Effect of Pregnancy on the Disposition of Valproate in Rats

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The disposition of sodium valproate in pregnant rats was studied comparing with nonpregnant (control) rats. In the pregnant rats, the total plasma clearance decreased significantly ($p < 0.05$) from 7.06 ml/min/kg of the control to 5.34 ml/min/kg, whereas the plasma elimination half-life of valproate did not change. The serum unbound fraction ($f_u$) of pregnant rats increased remarkably. The $f_u$ of the fetal plasma was lower than that of the maternal serum in spite of the lower albumin (main binding protein for valproate) concentration in the fetal plasma. A non-linear serum (plasma) protein binding was observed both in the control and the fetal rats, but not observed in the pregnant rats. In the pregnant rats, the tissue-to-plasma concentration ratio ($K_p$) of the brain was higher than that in the control rats, whereas the $K_p$ values of the liver and lung were lower than those in the control rats. In other tissues, the $K_p$ values did not show a significant difference. Rapid placental transfer was observed and the $K_p$ value of the fetus was 0.43.

**Keywords** — valproate; disposition; tissue distribution; non-linear kinetics

Introduction

It has been reported that the pharmacokinetics of many drugs are affected by the physiological changes associated with pregnancy.1–9 Significant changes in their plasma (serum) concentrations during pregnancy have been reported in humans10,11 and in experimental animals.12,13 The plasma (serum) protein binding of some drugs was decreased during pregnancy.14,15 In rats, extreme increases in the serum unbound fraction ($f_u$) of acidic drugs such as salicylate and sulfisoxazole, were observed in the 20th day pregnant rats.16–18

Sodium valproate (VPA) is a fatty acid with a simple branched chain and is used clinically in the treatment of both generalized and partial seizures. Although several studies on the pharmacokinetics of VPA in humans19,20 and experimental animals have been reported,21,22 there is limited information on the relationship between its pharmacokinetics and the physiological changes in pregnancy. The purpose of this study is to investigate the effect of pregnancy on the disposition of VPA, and to assess the factors which are responsible for the changes of its disposition in pregnancy.

Materials and Methods

Chemicals — VPA, Sodium valproate was supplied by Kyowa Hakko Co. (Tokyo, Japan). [Carbonyl-14C]VPA (54.9 mCi/mmole) was supplied by Sanofi Co. (Paris) and was found to be 99% pure by gas chromatography. All other chemicals were commercially available and of analytical grade.

Animals — Adult female rats (Wistar, Nihon Rat Co., Tokyo) weighing between 170 and 200 g were mated with males overnight. The day when the spermatozoa was first found in vaginal smear was counted the 1st day of pregnancy. The animals were housed under conditions of controlled temperature and lighting (12 h) with free access to food and water at all times. All non-pregnant rats, which were as old as pregnant rats, were used as the control.

In Vivo Experiments — All rats were fasted for 16–18 h before operation. Body weights of the 20th day-pregnant rats were between 290 and 420 g and those of the control rats were between 195 and 250 g. Under light ether anesthe-
sia, the bile duct was cannulated with PE-10 polyethylene tubing to exclude the enterohepatic 
circulation of VPA.\textsuperscript{21,22} The femoral vein 
and artery were cannulated with PE-50 tubing. 
Cannulated rats were kept in the supine position 
on a board and recovered from anesthesia prior 
to the injection of VPA. Each rat received a sin-
gle dose of 10, 50 and 100 mg/kg of VPA in 
physiological saline through the femoral vein 
cannula. Blood samples were obtained at 1, 3, 
5, 10, 15, 30 and 45 min, and 1, 2, 3 and 4 h in 
heparinized capillary tubes (Drummond Sci. Co., 
CA). Plasma was separated by centrifugation at 
3000 rpm for 10 min in a table-top centrifuge 
(Kubota, Tokyo) for the determination of the 
VPA concentration by gas-liquid chromatogra-
phy (GLC).

