Inhibitory Effect of Valproic Acid on Metabolic Clearance of Carbamazepine Epoxide

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In order to clarify the inhibitory effect of valproic acid (VPA) on the metabolic clearance of carbamazepine-10,11-epoxide (EPO), an active metabolite of carbamazepine, in rat, the disposition of EPO from plasma and the \textit{in vitro} hydration of EPO were measured in the presence of VPA, in addition to estimating the effect of the antiepileptic on uridine 5'-diphosphoglucuronyltransferase (UDPGT) activity. It was found that the elimination of EPO from plasma was hardly affected by the pretreatment of VPA (100 mg/kg/d for 7 d). However, the elimination was significantly delayed by VPA at steady-state concentration, maintained by the repeated oral administration of the drug, with a 38% decrease in total clearance and a 52% increase in the fraction of EPO eliminated unchanged in urine. The \textit{in vitro} hydration of EPO was inhibited competitively by VPA at the concentrations comparable to the \textit{in vivo} levels. The UDPGT activity of hepatic microsomes was also partly inhibited by VPA. These results suggest that VPA at steady state interferes with the metabolic clearance of EPO, by inhibiting the hydration of EPO and glucuronidation of carbamazepine-\textit{trans}-diol, a hydrated metabolite.

**Keywords** — valproic acid-carbamazepine epoxide interaction; carbamazepine epoxide elimination; hydration inhibition; glucuronidation inhibition; decreased epoxide clearance; valproic acid; rat

**Introduction**

Carbamazepine (CBZ) is now a widely used anticonvulsant drug. CBZ gives rise to an epoxide metabolite, carbamazepine-10,11-epoxide (EPO) in man and animals, which has anticonvulsant properties comparable to the parent drug in experimental animals.\textsuperscript{1–3} EPO is further transformed to dihydrocarbamazepine-\textit{trans}-diol (CBZ-diol) (Fig. 1).\textsuperscript{4} Variation in therapeutic response to a given concentration of CBZ in serum is explained in part by the presence of steady-state concentrations of the epoxide metabolite.\textsuperscript{5} It is therefore important to measure the kinetic behavior of EPO in addition to CBZ.

Valproic acid (VPA) is a broad spectrum antiepileptic agent. VPA and CBZ are often used in combination for the treatment of epileptic patients. A few studies have indicated that VPA may have a clinically significant effect upon CBZ disposition.\textsuperscript{6–8} It has been shown that inclusion of VPA in the drug regimen results in higher plasma levels of EPO while CBZ levels are not significantly affected\textsuperscript{9} and that VPA reduces the binding of CBZ to plasma proteins.\textsuperscript{10} Although

![Fig. 1. Metabolism of Carbamazepine by the Epoxide-Diol Pathway](image-url)
these observations suggest that VPA may affect the activity of the epoxide hydrolase, the essential effect on the enzyme remains unclear. Additionally, the drug interactions between VPA and EPO are not well understood.

The present study had two objectives: 1) to determine whether VPA inhibits the metabolism of EPO in the rat in vivo; 2) to find out whether VPA decreases the non-renal clearance of EPO in the same animal species.

Materials and Methods

Materials — Sodium salt of VPA (VPA-Na) was a generous gift of Kyowa Hakko Kogyo Co., Ltd. CBZ was synthesized from Tokyo Kasei Co., Ltd. EPO was synthesized from CBZ by the method of Frigerio et al.\textsuperscript{1,12} 5-(p-Methylyphenyl)-5-phenylhydantoin (methylphenytoin), an internal standard for high performance liquid chromatography (HPLC), was purchased from Aldrich Chemical Co. Uridine 5'-diphosphoglucuronic acid (ammonium salt) and p-nitrophenol were obtained from Sigma Chemical Co. and Kishida Chemical Co., respectively. All other chemicals used were of reagent grade.

Treatment of Animals — Male Wistar rats (Japan SLC Inc.), weighing 200—280 g, were used throughout this study.

