Pharmacokinetics and Enterohepatic Circulation of \((E)-2\)-Ene Valproic Acid in the Rat

Kuldeep SINGH,* James M. ORR** and Frank S. ABBOTT**

Clinical Biology V-33, CIBA-GEIGY Corporation,* Summit, NJ, 07901 USA and Faculty of Pharmaceutical Sciences, 2146 East Mall, University of British Columbia, ** Vancouver, B.C., Canada V6T 1W5

(Received May 9, 1990)

A pharmacologically active monounsaturated metabolite of valproic acid (VPA), \((E)-2\)-ene VPA, was administered by an intravenous bolus dose of 20 mg/kg to normal and bile-exteriorized rats. The total plasma clearance of \((E)-2\)-ene VPA in normal rats was 4.9 ml/min/kg and in bile-exteriorized rats, 7.7 ml/min/kg. \((E)-2\)-ene was recycled in the plasma of normal rats due to enterohepatic circulation. Approximately 32% of the dose was excreted in the urine of normal rats. Of the administered dose to bile exteriorized rats, approximately 27% was excreted in the urine and 38% in the bile. Administration of \((E)-2\)-ene increased bile flow rate, and the induced choleretic lasted for 3–4 h. \((E)-2\)-ene VPA was largely excreted in apparently conjugated form in the urine and bile. The pharmacokinetics of \((E)-2\)-ene VPA were similar to that of the parent drug VPA.

Keywords — \((E)-2\)-ene valproic acid; pharmacokinetics; rat; enterohepatic circulation; biliary excretion

Introduction

Valproic acid (VPA) is a broad spectrum anticonvulsant widely used in the treatment of generalized absence and tonic-clonic seizures.\(^{1,2}\) VPA (I) is metabolized to several metabolites\(^{3}\) in man. The major routes of I metabolism are conjugation, which produces I glucuronide\(^{4,5}\) and \(\beta\)-oxidation, that forms \((E)-2\)-ene VPA (II).\(^{6}\) The steady state serum levels of II have been reported to be 12% of that of I in pediatric patients.\(^{3}\) The major interest in II emerges from the fact that II is a potent anticonvulsant\(^{7,8}\) in a variety of seizure models including mice, rats and gerbils. Based on the brain levels, the relative pharmacologic activity of II is 1.3 times higher than that of I.\(^{9}\) The anticonvulsant activity of II may be attributed to its ability to elevate \(\gamma\)-aminobutyric acid (GABA) levels in the brain.\(^{10}\) Moreover, II is free of teratogenic side effects at concentrations higher than I.\(^{7,10}\) Kesterson et al.\(^{12}\) have shown that II, unlike I, is not hepatotoxic in the rat. These results suggest that II may be suitable alternate antiepileptic agent that is free of serious side effects of I, namely embryotoxicity and hepatotoxicity.

The pharmacokinetics of II have not been well characterized. In some studies, the parent drug I was administered, and the plasma or tissue levels of II were monitored.\(^{13,14}\) After an i.p. dose of I to mice, the plasma elimination \(t_{1/2}\) of II was 130 min.\(^{13}\) In man, the apparent elimination \(t_{1/2}\) of II has been reported to be 43 h following I administration.\(^{14}\) In a few studies, II was administered to mice\(^{15,16}\) and its plasma levels were measured. After a constant rate administration of II to mice, the elimination \(t_{1/2}\) was found to be 70 min.\(^{15}\) In a short abstract, O'Connors et al.\(^{17}\) had reported only the serum clearance of II in the rat. In this report, the pharmacokinetics and enterohepatic circulation of II in the rat are described.

Materials and Methods

Materials — II and the internal standard, di-\(N\)-butyric acid, were synthesized and purified as described earlier.\(^{13}\) All other chemicals and solvents were of analytical grade.