The rats were sacrificed by exsanguination 
from the femoral artery at 1, 2, 3 and 4 h after 
intravenous injection of VPA. After sacrifice of 
the pregnant rats, approximately 12 fetuses were 
obtained from the mother. Plasma and tissues 
from these fetuses were pooled and used for 
measuring the VPA concentrations. Each tissue 
of the pregnant and control rats was immediately 
excised, rinsed with physiological saline and 
weighed. The visceral organs except the skin, gut, 
fat and muscle were homogenized with an equal 
volume of physiological saline. The gut, fat and 
muscle were homogenized with a 3 fold excess 
volume of physiological saline. Sections of skin 
were cut into small pieces and crushed. One gram 
of skin was then extracted with 1 ml of physio-
logical saline by shaking for 12 h at room tem-
perature. The VPA concentration in the tissues 
of the control, pregnant, and fetal rats was mea-
sured by the method described below.

The tissue-to-plasma concentration ratio ($K_p$) 
was calculated using the plasma and tissue con-
centrations of VPA at 3 and 4 h post-intravenous 
injection and was corrected according to the 
method of Terasaki et al.\textsuperscript{24}

**Serum Protein Binding** — Serum protein 
binding of VPA was determined over wide 
ranges of $[^{14}C]$VPA concentrations (0.1—2000 
$\mu g/ml$) by an equilibrium dialysis method. Se-
rum obtained from control and pregnant rats and 
plasma from fetal rats (from the same pregnant 
rats) were used in the equilibrium dialysis at 37

\[ {C_b} = \frac{(P_t)}{K_{d1} + C_f} \left( \frac{n_1 C_f}{K_{d1} + C_f} + \frac{n_2 C_f}{K_{d2} + C_f} \right) \]  

where $C_b$, $C_f$, $P_t$, $n$, and $K_d$ are the concentra-
tion of bound drug in serum (plasma), the con-
centration of unbound drug in serum (plasma), 
the concentration of albumin in serum (plasma), 
the number of binding sites on albumin, and the 
dissociation constant of serum (plasma) protein 
binding, respectively.

**Analytical Procedures**

**Sample Preparation** — The VPA in biologi-
cal materials was determined by a GLC using 
flame ionization detection (FID). For the deter-
mination of VPA concentration in plasma, 0.2 
ml of plasma was added to a centrifuge tube (10 
ml) containing 0.4 ml of 2 N HCl solution and 
0.2 ml of chloroform which contained diphenyl 
as an internal standard. The mixture was shaken 
with a mixer for 1 min and was centrifuged at 
3500 rpm for 5 min. Then, an aliquot (1—5 $\mu l$) 
of the chloroform layer was injected into a gas 
chromatograph. For the determination of the
VPA concentration in the visceral tissues except the skin, 1 ml of homogenized tissues was added to a centrifuge tube (50 ml) containing 1 ml of 0.25 M NaH₂PO₄ and 20 ml of l-chlorbutane. The mixture was shaken for 10 min and centrifuged at 4000 rpm for 20 min. Then, 15 ml of the organic solvent was shaken with 3 ml of 0.5 N NaOH and centrifuged. Two ml of the aqueous layer was added to a centrifuge tube (10 ml) containing 2 ml of aqueous solution (4 M NaCl–1 N HCl) and 0.2 ml of chloroform which contained diphenyl as an internal standard. The mixture was treated in the same manner as described above. For the skin, the solution extracted was added to a centrifuge tube containing 1 ml of 2 N HCl and 25 ml of chlorobutane. Then, the same procedures as described above were followed. The detection limit of this method was 0.2 μg/ml for plasma and 0.2 μg/g for tissues.

