (LOQ) of the assay (see footnote b in Table 1 for exceptions). Also, the 2-PGA metabolite was below the LOQ in all fetal plasma samples. During fetal infusion experiments, measurable concentrations of 2-PGA were detected in maternal plasma samples from only one animal. Concentrations of all VPA metabolites, except (E)-2-ene and 3-keto VPA, appeared to be relatively stable in maternal as well as in fetal plasma during the 20- to 24-h period of infusion and declined relatively rapidly to below LOQ levels within 12 to 24 h postinfusion (the figures of plasma profiles of these metabolites are not shown due to an apparent similarity between their shapes and time course). However, plasma concentrations of (E)-2-ene and 3-keto VPA were still increasing at the end of the infusion period and the maximal concentrations were typically observed a few hours later (Fig. 2). These two metabolites were detectable in maternal and fetal plasma for up to 36 to 72 h after the end of infusion (Fig. 2). Table 1 presents the average maternal and fetal Cmax values of all metabolites during maternal and fetal administration experiments. Maternal plasma Cmax of VPA after maternal administration occurred at 5 min (i.e., in the first sample after the i.v. bolus loading dose), whereas that after fetal administration occurred within 1 to 3 h. Similarly, the fetal plasma Cmax of VPA after fetal administration occurred at 5 min, whereas that after maternal administration occurred within 1 to 3 h. Maternal and fetal Cmax values of all metabolites, except (E)-2-ene and 3-keto VPA, occurred during the 20- to 24-h period of infusion; the Cmax values of these two metabolites occurred within 1 to 12 h after the end of VPA infusion (Fig. 2).

Plasma concentrations of VPA metabolites were also measured in samples obtained from four newborn lambs; samples could not be
collected in the 5th animal due to catheter failure. Although steady state could not be reached in newborn lambs during the experimental period, the plasma concentrations of VPA were either higher than or within the range observed in the mother and fetus (Table 1). Thus, valid comparisons of metabolite concentrations can be made between the mother, fetus, and newborn to assess their relative metabolite exposure. In addition, the ability of the newborn to form these metabolites can be clearly identified and compared with that of the mother. Table 1 also presents the $C_{\text{max}}$ of VPA and various metabolites in newborn plasma and the corresponding times of their occurrence ($t_{\text{max}}$). As in the fetus, plasma concentrations of 2-PSA, 2-PGA, and 3-OH VPA metabolites were below the LOQ in newborn lambs. Newborn plasma concentrations of the majority of the detected metabolites increased continuously for prolonged periods of time postinfusion, with $C_{\text{max}}$ values occurring 0.5 to 54 h after the end of infusion (Table 1). In contrast to the mother and the fetus, low concentrations

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Maternal plasma (n = 5)</th>
<th>Fetal plasma (n = 5)</th>
<th>Newborn plasma (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cmax</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VPA</td>
<td>135.04 ± 51.22</td>
<td>87.12 ± 54.50</td>
<td>150.04 ± 50.76</td>
</tr>
<tr>
<td>$(E)$-2-En</td>
<td>0.06 ± 0.11</td>
<td>0.05 ± 0.21</td>
<td>0.04 ± 0.14</td>
</tr>
<tr>
<td>$(E)$-3-En</td>
<td>0.13 ± 0.05</td>
<td>0.13 ± 0.08</td>
<td>0.07 ± 0.05</td>
</tr>
<tr>
<td>3-keto</td>
<td>0.48 ± 0.29</td>
<td>2.10 ± 1.05</td>
<td>1.66 ± 0.57</td>
</tr>
<tr>
<td>4-ene</td>
<td>0.40 ± 0.37</td>
<td>0.32 ± 0.35</td>
<td>0.11 ± 0.15</td>
</tr>
<tr>
<td>4-keto</td>
<td>0.24 ± 0.11</td>
<td>0.10 ± 0.08</td>
<td>0.02 ± 0.02</td>
</tr>
<tr>
<td>4-OH</td>
<td>3.91 ± 2.87</td>
<td>2.59 ± 1.65</td>
<td>0.61 ± 0.44</td>
</tr>
<tr>
<td>5-OH</td>
<td>0.88 ± 0.85</td>
<td>1.00 ± 0.86</td>
<td>0.38 ± 0.27</td>
</tr>
<tr>
<td>2-PGA</td>
<td>0.17 ± 0.11</td>
<td>BLOQ</td>
<td>BLOQ</td>
</tr>
</tbody>
</table>

*The 3-OH metabolite was below the LOQ in maternal, fetal, and newborn plasma. The 2-PSA metabolite was below the LOQ in all maternal, fetal, and newborn plasma samples, except in two animals where maternal plasma $C_{\text{max}}$ values during maternal administration were 0.05 and 0.03 μg/ml, respectively.