Animal Experiments — Male Sprague-Dawley rats weighing 270—320 g were used. The animals were divided into two groups, normal and bile-exteriorized. The jugular vein of normal rats was cannulated with polyethylene tub-
ing. The jugular vein and bile-duct of bile-exteriorized rats were cannulated. Rats from each group were given an i.v. dose of 20 mg/kg of II. Plasma, urine and bile samples were collected as detailed earlier.19

Assay Procedure — Plasma, urine and bile were analyzed for II by a capillary gas chromatograph-mass spectrometer assay method identical to that reported for 4-ene VPA.19,20 For the analysis of conjugates of II, an aliquot of urine or bile was heated with 3N NaOH, acidified with 4N HCl and extracted as described earlier.20

Pharmacokinetic Analysis — The terminal elimination rate constant, K, was calculated from the linear portion of the log plasma concentration-time plot. The apparent $t_{1/2}$ was determined as 0.693/K. Volume of distribution of central compartment, $V_1$, was calculated as $D/C_0$, where $D$ is the dose and $C_0$ is the initial plasma concentration at time zero. Total area under the plasma concentration ($AUC_{0-\infty}$) was calculated as $AUC_{0-1} + C_t/K$. $AUC_{0-1}$ was obtained by trapezoidal rule from time zero to time $t$. $C_t$ is the plasma concentration at time $t$. The total body plasma clearance, $Cl_{1r}$, was calculated as $D/AUC_{0-\infty}$. Renal and biliary clearances, $Cl_{1r}$ and $Cl_{1b}$, were determined as the fraction of dose excreted unchanged (unchanged II) in urine and bile, respectively, multiplied by $Cl_{1r}$. The clearance due to the formation of conjugates, $Cl_{1con}$, was determined as a sum of the fractions of dose recovered collectively as conjugated II in urine and bile, multiplied by $Cl_{1r}$. The metabolic clearance, $Cl_{m}$, was obtained as the difference between $Cl_{1r}$ and $Cl_{1r}$ in normal rats.

For normal rats, a simplified version of the time-lag pharmacokinetic model,21 as shown in Fig. 1, was applied to describe the plasma profile in normal rats. The differential equations for this model were solved by MULTI(RUNGE), a non-linear regression computer program.22 For bile-exteriorized rats, the terminal plasma elimination rate constant ($K$) was equal to the sum of microparameters $k_{10} + k_{12}$. The microparameter $k_{12}$ was calculated as fraction of the dose eliminated in bile times $K$, and $k_{10}$ was $K - k_{12}$.

Results

Assay Method

Calibration curves for II were linear in the concentration range of 0.4—35 µg/ml in plasma, 2—200 µg/ml in urine and 1—150 µg/ml in bile. For all the calibration curves, the coefficient of correlation, $r$, was >0.997, within day coefficient of variation (CV) <5% and between days CV <7%. The lowest detection limit for an 80 µl plasma sample was 60 ng/ml with a signal-to-noise ratio of 4. The extraction efficiency was essentially 100%.

Pharmacokinetics in Normal Rats

Figure 2 shows plasma profile of II in normal rats following an i.v. dose of 20 mg/kg. The initial plasma level, of $85 \pm 14$ µg/ml at time zero, declined rapidly with a $t_{1/2}$ of $23 \pm 4.1$ min dur-

![Fig. 1. Time-Lag Pharmacokinetic Model](image)

Compartment 1, sampling compartment (blood); compartment 2, bile; $\tau$, time lag; $k_{10}$, $k_{12}$ and $k_{21}$, first order rate constants.

![Fig. 2. Average Plasma Levels of (E)-2-Ene VPA in Normal Rats after an i.v. Bolus Dose of 20 mg/kg](image)