1) Pretreatment of Animals with VPA and Single Administration of EPO: The animals were divided at random into 2 groups, each consisting of 4—6 rats. One group was treated for 7 d with daily oral administration of VPA-Na (100 mg/kg, VPA equivalent) as 2% acacia solution and on the day 6 the rat jugular vein was cannulated with a polyethylene tubing.\textsuperscript{13,14} At 2 h after the final dosing of VPA-Na, EPO (15 mg/kg), dissolved in propylene glycol–ethanol–H\textsubscript{2}O (5:2:3, by volume), was injected into the jugular vein through the tubing. The second group was treated for 7 d with daily oral dosing of 2% acacia and followed by intravenous (i.v.) administration of EPO as mentioned above (control). Blood samples (0.25 ml) were collected periodically after dosing through the tubing. The urine was collected in 6-h intervals up to 48 h after dosing of EPO, by the bladder cannulation method.\textsuperscript{15}

2) Single Administration of EPO under Steady-State Plasma Concentrations of VPA: VPA-Na (the first dose 200 mg/kg and then 100 mg/kg, VPA equivalent) as 2% acacia solution was orally administrated every 3 h up to 15 h. At 1 h after the second dosing of VPA, EPO (15 mg/kg) was injected intravenously under ether anesthesia. The second group was treated with 2% acacia solution alone every 3 h (control). These rats were kept in metabolic cages separately and urine was collected in 12-h intervals up to 48 h after administration of EPO. After 7 d rest, the same rats were treated with both VPA and EPO in the same manner as mentioned above and the blood samples (0.3 ml) were collected periodically after dosing of EPO. The urine and blood samples were frozen until assays.

 Determination of EPO in Plasma and Urine — EPO in plasma was determined by the method of Sumi et al.\textsuperscript{16} and McKague et al.,\textsuperscript{9} with slight modifications; acetonitrile (0.2 ml) containing methyl phenytoin (2.5 \mu g/ml) was added to the plasma (0.1 ml) and centrifuged. The sample reconstituted in 100 \mu l of methanol was injected into a column (4.6 mm i.d. \times 15 cm) packed with Inertsil ODS (5 \mu m particle size, Gasukuro Kogyo Co.). EPO in urine was determined by the method of Sumi et al.,\textsuperscript{16} except that 100 \mu l of water was first added to the urine sample.

 Determination of VPA in Plasma — VPA concentration in plasma was determined by the method as described previously.\textsuperscript{17b}

Protein Determination — Protein concentration was determined by the method described by Lowry et al., with bovine serum albumin as a standard.\textsuperscript{18b}

Preparation of Hepatic Microsomal Fractions — The microsomes were prepared from the rat pretreated with phenobarbital (40 mg/kg/d) for 4 d according to the procedure of Omura and Sato.\textsuperscript{19b}

In Vitro Epoxide Hydration of EPO and Effect of VPA — EPO hydration was measured by the method of Tybring et al.,\textsuperscript{20b} using rat hepatic microsomes (5 mg protein/ml) in the presence of 1 and 2 mM VPA. The determination of CBZ-diol was carried out according to the
same method as described by Tybring et al. 20)

Assay of Uridine 5'-Diphosphoglucuronyltransferase (UDPGT) Activity — UDPGT activity was estimated using p-nitrophenol (0.6 mM) as a substrate by the method described by Mulder, 21) in the presence of 0.35—1.40 mM VPA.

Pharmacokinetic and Statistical Analyses — Kinetic parameters were calculated by using the least-squares fit program, MULTI. 22) The elimination rate constant (k_e) was calculated from the slope of terminal elimination phase. The half-life (t_{1/2}) was calculated as t_{1/2} = 0.693/k_e. The area under the plasma concentration-time curve (AUC), the mean residence time (MRT), the area under the first moment curve (AUMC) and the apparent distribution volume at steady state (V_{ds}) were calculated by means of following equations:23, 24)

\[ AUC = \int_0^\infty C_p \cdot dt \]
\[ AUMC = \int_0^t t \cdot C_p \cdot dt \]
\[ MRT = AUMC / AUC \]
\[ V_{ds} = D \cdot AUMC / (AUC)^2 \]

where C_p and D are the plasma concentration and the dose, respectively. The total clearance (Cl_{tot}), renal clearance (Cl_r) and non-renal clearance (Cl_{nr}) were calculated by the following equations:

\[ Cl_{tot} = D / AUC \]
\[ Cl_r = Cl_{tot} \cdot F_{EPO} \]
\[ Cl_{nr} = Cl_{tot} - Cl_r \]

where F_{EPO} is the fraction of EPO dose which was eliminated unchanged in urine collected for 48 h after dosing of EPO.