GLC Condition — The gas chromatograph (Model GC-6A, Shimadzu, Kyoto, Japan) was equipped with a flame ionization detector (FID) and a 2.1 m × 3 mm glass column packed with DEGA on 60—80 mesh Chromosorb W for plasma and with Termon-1000 (+ H₃PO₄:5 + 0.5%) on 80—100 mesh Chromosorb W for tissue samples. The temperature of injection port was 230 °C. The column oven temperature was 190 °C. The nitrogen flow rate was 80 ml/min.

Blood-to-Plasma Concentration Ratio of VPA — The blood from five pregnant or control rats was combined. The pooled blood of the pregnant or control rats was incubated with [¹⁴C]VPA (5—500 µg/ml) at 37 °C for 30 min. After centrifugation an aliquot of plasma was removed and the concentration of [¹⁴C]VPA was determined as described above.

Data Analysis — The data were analyzed by an iterative least squares method with a digital computer using MULTII program (Yamaoka et al.20). Model independent analysis was used for pharmacokinetic parameters.

Statistical Analysis — All means are presented ± S.E. Student’s t-test was utilized to estimate a significant difference between the pregnant and control or fetal rats.

**Results**

**Plasma Concentrations**

The plasma concentration–time profile of VPA after intravenous administration of 10, 50 and 100 mg/kg in the control and the 20th day-pregnant bile-exteriorized rats are shown in Fig.

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**Fig. 1.** Plasma Concentration–Time Courses of VPA in Pregnant Rats and Control Rats Following Various Intravenous Doses (A) Control, ○, 100 mg/kg; □, 50 mg/kg; Δ, 10 mg/kg. (B) Pregnant, ●, 100 mg/kg; □, 50 mg/kg; ▲, 10 mg/kg. Each point and vertical bar represents the mean ± S.E. of five rats. a) Significantly different from the control (p < 0.05).
### Table 1. Pharmacokinetic Parameters of VPA after Intravenous Administration of 10 mg/kg in Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta$ (min$^{-1}$)</td>
<td>0.0121 ± 0.0008</td>
<td>0.0109 ± 0.0008</td>
</tr>
<tr>
<td>$t_{1/2}$ (min)</td>
<td>58.8 ± 4.6</td>
<td>64.7 ± 3.9</td>
</tr>
<tr>
<td>$V_{ds}$ (ml/kg)</td>
<td>263 ± 26</td>
<td>260 ± 17</td>
</tr>
<tr>
<td>$CL_{int}$ (ml/min/kg)</td>
<td>7.06 ± 0.66</td>
<td>5.34 ± 0.32</td>
</tr>
<tr>
<td>AUC$_w$ (µg-min/ml)</td>
<td>1471.3 ± 145.9</td>
<td>1896.8 ± 101.0</td>
</tr>
<tr>
<td>MRT (min)</td>
<td>37.3 ± 0.9</td>
<td>49.0 ± 3.2</td>
</tr>
</tbody>
</table>

The results are presented as the mean ± S.E. of five animals. Abbreviations used are: $\beta$, disposition rate constant in the terminal phase; $t_{1/2}$, half-life in the terminal phase; $V_{ds}$, distribution volume at steady state; $CL_{int}$, total plasma clearance; AUC, the area under the plasma concentration–time curve; MRT, mean residence time; NS, not significant.

1. The plasma VPA disappeared biphasically in both the control and pregnant rats at 10 mg/kg. Several pharmacokinetic parameters of VPA (model independent) after intravenous administration of 10 mg/kg are also shown in Table 1. The elimination half-lives ($t_{1/2}$) in both the control and pregnant rats were almost the same. In the pregnant rats, the significant increases in the infinite area under the time–concentration curve (AUC$_w$) and the mean residence time (MRT) were observed (Table 1). At the dose of 50 mg/kg, although weak saturation in the elimination from plasma was observed in the control rats, distinct saturation was found in the pregnant rats (Fig. 1). At 100 mg/kg, remarkable saturation in the elimination from plasma was recognized in both groups (Fig. 1).