Solid line —, model predicted. Standard deviations are shown by vertical lines. ($N = 4$).
TABLE I. Pharmacokinetic Parameters of (E)-2-Ene VPA in Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Bile-exteriorized&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{1/2} ) min</td>
<td>23 ± 4.1</td>
<td>20 ± 3.6</td>
</tr>
<tr>
<td>( V_1 ), ml/kg</td>
<td>240 ± 43</td>
<td>220 ± 26</td>
</tr>
<tr>
<td>( AUC_{0-\infty}, \mu g \cdot \text{min/ml} )</td>
<td>4630 ± 1980</td>
<td>2750 ± 732</td>
</tr>
<tr>
<td>% Dose excreted in urine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unconjugated</td>
<td>5.9 ± 3.2</td>
<td>3.5 ± 1.6</td>
</tr>
<tr>
<td>Conjugated</td>
<td>26 ± 6.4</td>
<td>24 ± 5.8</td>
</tr>
<tr>
<td>Total</td>
<td>32 ± 6.0</td>
<td>28 ± 7.9</td>
</tr>
<tr>
<td>% Dose excreted in bile</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unconjugated</td>
<td></td>
<td>8.2 ± 2.8</td>
</tr>
<tr>
<td>Conjugated</td>
<td></td>
<td>30 ± 7.0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>38 ± 9.8</td>
</tr>
<tr>
<td>Apparent clearance (ml/min/kg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( C_l )</td>
<td>0.27 ± 0.13</td>
<td>0.28 ± 0.13</td>
</tr>
<tr>
<td>( C_{lb} )</td>
<td></td>
<td>0.64 ± 0.28</td>
</tr>
<tr>
<td>( C_{l_{(con)}} )</td>
<td></td>
<td>4.1 ± 0.99</td>
</tr>
<tr>
<td>( C_l )</td>
<td>4.9 ± 1.7</td>
<td>7.7 ± 1.8</td>
</tr>
<tr>
<td>Microparameters (min⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( k_{10} )</td>
<td>0.017 ± 0.005</td>
<td>0.022 ± 0.004</td>
</tr>
<tr>
<td>( k_{12} )</td>
<td>0.011 ± 0.002</td>
<td>0.013 ± 0.005</td>
</tr>
<tr>
<td>( k_{21} )</td>
<td>0.021 ± 0.005</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> N = 4; <sup>b</sup> initial plasma decline, 0—1 h.

The trough levels of 5.7 ± 5.6 μg/ml were reached at approximately 2 h. Thereafter, the plasma levels started to rise to achieve a secondary peak at approximately 4 h. This secondary plasma peak was attributed to enterohepatic circulation (EHC) of II in the rat. After 4 h, II was eliminated from plasma with an apparent \( t_{1/2} \) of 55 min. The plasma data of II was fitted to the time-lag model (Fig. 1) using an optimal time-lag (\( \tau \)) value of 1.75 h. The values of first-order transfer rate constants \( k_{10} \), \( k_{12} \) and \( k_{21} \) were 0.017 ± 0.005, 0.011 ± 0.002 and 0.021 ± 0.005 min⁻¹, respectively. The model generated curve (solid line) is shown in Fig. 2. Various pharmacokinetic parameters for II in normal rats are shown in Table I.

**Pharmacokinetics in Bile-Exteriorized Rats**

The plasma levels of II in bile exteriorized rats are shown in Fig. 3. Following an i.v. dose of 20 mg/kg, the plasma profile follows an open one-compartment model with first-order elimination \( t_{1/2} \) of 20 ± 3.4 min. No secondary plasma peaks were seen in the bile-exteriorized rats. The pharmacokinetic parameters of II in bile-exteriorized rats are shown in Table I.

**Choleretic Effect**

A typical choleretic effect of II is shown in Fig. 4. The normal bile flow rate of 0.82 ml/h increased rapidly to maximal value of 2.3 ml/h during the first 0.5 h of II administration. Thereafter, the bile flow rate declined gradually to reach normal values within 4 h.
Pharmacokinetics of (E)-2-ene VPA in Rat

Fig. 4. Biliary Excretion and Choleretic Effect of (E)-2-Ene VPA in a Rat after an i.v. Bolus Dose of 20 mg/kg
- bile flow rate (ml/h); ○, conjugated and ○, unconjugated (E)-2-ene VPA (mg/ml), respectively, in bile.

The concentration of unconjugated, and conjugated II in bile samples is plotted against the mid-point of the time interval (Fig. 4). The peak concentration of conjugated II, 1.1 mg/ml, was achieved within the first half an hour. The concentration of conjugated II declined parallel to the bile flow rate. Only low levels of unconjugated (E)-2-ene VPA were found in the bile.