The means of all data are presented with their standard deviation (mean ± S.D.). Statistical analysis was performed using the non-paired Student's t-test and a p value of 0.05 or less was considered to be significant.

![Graph](image)

Fig. 2. Effect of Repeated Oral Administration of VPA on Plasma Concentration of EPO

○, control; ●, pretreatment with VPA (100 mg/kg/d) for 7 d. EPO (15 mg/kg) was injected intravenously 2 h after the final dosing of VPA. Solid line represents a simulation curve and each point represents the mean ± S.D. of 5 experiments.

Results

Effect of Pretreatment with VPA on Disposition of EPO

The plasma concentration-time curves for EPO after pretreatment with VPA (100 mg/kg/d for 7 d) are depicted in Fig. 2. The elimination of EPO from plasma was slightly decreased by the pretreatment, but not significantly, compared with the control. Some pharmacokinetic parameters calculated are shown in Table I. No significant differences in the parameters were seen between both groups. The elimination parameters of EPO, obtained from the urinary data, are shown in Table II. VPA pretreatment decreased these values slightly compared with the control, but not significantly except for the Cl_r.

Effect of VPA Steady State on Disposition of EPO

In order to estimate the effect of VPA under steady state on the elimination of EPO, VPA was orally administered repeatedly every 3 h. The plasma concentrations of VPA were between 60 and 100 ng/ml, which were within the therapeutic range, 25) and the concentrations ap-
TABLE I. Pharmacokinetic Parameters of EPO Following Repeated Oral Administration of VPA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$t_{1/2}$ (h)</th>
<th>Vdss (l/kg)</th>
<th>AUC (µg·h/ml)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.12 ± 0.45</td>
<td>2.94 ± 0.85</td>
<td>20.45 ± 3.50</td>
<td>3.89 ± 0.84</td>
</tr>
<tr>
<td>Pretreatment with VPA</td>
<td>3.80 ± 0.46</td>
<td>3.00 ± 1.05</td>
<td>27.43 ± 6.29</td>
<td>5.15 ± 1.17</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 5 experiments.

TABLE II. Renal and Non-renal Clearances of EPO Following Repeated Oral Administration of VPA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cl_{tot} (l/h/kg)</th>
<th>$F_{EPO}^a$</th>
<th>Cl_{r} (l/h/kg)</th>
<th>Cl_{nr} (l/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.737 ± 0.150</td>
<td>0.075 ± 0.010</td>
<td>0.055 ± 0.011</td>
<td>0.682 ± 0.141</td>
</tr>
<tr>
<td>Pretreatment with VPA</td>
<td>0.587 ± 0.125</td>
<td>0.056 ± 0.012</td>
<td>0.037 ± 0.011 b</td>
<td>0.554 ± 0.116</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 5 experiments. a) The fraction of EPO dose eliminated unchanged in urine. b) $p < 0.005$ compared with the control.

TABLE III. Pharmacokinetic Parameters of EPO under Steady-State Concentration of VPA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$t_{1/2}$ (h)</th>
<th>Vdss (l/kg)</th>
<th>AUC (µg·h/ml)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.83 ± 0.55</td>
<td>3.56 ± 0.14</td>
<td>16.74 ± 2.82</td>
<td>3.99 ± 0.78</td>
</tr>
<tr>
<td>VPA treatment</td>
<td>4.95 ± 0.56 b</td>
<td>3.93 ± 0.35</td>
<td>26.67 ± 4.12 a</td>
<td>6.93 ± 0.83 b</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 5 experiments. a) $p < 0.005$ and b) $p < 0.001$ respectively compared with the control.

Fig. 3. Effect of Steady-State Concentration of VPA on Plasma Concentration of EPO

○, control; ●, under steady-state concentration of VPA. ■, VPA concentration. EPO (15 mg/kg) was injected intravenously 1 h after the second dosing of VPA. Solid line represents a simulation curve and each point represents the mean ± S.D. of 5 experiments.