Significantly lower than the corresponding concentration in maternal plasma ($P < 0.05$).

Significantly higher than the corresponding concentration in maternal plasma ($P < 0.05$).

Significantly higher than fetal $C_{\text{max}}$ during maternal infusion ($P < 0.05$).

$C_{\text{max}}$ data are presented as median.

Significantly higher than fetal $C_{\text{max}}$ during fetal infusion ($P < 0.05$).

Significantly higher than the corresponding concentration in maternal plasma ($P < 0.05$).

Significantly higher than fetal $C_{\text{max}}$ during fetal infusion ($P < 0.05$).

Below the limit of quantitation of the assay.

**FIG. 2.** Mean maternal, fetal, and newborn plasma concentration versus time profiles of VPA metabolites.
of many VPA metabolites [(E)-3-ene, 4-ene, 4-keto, and 4-OH VPA] were detectable in newborn plasma until 54 to 66 h after the end of infusion. (E)-2-ene, 3-keto, and 5-OH VPA metabolites were still detectable in newborn plasma in significant concentrations (12.7 ± 7.9, 24.4 ± 16.0, and 7.0 ± 7.3% of their respective C<sub>max</sub> at the end of the 96-h experimental protocol (Fig. 2).

Fetal plasma concentrations of the 3-keto and 5-OH VPA metabolites increased steadily during the infusion, and were higher compared with maternal plasma concentrations during the majority of the experimental period (Fig. 2). Also, in contrast to the mother, fetal plasma concentrations of 3-keto VPA increased continuously and considerably for plasma concentrations of 3-keto VPA increased continuously and considerably for −12 h after the end of infusion (Fig. 2). Maternal and fetal C<sub>max</sub> values of the (E)-2-ene and (E)-3-ene VPA during maternal infusion were not significantly different; however, during fetal infusion, maternal C<sub>max</sub> of these metabolites was significantly higher. The C<sub>max</sub> values of (E)-2-ene and (E)-3-ene VPA in newborn lambs were significantly higher relative to both the mother and the fetus during maternal as well as fetal drug infusion. Fetal C<sub>max</sub> of 3-keto VPA during both maternal and fetal VPA infusions was significantly higher than the maternal C<sub>max</sub> of this metabolite. However, the C<sub>max</sub> of 3-keto VPA in the newborn lamb was significantly higher compared with both the ewe and fetus in all cases. In addition, fetal C<sub>max</sub> of 5-OH VPA was significantly higher compared with the mother during fetal drug infusion. During maternal infusion, fetal 5-OH VPA C<sub>max</sub> was higher in four of five animals, but the means were not statistically different. In the newborn, the C<sub>max</sub> of this metabolite was significantly higher than in both the ewe and fetus after maternal as well as fetal VPA infusion. Maternal, fetal, and newborn C<sub>max</sub> values of 4-OH, 4-ene, and 4-keto VPA generally appear to be similar. However, small statistically significant differences were observed in a few cases (Table 1).

In contrast to the human, the diunsaturated VPA metabolites [e.g., (E,E)-2,3-diene VPA, and (E)-2,4 diene VPA] were either absent or present only in trace amounts in maternal, fetal, and newborn plasma. Also, the 3-OH VPA metabolite was below the LOQ of our assay (0.1 µg/ml) in contrast to the human, where significant plasma concentrations of this metabolite are observed (Rettenmeier et al., 1989; Kasahun et al., 1990).

**Excretion of VPA Metabolites in Maternal, Fetal, and Newborn Urine.** Table 2 presents the percent fractions of the VPA dose excreted as unchanged VPA and various VPA metabolites in maternal plasma after maternal and newborn VPA infusions, respectively.