Discussion

The pharmacokinetics of II were similar to those reported for I,23 and a putative hepatotoxic metabolite 4-ene VPA19 in the rat. II was recirculated in normal rats due to EHC. II was largely excreted as conjugates in urine and bile.

In normal rats, the plasma level of II during the first h of dose declined with a $t_{1/2}$ of 23 ± 4.1 min, compared to 11.7 ± 0.5 min for I in the rat.23 There was a time-lag of approximately 1.5—2 h before the plasma levels of II started to rise due to EHC. After the secondary peak, the apparent elimination half-life of II (38.3 ± 15.9 min) was longer than that (23 ± 4.1 min) of plasma decline during the initial phase between 0—1 h. The reason for slower terminal elimination is that it is not “true” elimination phase, since absorption would still be occurring of the unconjugated II from the gastrointestinal tract (GIT) of the rat. Experiments with bile-exteriorized rats confirmed that if II was prevented from enterig the GIT via bile, secondary plasma peaks were abolished. EHC of several drugs following conjugation with glucuronic acid has been reported in the literature.24 It is generally believed that the glucuronide is transported via bile into the GIT, deconjugated, and the unconjugated drug is reabsorbed through the intestinal wall.24 The possible reasons for a delay in the appearance of secondary plasma peak, in spite of a lack of gall bladder in the rat, have been discussed by Singh et al.19 and Ogiso et al.25 These factors include delays due to the time required for bile to reach site of deconjugation, lack of β-glucuronidase activity at the proximal part of the intestine, time required for deconjugation, and absorption of unconjugated drug. Ogiso et al.25 have shown that deconjugation is the rate limiting step for VPA during its EHC in the rat.

The plasma data obtained from normal rats was fitted to a time-lag pharmacokinetic model.19 The differential equations were solved by MULTI(RUNGE). The time-lag value was chosen to provide the best fit of the model-predicted curve to actual plasma levels, as measured by the least sum of residual squares. The calculated values of microconstants $k_{10}$, $k_{12}$ and $k_{21}$ were used to determine effective clearance (Cl_{eff}) and net clearance (Cl_{net}) as reported by Colburn.21 Cl_{eff}, determined as $V_1(k_{10} + k_{12})$ describes the intrinsic ability of liver to remove the drug. Cl_{net}, estimated as $V_1k_{10}$, describes the permanent elimination from the body. The Cl_{eff} for II was 6.3 ± 1.1 ml/min/kg and Cl_{net}, 3.8 ± 0.78 ml/min/kg.

The total plasma clearance of II in normal rats, 4.9 ± 1.7 ml/min/kg, was comparable to that of serum clearance, 4.0 ml/min/kg reported by O’Connors et al.17 Both these studies indicate that the plasma clearance of II is similar to that of I, 4.17 ml/min/kg, in normal rats.26 The total plasma clearance of II was, however, only 56% of that of another monounsaturated metabolite of VPA, 4-ene VPA, in the rat.19

The volume of distribution of II, 220 ml/kg, was almost half of that of I, 430 ml/kg, in the rat.23 The smaller volume of distribution may be due to higher plasma protein binding of II (>90%) than I (<80%) in the rat.27

The total biliary excretion of II, 38% of the dose, was markedly smaller than that of I, which
was approximately 60% of the low dose of I.\textsuperscript{23})
Approximately 53\% of II was recovered as conjugates in the bile and urine, collectively, compared to 62\% for I.\textsuperscript{23}) These results were similar to those observed for another monounsaturated metabolite, 4-ene VPA.\textsuperscript{19}) Thus, introduction of a double bond appears to decrease conjugation and biliary excretion of the branched chain aliphatic acid, I.

In conclusion, the pharmacokinetics of II in the rat were similar to that of the parent drug I. II exhibited EHC in normal rats, induced choleretic effect, and was eliminated largely in conjugated form in urine and bile.

Acknowledgements

The authors thank Mr. Roland Burton for his technical assistance on the GCMS and Mr. Peter Phillips for his assistance on the computer programming.

References

22) K. Yamaoka and T. Nakagawa: A nonlinear least