**Serum (Plasma) Protein Binding**

Serum (plasma) protein bindings were determined over the wide ranges of $[^{14}C]$VPA concentrations (0.1—2000 µg/ml) by the equilibrium dialysis method in order to compare the binding isotherms among sera of the control and pregnant rats and fetal plasma. As shown in Fig. 2, the serum or plasma unbound fraction ($f_u$) of VPA in the control rats and fetuses increased with the increased concentrations. However the serum unbound fraction ($f_u$) in the pregnant rats was almost constant and independent of the concentrations. The Scatchard plots of the binding data suggested two independent classes of binding sites (Fig. 3). All parameters given in Table II were calculated by the non-linear least-squares method using MULTI program$^{26}$ after correction by the concentration of serum (plasma) albumin, which was the main binding pro-

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**Fig. 2.** Serum (Plasma) Protein Binding of VPA in Control (○), Pregnant (●) and Fetuses of the Same Pregnant Rats (△)

The points represent the experimental values as the unbound fraction of the total serum (plasma) concentration. The lines were calculated by fitting to a Langmuir-type equation (see text for details).

**Fig. 3.** Scatchard Plots of Data for the Binding of VPA to Serum (Plasma) Protein

Key: ○, control; ●, pregnant; △, fetal; $r$, number of mol of VPA bound per mol of albumin; $C_r$, molar concentration of unbound VPA.
TABLE II. Parameters for Serum (Plasma) Protein Binding of VPA

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pregnant</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Maternal</td>
<td>Fetal</td>
<td></td>
</tr>
<tr>
<td>$K_{d1}$ (mm)</td>
<td>0.165</td>
<td>0.205</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$n_1$</td>
<td>0.950</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{d2}$ (mm)</td>
<td>2.54</td>
<td>5.16</td>
<td>2.68</td>
<td></td>
</tr>
<tr>
<td>$n_2$</td>
<td>2.13</td>
<td>1.82</td>
<td>2.36</td>
<td></td>
</tr>
</tbody>
</table>

Values are the mean value of two independent experiments. Abbreviation used are: $n$, number of binding sites, $K_d$, the dissociation constant for the drug: albumin complex.

Table of VPA. The dissociation constants ($K_{d1}$, $K_{d2}$) and the numbers of binding sites ($n_1$, $n_2$) in the control and fetal rats were similar each other. In the pregnant rats, the dissociation constant calculated was comparable to those at the low affinity binding sites in the control and fetal rats.

Blood-to-Plasma Concentration Ratio of VPA

The ratios of blood-to-plasma concentration ($R_B$) of $[^14]$C-VPA over the wide range of the blood concentration ($5 - 500 \mu g/ml$) were determined in the control and pregnant rats (Fig. 4). The ratios increased linearly to around $100 \mu g/ml$ in both groups, then the increase became small. The $R_B$ values of the pregnant rats exceeded always those in the control rats, its ratio to that of the control was 1.1 to 1.2. At $5 \mu g/ml$, the $R_B$ values of the control and pregnant rats were 0.62 and 0.74, respectively.

Biochemical Variables

Biochemical variables of freshly isolated serum (plasma) from the control, pregnant and fetal rats are shown in Table III. The pH of plasma from the fetal rats was significantly lower compared to the pregnant rats ($p < 0.01$). Hematocrit value of the blood from the pregnant rats was significantly lower than the control rats. The total protein concentration in serum from the pregnant rats was significantly lower than the control rats ($p < 0.05$) and that of the fetal plasma was the lowest. The plasma albumin concentration of the pregnant rats was significantly lower than that of the control rats and both were far above that of the fetus. Significantly higher levels of triglycerides were found in both the pregnant rat serum and fetal plasma. The free fatty acid level of serum from the pregnant rats was almost three times higher than that of the control rat serum. However, the free fatty acid level in fetuses was only one tenth that of the control ($p < 0.001$).