<table>
<thead>
<tr>
<th>Drug or Metabolite</th>
<th>Percentage of Administered Dose in Maternal Urine (n = 5)</th>
<th>Percentage of Administered Dose in Newborn Urine (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unchanged VPA</td>
<td>19.30 ± 5.81</td>
<td>10.98 ± 5.87</td>
</tr>
<tr>
<td>VPA-Glucuronide</td>
<td>76.96 ± 7.81</td>
<td>28.30 ± 11.98</td>
</tr>
<tr>
<td>(E)-2-ene VPA</td>
<td>0.02 ± 0.01</td>
<td>0.11 ± 0.04</td>
</tr>
<tr>
<td>(E)-3-ene VPA</td>
<td>0.01 ± 0.03</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>3-keto VPA</td>
<td>1.56 ± 0.78</td>
<td>11.60 ± 3.48</td>
</tr>
<tr>
<td>4-ene VPA</td>
<td>0.03 ± 0.02</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>4-keto VPA</td>
<td>0.42 ± 0.12</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>3-OH VPA</td>
<td>0.20 ± 0.04</td>
<td>0.66 ± 0.10</td>
</tr>
<tr>
<td>4-OH VPA</td>
<td>2.31 ± 1.26</td>
<td>6.13 ± 3.19</td>
</tr>
<tr>
<td>5-OH VPA</td>
<td>0.76 ± 0.55</td>
<td>2.15 ± 0.55</td>
</tr>
<tr>
<td>2-PGA</td>
<td>0.09 ± 0.03</td>
<td>0.08 ± 0.09</td>
</tr>
<tr>
<td>2-PGA</td>
<td>0.85 ± 0.44</td>
<td>0.74 ± 0.50</td>
</tr>
<tr>
<td>Total</td>
<td>102.52 ± 7.00</td>
<td>60.85 ± 11.72</td>
</tr>
</tbody>
</table>

*Significantly lower compared to the percentage of dose excreted as this metabolite in maternal urine during maternal infusion (P < .05).

*Significantly higher compared to the percentage of dose excreted as this metabolite in maternal urine during maternal infusion (P < .05).

Profound differences in the kinetics of urinary excretion of these two compounds relative to the duration of VPA infusion in the ewe and the newborn lamb are evident. Excreted as unchanged VPA and various VPA metabolites in maternal urine during maternal VPA infusion. The majority of VPA metabolites detected in maternal plasma were also excreted in maternal urine in significant quantities. However, the excretion of unchanged VPA and VPA-glucuronide alone accounted for −95% of the administered dose (Table 2). Unchanged VPA, VPA-glucuronide, and all other VPA metabolites were also detectable in fetal urine, albeit in much lower amounts compared with the mother. Cumulative collection of fetal urine was not performed, hence, the total amounts of these compounds excreted as a fraction of the administered dose could not be estimated.

Cumulative urine samples were collected in four newborn lambs. Urine could not be collected in one animal due to catheter failure. Table 2 also presents the average percent fractions of the VPA dose recovered as unchanged VPA and various VPA metabolites in these four lambs. Figure 3 shows a representative example of the time course of unchanged VPA and VPA-glucuronide (the major urinary metabolites) excretion in maternal and newborn urine. In the mother,
the majority of the glucuronide was recovered in urine within 12 h after the end of VPA infusion (Fig. 3). In contrast, in the lamb, significant amounts were still detectable in urine at the end of the 96-h experimental protocol (i.e., 90 h after the end of infusion) (Fig. 3). The percentage of dose excreted in newborn urine as unchanged VPA, VPA-glucuronide, and 4-keto VPA was significantly smaller compared with the mother (Table 2). In contrast, significantly larger percentages of the dose were excreted in newborn urine as (E)-2-ene, (E)-3-ene, 3-keto, 3-OH, 4-OH, and 5-OH VPA. There was no significant difference between the percent fractions of dose excreted as 4-ene VPA, 2-PSA, and 2-PGA metabolites in newborn and maternal urine. Percentage of the administered dose recovered in newborn urine as VPA and the measured VPA metabolites was only 60.8 ± 11.7%, compared with the value of 102.5 ± 7.0% in the ewe. As in plasma, the diunsaturated VPA metabolites [(E)-2,4-diene and (E,E)-2,3'-diene VPA] were either absent or detectable only in trace amounts in maternal, fetal, or newborn urine. This appears to be a species difference compared with the human.