Peared to be at steady state. The elimination of EPO from plasma was significantly delayed by repeated dosing of VPA compared with the control without VPA, as shown in Fig. 3. The calculated parameters are listed in Table III. There were significant differences between the parameters of both groups, with the exception of the $Vd_{ss}$. The urinary excretion parameters of EPO in the presence or absence of VPA are shown in Table IV. The $Cl_{tot}$ was decreased by 38% and $F_{EPO}$ was increased by 52% in the presence of VPA, compared with the control, without a significant change of $Cl_r$. Consequently, the $Cl_{nr}$ was diminished by 33% under steady state of VPA. These results indicate that VPA at steady state diminished the elimination of EPO, probably due to the decreased metabolic conversion of the epoxide.

**In Vitro Hydration of EPO and Effect of VPA**

In order to examine *in vitro* the interactions between EPO and VPA which were observed in *in vivo* studies, the *in vitro* hydration of EPO
TABLE IV. Renal and Non-renal Clearances of EPO under Steady-State Concentration of VPA

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cl&lt;sub&gt;tot&lt;/sub&gt; (l/h/kg)</th>
<th>F&lt;sub&gt;EPO&lt;/sub&gt;</th>
<th>Cl&lt;sub&gt;r&lt;/sub&gt; (l/h/kg)</th>
<th>Cl&lt;sub&gt;nr&lt;/sub&gt; (l/h/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.919 ± 0.169</td>
<td>0.043 ± 0.002</td>
<td>0.040 ± 0.007</td>
<td>0.879 ± 0.161</td>
</tr>
<tr>
<td>VPA treatment</td>
<td>0.574 ± 0.097&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.089 ± 0.020&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.047 ± 0.004</td>
<td>0.528 ± 0.099&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 5 rats. <sup>a</sup>) p < 0.005 compared with the control.

was measured in the presence or absence of VPA. The results are shown as the Lineweaver-Burk plots in Fig. 4. The K<sub>m</sub> value was 0.29 mM and the apparent Michaelis constant, K<sub>p</sub>, was 0.41 and 0.49 mM in the presence of VPA 1.0 and 2.0 mM, respectively. The kinetic plots showed the competitive inhibition of EPO hydration by VPA. The mean K<sub>i</sub> value calculated from the formula, K<sub>i</sub> = 1 + (V/K<sub>m</sub>), where i is the concentration of inhibitor, was 2.77 ± 0.29 mM in the presence of VPA. Since the concentrations of VPA used in the in vitro study approximated to the steady-state concentration (100 μg/ml = 0.69 mM) in the in vivo experiment, it is suggested that the inhibitory effect of VPA observed in vivo would be partly due to the inhibition of EPO hydration.

**Effect of VPA on UDPGT Activity**

The hydrated metabolite of EPO, CBZ-diol, would be conjugated to glucuronide and excreted in urine and bile, since O-glucuronides of various hydroxylated metabolites are identified after dosing of CBZ so far. To further obtain insight into the mechanism of VPA inhibition, the glucuronidation of p-nitrophenol was estimated in the presence of VPA. The results obtained are shown in Table V. VPA partly inhibited the in vitro glucuronidation of p-nitrophenol, suggesting that VPA may interfere, at least partly, with the glucuronidation of CBZ-diol in vivo. The in vitro glucuronidation of CBZ-diol was not measured because of the difficulty of obtaining the agent.

**Discussion**

In our previous paper, we showed that VPA, even after repeated administration, at high doses (100 and 200 mg/kg/d for 7 d) could not inhibit the hepatic drug metabolizing enzyme activities in rats. Therefore, in the design of the present study, the effect of VPA on EPO kinetics was examined under steady-state concentration of VPA. VPA at steady-state concentration delayed significantly the elimination of EPO from the systemic circulation and

<table>
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<tr>
<th>VPA (mm)</th>
<th>UDPGT activity (μmol/min/mg protein)</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.12 ± 0.08</td>
<td>100.0</td>
</tr>
<tr>
<td>0.35</td>
<td>1.99 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.9</td>
</tr>
<tr>
<td>0.69</td>
<td>1.91 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.1</td>
</tr>
<tr>
<td>1.40</td>
<td>1.76 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.0</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 3 experiments.