TABLE III. Biochemical Variables of Pregnant and Non-pregnant (Control) Female Rats and Fetal Rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pregnant</th>
<th>Fetus</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.53 ± 0.04</td>
<td>7.57 ± 0.04</td>
<td>7.36 ± 0.02 e</td>
</tr>
<tr>
<td>Hematocrit value</td>
<td>0.54 ± 0.00</td>
<td>0.43 ± 0.00 b</td>
<td>0.50 ± 0.02 c</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>8.11 ± 0.04</td>
<td>6.21 ± 0.02 a</td>
<td>2.41 ± 0.17 a</td>
</tr>
<tr>
<td>Albumin (mm)</td>
<td>0.622 ± 0.008</td>
<td>0.567 ± 0.012 w</td>
<td>0.195 ± 0.004 y</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>49.0 ± 3.6</td>
<td>787 ± 37 b</td>
<td>106 ± 4</td>
</tr>
<tr>
<td>Free fatty acid (meq/dl)</td>
<td>0.556 ± 0.064</td>
<td>1.85 ± 0.06 b</td>
<td>0.0560 ± 0.0072 y</td>
</tr>
</tbody>
</table>

Results are presented as the mean ± S.E. of five samples. a) Significantly different from the control rats ($p < 0.05$). b) Significantly different from the control rats ($p < 0.001$). c) Significantly different from the pregnant rats ($p < 0.05$). d) Significantly different from the pregnant rats ($p < 0.01$). e) Significantly different from the pregnant rats ($p < 0.001$).
Tissue Distribution of VPA

The tissue concentrations of VPA after intravenous administration (10 mg/kg) to the control and pregnant rats are shown in Fig. 5. Except for the kidney, almost all tissues in the pregnant rats exhibited higher concentrations of VPA. The $K_p$ values calculated using the concentrations in plasma and tissues at 3 and 4 h ($\beta$-phase) after intravenous injection of 50 mg/kg VPA, are listed in Table IV. In the brain (the target organ of VPA), the $K_p$ value in the pregnant rat was significantly higher, whereas significantly low values were observed in the liver and lung of the pregnant rats. Similar $K_p$ values were calculated in other tissues of both groups. The $K_p$ value of the fetus was 0.43, suggesting the placental transfer of VPA.¹⁹,²⁷

**Table IV. Tissue-to-Plasma Concentration Ratios ($K_p$) of VPA in Rats after Intravenous Administration of 50 mg/kg**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Pregnant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>0.07 ± 0.00</td>
<td>0.19 ± 0.04 ¹⁰</td>
</tr>
<tr>
<td>Liver</td>
<td>1.76 ± 0.22</td>
<td>1.03 ± 0.11 ¹⁰</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.54 ± 0.46</td>
<td>0.83 ± 0.14</td>
</tr>
<tr>
<td>Lung</td>
<td>0.42 ± 0.09</td>
<td>0.13 ± 0.04 ¹⁰</td>
</tr>
<tr>
<td>Heart</td>
<td>0.43 ± 0.13</td>
<td>0.22 ± 0.02</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.49 ± 0.15</td>
<td>0.47 ± 0.15</td>
</tr>
<tr>
<td>Intestine</td>
<td>0.45 ± 0.22</td>
<td>0.47 ± 0.19</td>
</tr>
<tr>
<td>Fat</td>
<td>0.15 ± 0.05</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.16 ± 0.11</td>
<td>0.18 ± 0.09</td>
</tr>
<tr>
<td>Skin</td>
<td>0.47 ± 0.09</td>
<td>0.43 ± 0.07</td>
</tr>
<tr>
<td>Placenta</td>
<td>0.48 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>Fetus</td>
<td>0.43 ± 0.03</td>
<td></td>
</tr>
</tbody>
</table>

Results are presented the mean ± S.E. of three to five animals.  a) Significantly different from the control rats ($p<0.05$).  b) Significantly different from the control rats ($p<0.01$).