Renal Clearance of VPA in Mother, Fetus, and Newborn. Table 3 presents the average renal clearance of the unbound and total VPA in maternal, fetal, and newborn sheep. Maternal renal clearances were determined during maternal drug infusion, whereas fetal renal clearances were determined during fetal drug administration. Maternal renal clearance data were available for all five animals; however, fetal renal clearance data were available for only four animals because bladder catheter was not implanted in one animal. Mean fetal renal clearance of the unbound and total VPA was significantly lower compared with the corresponding maternal value. Paired plasma and urine data were available in only three newborn lambs, hence, renal clearance could be calculated only in these three animals. Similar to the fetus, renal clearance of the unbound as well as total VPA in newborn lambs was much lower compared with the corresponding values in the mother (Table 3). Renal clearance of the unbound as well as total VPA in newborn lambs appears to be somewhat higher compared with the fetus (Table 3); however, due to a low n value for newborn lambs, a meaningful statistical comparison is difficult.

**Table 3: Pharmacokinetic parameters of renal elimination of VPA in maternal, fetal, and newborn sheep**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Maternal (n = 5)</th>
<th>Fetal (n = 4)</th>
<th>Newborn (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal clearance of total VPA</td>
<td>0.26 ± 0.08</td>
<td>0.01 ± 0.00*</td>
<td>0.03 ± 0.02*</td>
</tr>
<tr>
<td>Renal clearance of unbound VPA (CLu)</td>
<td>0.94 ± 0.40</td>
<td>0.02 ± 0.01*</td>
<td>0.08 ± 0.06*</td>
</tr>
</tbody>
</table>

* Significantly lower than the corresponding maternal renal clearance (P < 0.05).

Discussion

Plasma Concentrations of VPA Metabolites in Maternal, Fetal, and Newborn Sheep. The average maternal plasma Cmax values of a number of VPA metabolites during maternal drug administration were within or close to the plasma concentration range encountered in human adult epileptics (Rettenmeier et al., 1989; Kassahun et al., 1990). These include 4-ene VPA (0.40 versus trace – 0.64 µg/ml; sheep versus human), 4-keto VPA (0.24 versus trace – 4.50 µg/ml), 3-OH VPA (3.91 versus trace – 2.97 µg/ml), 5-OH VPA (0.88 versus trace – 1.06 µg/ml), and 2-PGA (0.17 versus trace – 0.22 µg/ml). Maternal plasma Cmax of (E)-2-ene VPA (0.63 µg/ml) was near the low end of the range encountered in human adults (0.55–4.66 µg/ml). Also, maternal plasma Cmax values of the other two metabolites formed via the β-oxidation pathway, i.e., (E)-3-ene and 3-keto VPA, appear to be lower than the range in humans (0.13 versus 0.41 – 1.68 µg/ml for (E)-3-ene VPA; 0.48 versus 2.26 – 14.7 µg/ml for 3-keto VPA). Although these differences may be related to interspecies variation in VPA metabolism, it has also been suggested that pregnancy is associated with a reduced fatty acid β-oxidation activity during the later part of gestation (Grimbert et al., 1993, 1995). In agreement with this, significantly higher plasma concentrations of β-oxidation VPA metabolites have been observed during our studies in nonpregnant sheep (H. Wong, S. Kumar, K. Wayn Rigg, and D.W. Rurak, unpublished data). Also, one report in humans showed lower maternal serum concentrations of (E)-2-ene, (E)-3-ene, and 3-OH VPA during the early second trimester compared with the late first trimester of pregnancy (Omtzig et al., 1992). Similar maternal plasma Cmax values of the β-oxidation products during maternal and fetal VPA infusions, in spite of ~3- to 4-fold different VPA plasma concentrations, indicate a possible saturation of the β-oxidation pathway (Table 1). However, maternal plasma concentrations of VPA metabolites formed via the cytochrome P-450 pathways (4-ene, 4-keto, 4-OH and 5-OH VPA, and 2-PGA) were proportionately lower during fetal infusion experiments, suggesting an overall linearity of these pathways. These observations are similar to the data on human VPA metabolism (Granneman et al., 1984).