<sup>a</sup>) p < 0.005 and <sup>b</sup>) p < 0.001 respectively compared with the VPA 0 mm.
dramatically decreased the non-renal clearance of EPO, consequently increasing the fraction of EPO excreted unchanged. Thus, it appears that the decrease in the non-renal clearance was responsible for the large reduction observed in the systemic clearance of EPO under steady-state concentration of VPA. These data indicate that VPA at steady state can inhibit the metabolic conversion of EPO.

On the other hand, the pretreatment with VPA for 7 d did not give the significant effect on the EPO disposition from plasma and the urinary excretion, although there was a partial decrease in the kinetic values after the pretreatment. A possible mechanism involved in the decrease (33%) in renal clearance is unclear. This result confirmed our previous suggestion that the reduced effect after the pretreatment with VPA was probably due to the rapid elimination of VPA from the circulation.\(^{17}\)

To date, no quantitative data are available concerning the conversion of CBZ epoxide to the corresponding trans-diol. In order to clarify the inhibitory mechanism by VPA, the in vitro experiment on the hydration of EPO and UDPGT activity were carried out. As a result, VPA in the concentrations comparable to the in vitro levels inhibited the hydration of EPO to CBZ-diol competitively and the \(\alpha\)-glucuronidation partly. It has been reported that EPO elimination is mainly mediated by epoxide hydrolase forming CBZ-diol (73 - 100% of epoxide dose) in man.\(^{4}\) Thus, the inhibitory effect observed in vivo was mainly due to the inhibition of epoxide hydrolase and UDPGT activities by VPA, although a possibility of the metabolic inhibition of \(N\)-glucuronidation of EPO remains.\(^{26,27}\) However, it is reported that \(N\)-glucuronide of EPO represents a very small fraction of the dose (< 5%) in rat urine.\(^{28}\) VPA is shown to deplete hepatic UDP-glucuronic acid significantly, by 90%, at dosages as low as 0.5 mmol (72.1 mg)/kg.\(^{29}\) Therefore, VPA may affect more dramatically the in vivo glucuronidation of CBZ-diol than that in vitro.

The distribution of hepatic epoxide hydrolase is in accord with the distribution of cytochrome P-450 (P-450) and UDPGT is present in the membrane of hepatic endoplasmic reticulum (ER) fractions.\(^{30}\) These three enzymes are therefore ideally positioned to biotransform the products of the enzyme reactions. VPA, incorporated into ER and the membrane at the higher levels, inhibits the epoxide hydrolase and UDPGT, but exhibits a less inhibitory effect on P-450 in the rat,\(^{17}\) and thus leads to the decreased biotransformation and higher plasma levels of EPO. The increase in the amount of urinary EPO may be partly due to an inhibitory effect of VPA on conjugation pathway.

VPA has been reported to be a competitive inhibitor of CBZ epoxidation in the isolated perfused liver.\(^{31}\) The findings are in keeping with our results that VPA inhibited the hydration of EPO competitively. Our estimated \(K_i\) value was 2.77 mM, while the \(K_i\) obtained with human liver microsomes was 0.55 mM.\(^{32}\) The discrepancies between our study and the study of Kerr et al.\(^{32}\) are most likely due to differences in the microsomes.

The hydration pathway, mediated by epoxide hydrolase, is an important detoxication mechanism for various epoxides in man and rat.\(^{33}\) Therefore, it is of significance to clarify the inhibitory effect of VPA on formation of the hydration product of EPO, in relation to the side effect of CBZ which is owing to EPO produced in vivo.\(^{34}\) It is also interesting to note that the decrease in epoxide clearance, observed in this study, is parallel to reports of increased EPO levels in patients on regimens of VPA and CBZ.\(^{9,35,36}\)

In conclusion, significant inhibition by VPA at steady state on the elimination of EPO was observed in rats with a 33% decrease in the non-renal clearance of EPO and a 52% increase in the fraction excreted unchanged. VPA inhibited the in vitro hydration of EPO competitively and the glucuronidation of p-nitrophenol partly. The inhibitory effect in vivo thus may be ascribed to the metabolic inhibition of the hydration of EPO and glucuronidation of CBZ-diol. Our results evoked the possibility that the inhibition spectrum of VPA includes epoxide hydrolase and UDPGT.
References


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