**Discussion**

In this study, we investigated the effect of pregnancy on the disposition of VPA. Since VPA is recycled in the serum (plasma) due to enterohepatic circulation,²¹,²³ we examined pharmacokinetic changes of this drug in the pregnant bile-exteriorized rats (late pregnancy) for more detailed comparative study.

The plasma elimination of VPA followed
biexponential curves after intravenous administration of 10 mg/kg (the lowest dose) in both the control and pregnant rats, whereas saturation in the plasma elimination was observed at the higher doses (50 and 100 mg/kg) in both groups of rats (Fig. 1). This dose dependent elimination of VPA has been reported previously in male rats. It seemed that the pregnant rats were more liable to be saturated in the elimination of VPA from plasma than the control rats on the body weight-adjusted basis. This suggested the decreased hepatic extraction of VPA in the pregnant rats, since the elimination of VPA is mainly due to the hepatic metabolism.

We calculated the pharmacokinetic parameters of VPA at the lowest dose (10 mg/kg), since nonlinearity was observed in the plasma elimination at the higher doses (50 and 100 mg/kg) as described above. The significant difference observed in the total plasma clearance (CL\text{tot}) and AUC\text{∞} between the control and pregnant rats may be due to the decreased hepatic extraction of VPA in the pregnant rats. If the total plasma clearance and the volume of distribution are expressed in absolute terms for the whole animal, then these results suggest that there is about a 40% increase (not significant) in the volume of distribution of VPA without any significant change in its clearance in the pregnant rats. Lin and Levy drew attention to the problem of explaining the results obtained because of the large increase in the body weight associated with pregnancy in the rat. However, at the 20th-day of gestation, pregnancy is associated with not only a significant increase in body weight but also an even greater relative increase in the liver weight, and VPA clearance is primarily controlled by the hepatic metabolism. It would appear that the body weight-normalized data reflect more accurate changes due to pregnancy than do the absolute values.

The value of \( f_s \) of VPA increased markedly in pregnancy as described below, whereas the CL\text{tot} of VPA decreased. Since the CL\text{tot} of VPA was smaller than the hepatic plasma flow, the elimination seems to be dependent upon the hepatic intrinsic clearance. It has been reported that at the low dose VPA was metabolized by β-oxidation, ω-oxidation, ω-1 oxidation and so on, whereas at the high dose it was metabolized mainly by glucuronic acid conjugation. In regard to the influence of pregnancy on glucuronic acid conjugation some reports indicated that glucuronyl transferase activity increased in pregnancy, but others described its decrease in pregnancy, suggesting the diversity of glucuronyl transferase. Decreased clearance of VPA may reflect decreased glucuronide formation.

Dickinson et al. reported VPA induced cholelithiasis. In this study, we also measured the bile flow rate for 4 h after intravenous injection of VPA. In the control rats, the bile flow rates were 12.1 ± 2.0, 15.0 ± 2.6 and 20.8 ± 2.0 μl/min (n = 5) after intravenous injection of 10, 50 and 100 mg/kg. In the pregnant rats, those were 17.9 ± 0.9, 22.5 ± 1.7 and 22.1 ± 2.8 μl/min (n = 5), respectively. The cholelithiasis induction by VPA was observed in both groups and its effect was more liable to saturate in the pregnant rats. Further study will be necessary in this matter.