Fetal exposure to VPA metabolites is high, with a number of metabolites detected at comparable [(E)-2-ene, (E)-3-ene, and 4-OH VPA] or higher (3-keto and 5-OH VPA) maximal concentrations in fetal plasma relative to the mother (Table 1). The longer residence and much higher concentrations of 3-keto VPA in fetal plasma may be related to its increased formation and/or reduced elimination in the fetus. It is interesting to note that higher concentrations of β-oxidation VPA metabolites compared with maternal serum are also found in cord serum samples obtained at birth from epileptic mothers (Nau et al., 1981, 1984; Kondo et al., 1987). A number of mechanisms based on the fetus acting as a deep compartment, active transport of metabolites across the placenta, and maternal and fetal differences in plasma protein binding of these compounds, have been proposed to explain the apparent fetal accumulation of these metabolites (Nau et al., 1981, 1984). However, our combined fetal and newborn metabolite data provide strong evidence that the apparent fetal accumulation of these metabolites may result from increased fetal metabolism of VPA via these pathways (see below).

The majority of VPA metabolites were also detectable in significant concentrations in newborn lamb plasma, suggesting that these pathways are likely functional in the late-gestational fetus as well. Perhaps the most significant observation with respect to newborn VPA metabolism was the detection of much higher plasma concentrations (up to 10-fold for 3-keto VPA) of many metabolites [i.e., (E)-2-ene, 3-ene, 3-keto, 5-OH VPA, and possibly 4-OH VPA] in the newborn compared with the fetus, and in some cases even compared with the mother. Apart from the above discussed isolated reports on VPA metabolite concentrations in cord serum samples, little data exist on VPA disposition in human neonates because of its limited clinical use in this population. However, VPA is commonly used to control seizures in epileptic children (Nau et al., 1991; Kondo et al., 1992; Siemes et al., 1993). The comparison of VPA metabolite serum profiles in human children with our newborn lamb data reveals some
striking similarities. Plasma concentrations of (E)-2-ene, (E)-3-ene, 3-keto, and 5-OH VPA metabolites are generally higher in children <2 years of age compared with those >2 years. In contrast, the plasma concentrations of 4-ene and 4-keto VPA and 2-PGA metabolites are lower in the younger age group (Nau et al., 1991). Hepatic fatty acid β-oxidation activity is low in utero and at birth in many species (Krahling et al., 1979; Duee et al., 1985; De Vivo et al., 1991). However, these activities increase dramatically during the first few hours after birth and may reach values higher than the adult within 1 day; subsequently the β-oxidation activities decline steadily to adult levels until weaning. This increased β-oxidation capacity after birth may be responsible for the higher plasma concentrations of VPA metabolites in newborn lambs and younger human children. However, as discussed above, our sheep data and limited cord serum human VPA metabolite concentration data provide strong evidence for significant fetal formation of the 3-keto metabolite (Nau et al., 1981), indicating that at least VPA β-oxidation is significantly functional in utero.

Excretion of Unchanged VPA and Its Metabolites in Maternal, Fetal, and Newborn Urine. During maternal drug infusion, the major components of the VPA urinary metabolite profile were unchanged VPA (∼19%) and VPA-glucuronide (∼77%), with all other metabolites collectively accounting for <5%. There appear to be a number of differences between the sheep and human VPA urinary metabolite profiles (Gugler et al., 1977; Dickinson et al., 1989; Levy et al., 1990; Levy and Shen, 1995). Firstly, renal excretion of the unchanged drug accounts for a much larger percentage of the dose in sheep (19%) as compared with the human (1–2%), possibly because of a higher renal clearance of VPA in sheep (0.94 versus 0.03–0.06 ml/min/kg). Secondly, the fraction of dose excreted as VPA-glucuronide in sheep (77%) was consistently near the high end of the range observed in humans (10–70%). The third major difference between maternal sheep and humans appears to be the much lower contribution of β-oxidation pathway to total VPA elimination in sheep. In addition to VPA-glucuronide, 3-keto VPA is a major human urinary metabolite and may account for 10 to 60% of the dose; (E)-2-ene and 3-OH VPA may each account for an additional 1 to 3% (Dickinson et al., 1989; Levy et al., 1990; Sugimoto et al., 1996). As discussed before, the lower contribution of β-oxidation to VPA metabolism in maternal sheep may be related to pregnancy-related reductions in β-oxidation activity. In agreement with this, we have recovered a larger percentage (10–20%) of the VPA dose as 3-keto VPA in nonpregnant sheep urine (H. Wong, S. Kumar, K. Wayne Riggs, and D.W. Rutak, unpublished data). The contribution of most other measured metabolites to total elimination of VPA appears to be close to the range in humans.

Presence of VPA-glucuronide in fetal urine likely indicates the fetal origin of this conjugate because of the very low permeability of the epitheliochorial sheep placenta to hydrophilic glucuronides (Wang et al., 1986). Similar to VPA, very small amounts of the glucuronide conjugate have also been found in fetal lamb urine for another carboxylic acid drug, indomethacin (Krishna et al., 1995). In contrast, the ability to glucuronidate alcoholic/phenolic moieties appears to be much more developed in the late gestation fetal lamb as ∼63, 22, and 40% of the fetal dose of morphine, ritodrine, and labetalol, respectively, is glucuronidated (Olsen et al., 1988; Wright et al., 1991; Yeleswaran et al., 1993). Significant amounts of acetaminophen and para-nitrophenol glucuronides are also formed by the fetal lamb in utero and by the isolated perfused fetal lamb liver in vitro, respectively (Wang et al., 1986; Ring et al., 1996). These data may indicate differential ontogenetic development of UDP-glucuronosyl transferases involved in the glucuronidation of acidic and alcoholic/phenolic compounds, as has been observed in the rat and the human fetus (Wishart, 1978; Burchell et al., 1989).

Although the fraction of dose recovered as unchanged VPA and VPA-glucuronide in newborn lamb urine is only ∼2- to 3-fold lower compared with that of the mother, the maternal-neonatal kinetic differences in these pathways are much more pronounced (Fig. 3). The continuous excretion of small amounts of VPA and its glucuronide in lamb urine indicates that these pathways are likely saturated over a prolonged period of time due to their low capacity. Also, in contrast to the adult, metabolites formed via the β-oxidation and cytochrome P-450 pathways [(E)-2-ene, (E)-3-ene, 3-keto, and 3-OH, 4-OH, and 5-OH VPA] appear to account for a much larger fraction of the VPA dose in newborn lambs. Interestingly, however, larger amounts of the potentially hepatotoxic cytochrome P-450 metabolite, 4-ene VPA, were not excreted in newborn lambs. Higher fatty acid β-oxidation capacity, combined with impaired VPA glucuronidation and renal excretion in the newborn lamb, is likely responsible for a larger fraction the VPA dose being metabolized via the cytochrome P-450 and β-oxidation pathways. However, these metabolic routes appear to be kinetically less efficient, resulting in apparent nonlinear kinetics with a significantly reduced clearance in newborn lambs (Kumar et al., 2000). In contrast to the mother, a significant portion of the VPA dose (∼30–50%) could not be accounted for in the newborn lamb, suggesting the possibility of additional routes of VPA metabolism during the newborn period.

Renal Clearance of VPA in Maternal, Fetal, and Newborn Sheep. The negligible renal clearance of VPA in the fetal lamb compared with maternal sheep is similar to the data for a number of other acidic compounds such as indomethacin (Krishna et al., 1995), diphenylmethoxyacetic acid (Kumar et al., 1997), and para-aminophenuric acid (Elbourne et al., 1990). Limited VPA renal excretion ability also appears to exist in the immediate newborn period, with the average unbound and total renal clearances being ∼10-fold lower compared with those of the mother (Table 3).

In conclusion, we have obtained strong evidence of in utero fetal formation of a number of VPA metabolites, such as 3-keto, 5-OH and 4-OH VPA, and VPA-glucuronide. Studies in newborn lambs indicate that all major pathways of VPA metabolism are functional in the immediate newborn period. Fetal and newborn lamb exposure to maternal VPA metabolites is comparable or greater than and prolonged relative to that in the mother. Similar to human newborns and children (<2 years of age), plasma concentrations of the β-oxidation VPA metabolites in newborn lambs are much higher compared with those of the mother, possibly because of a high β-oxidation capacity at birth. Glucuronidation and renal excretion of unchanged VPA are the major determinants of VPA elimination in maternal sheep. Both of these routes are significantly underdeveloped in the newborn lamb and lead to a slower VPA elimination. This also results in a larger percentage of the dose being metabolized via the β-oxidation and cytochrome P-450 pathways in newborn lambs. Because a number of similarities have been observed in the pharmacokinetics and metabolism of VPA in human neonates and newborn lambs, it is tempting to speculate that reduced VPA glucuronidation and/or renal clearance may also underlie the slow elimination of VPA observed in human newborns.

References