The most remarkable change in pregnancy was the decrease in the protein binding of VPA in plasma (Fig. 3), which brought about a remarkable increase in the \( f_s \) of the pregnant rats. At the low concentration (1 μg/ml) of VPA in serum, the \( f_s \) was 0.24 in the control rats, while that of the pregnant rats was 0.88. This phenomenon was coincident with the report by Stock et al. and Yoshikawa et al. Decreased affinity to the protein binding was indicated, since plasma albumin (main binding protein for VPA) concentrations did not significantly decrease (reduction by 8.8%). The Scatchard plots (Fig. 3) revealed no binding to the high affinity site in the pregnant rats; therefore the high affinity site may be occupied by some endogenous substances. Rai et al. reported that a part of albumin was alkylated during pregnancy, so we cannot deny the possibility that the binding sites of albumin itself may change. Yoshikawa et al. have shown that the \( f_s \) of salicylate increased with increased free fatty acids. Therefore we examined the protein binding to the control rat serum to which was added the same equivalent amount of oleic acid as free fatty acids determined. However, this could not fully explain the remarkably high un-
bound fraction in the pregnant rat serum (data not shown). So we assumed that there were still other influencing intrinsic factors. Non-linear blood cell transfer of VPA was observed both in the control and pregnant rats (Fig. 4). However the concentrations of the blood cell were less than those of plasma in the two groups. The $R_B$ values of pregnant rats always exceeded those in the control rats, reflecting the marked increase in $f_s$.

Yoshikawa et al. reported that the $K_p$ values of salicylate rose with increase of the $f_s$ in pregnancy. In this study, at 10 mg/kg, higher concentrations were observed in most organs in the pregnant rats than in the control rats except for the kidney (Fig. 5). However, the $K_p$ values could be calculated only in the liver ($2.22 \pm 0.23, 1.48 \pm 0.34$) and kidney ($3.04 \pm 0.64, 1.22 \pm 0.25$) of the control and pregnant rats. In the pregnant brain, the $K_p$ was $0.18 \pm 0.02$.

In this study, we determined the $K_p$ values from the concentrations at 3 and 4 h ($\beta$-phase) after intravenous administration of 50 mg/kg. It was assumed that the $K_p$ values might reflect serum (plasma) unbound fraction if the drug was distributed into organs by passive transport. However, the $K_p$ value reflective of the increased unbound fraction in pregnancy was observed only in the brain (the target organ of VPA) (2.7 times that of the control value), whereas significantly lower values were calculated in the liver (approximately half of the control value) and lung (approximately one third of the control value). Although the free fatty acids or other displacing factors may make the $K_p$ values of the liver and lung in pregnant rats lower in a manner similar to that of serum protein binding, the details remain to be elucidated. There was no significant change in other organs, (Table IV). VPA was distributed into the fetuses across the blood-placental barrier as reported by Dickinson et al. Changes of physiological factors in pregnancy may be responsible for the unexpected results.

The protein binding ratio in fetal plasma was higher than that in the maternal serum, but the concentration ratio of the fetal plasma to the maternal plasma ($C_f/C_m$) was approximately 0.9. It is said that the possible dominating factors of the placental transfer are lipophilicity, molecular weight (the molecule with less than 1000 Da is easy to pass), and protein binding. VPA is a weak organic acid ($pK_a = 4.56$) and is almost completely ionized at the physiological pH. The pH value of the pregnant rat serum was 7.53 and that of fetal plasma was 7.36 (Table III). If only the non-ionized form of VPA can pass through the blood-placental barrier, the ratio of $C_f/C_m$ will be calculated according to the following equation:

$$\frac{C_f}{C_m} = \frac{1 + 10 \cdot \text{pH}_f \cdot pK_a}{1 + 10 \cdot \text{pH}_m \cdot pK_a}$$

where $C_f$, $C_m$, pH$_f$ and pH$_m$ are the unbound concentration of VPA in the fetal plasma, the unbound concentration of VPA in the maternal serum, the pH of the fetal plasma, and the pH of the maternal serum, respectively. If we consider the difference in the serum (plasma) unbound fraction between the maternal serum and fetal plasma, the serum (plasma) concentration ratio of VPA (fetal/maternal) could be calculated by $(0.677/0.615)/(1/0.86) = 0.947$. Since this value approximates to 0.9, it appears that the ratio of maternal serum concentration to the fetal plasma concentration could be explained by the pH and protein binding.

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References


