PROBENECID-ASSOCIATED ALTERATIONS IN VALPROIC ACID PHARMACOKINETICS IN RATS: CAN IN VIVO DISPOSITION OF VALPROATE GLUCURONIDE BE PREDICTED FROM IN VITRO FORMATION DATA?

E. STACY WARD, GARY M. POLLACK, AND KIM L. R. BROUWER

Curriculum in Toxicology, School of Medicine, and Division of Drug Delivery and Disposition, School of Pharmacy, The University of North Carolina at Chapel Hill, North Carolina

(Received May 17, 2000; accepted August 18, 2000)

This paper is available online at http://www.dmd.org

ABSTRACT:

Previous investigations have suggested that probenecid (PRB) alters the in vivo disposition of valproic acid (VPA), perhaps by inhibiting hepatic formation of valproate glucuronide (VG). Because VPA and PRB bind moderately to plasma proteins, protein binding also is a potential locus of interaction. The purpose of this investigation was to determine whether in vitro systems could accurately predict PRB-associated perturbations in the hepatobiliary disposition of VPA and VG in vivo. VPA and PRB were coadministered to rats for 60 min at various infusion rates to examine steady-state VPA disposition. PRB did not alter the binding of VPA in serum or hepatic cytosol. However, PRB decreased the apparent intrinsic clearance of VPA (1.81 ± 0.58 versus 1.23 ± 0.23 ml/min; P = .025) by competitively inhibiting VPA elimination. In a separate study, rat hepatic S9 fractions were incubated with VPA (7.2–721 μg/ml) and PRB (0–2850 μg/ml). VG formation (Vmax = 0.80 ± 0.06 μg/min/mg of protein; Km = 173 ± 28.8 μg/ml) was impaired by PRB in a competitive manner (K = 876 ± 559 μg/ml), consistent with the in vivo data. Despite inhibition of phase II metabolism of VPA to VG by PRB, the VG biliary excretion rate at similar unbound VPA concentrations in hepatic cytosol was not lower in PRB-treated rats. These results indicate that VG disposition in the presence of PRB cannot be predicted accurately based solely on in vitro inhibition of glucuronidation and emphasize the complexity of processes associated with the hepatobiliary system.

Hepatic disposition of xenobiotics may be modulated by a number of mechanisms, including alterations in hepatocellular uptake, hepatocellular binding, metabolism, hepatic egress, and biliary excretion. Perturbations in the hepatobiliary disposition of drugs, toxicants, and endogenous substrates may alter pharmacologic and toxic responses. In vitro systems may be useful in predicting xenobiotic interactions at specific sites in the liver. However, the complexity of the in vivo system may require a multiexperimental approach to accurately predict a priori perturbations in the disposition of xenobiotics and metabolites secondary to drug interactions, chemical exposure, or physiologic variations.

The anticonvulsant valproic acid (VPA) is a useful model substrate for studying hepatic translocation processes. VPA is relatively lipophilic [octanol:water partition coefficient of ~900 at pH 7.2 (Liu, 1991)] and exists predominantly in the ionized form at physiologic pH (Bellringer et al., 1988). The nonlinear relationship between VPA plasma concentrations and dose in rats has been attributed, in part, to saturable hepatic clearance (Dickinson et al., 1979). Although the VPA elimination rate does not increase proportionately with steady-state concentrations of unbound VPA in serum, the relationship between elimination rate and unbound concentration in hepatic cytosol is linear (Brouwer et al., 1993). These data support the hypothesis that the nonlinear elimination of VPA in rats may be due to concentration-dependent distribution of VPA between the hepatocyte and blood. However, studies in freshly isolated rat hepatocytes indicated that hepatocellular uptake of VPA occurred rapidly by passive diffusion (Booth et al., 1996); no evidence for a saturable translocation process was observed. The mechanism(s) underlying the nonlinear hepatic distribution of VPA have yet to be elucidated completely.

VPA is metabolized by β- (in hepatic mitochondria) and ω- (by microsomal enzymes) oxidation, as well as by acyl glucuronidation [mediated by uridine diphosphate glucuronosyltransferase (Schobben and van der Kleijn, 1982)]. After a 75- to 300-mg/kg dose of VPA, approximately 50% of the administered dose undergoes glucuronidation in male rats (Pollack and Brouwer, 1991) with somewhat more extensive glucuronidation in female animals (Liu and Pollack, 1993). After a similar dose of VPA in female rats, 29% of the dose was excreted in urine, and 53% in bile, as valproate glucuronide (VG) (Liu et al., 1992). The mechanism(s) involved in the formation and biliary excretion of VG, and their contribution to the nonlinear disposition of VPA, have not been investigated.

VPA is bound moderately and in a concentration-dependent fashion to proteins in rat serum and hepatic cytosol (Brouwer et al., 1993). The degree of VPA binding was lower ex vivo than in vitro, and VG
was bound more highly than VPA ex vivo in serum. These data are consistent with the hypothesis that VG may displace VPA from protein binding sites. The intrahepatocyte protein binding may alter hepatic translocation of VPA and should be considered as a potential site of interaction/regulation of hepatobiary VPA disposition.

Previous investigations have suggested that probenecid (PRB) alters the hepatobiary disposition of VPA, perhaps by inhibiting VG formation (Golden et al., 1993). PRB is an inhibitor of several renal and hepatic transport systems and undergoes extensive phase I and phase II metabolism in the rat (Dayton et al., 1973). PRB ultimately is metabolized to an acyl and two ether glucuronides (Guarino et al., 1969). PRB decreases the clearance of numerous substrates of uridine diphosphoglucuronosyltransferase (Smith et al., 1985) and also directly inhibits glucuronidation of various substrates (Mays et al., 1991). In addition to directly inhibiting glucuronidation, PRB may deplete cofactor supply or inhibit cofactor transport to the enzymatic site (Hauser et al., 1988). PRB binds significantly (~90%) in human serum (Vee et al., 1993), and in vitro studies have shown that PRB glucuronide (a major metabolite of PRB) is ~75% bound in serum (Vee et al., 1992). PRB is transported actively into bile (Guarino and Schanker, 1968) and may impair the biliary excretion of other xenobiotics (Savina and Brouwer, 1992). PRB, a classic inhibitor of organic anion transport, has been shown to reverse multidrug resistance in multidrug-resistant-associated protein (MRP) overexpressing cell lines (Gollapudi et al., 1997).

The current investigation characterizes the effects of PRB on the in vivo disposition of VPA in rats. To determine whether in vitro systems could accurately predict PRB-associated perturbations in the hepatobiary disposition of VPA and VG in vivo, the effects of PRB on the ex vivo protein binding and in vitro hepatic glucuronidation of VPA were examined.

Materials and Methods

Chemicals. VPA was obtained from Aldrich Chemical Co. (Milwaukee, WI), and PRB was purchased from Sigma Chemical Co. (St. Louis, MO); all other reagents were purchased from standard commercial sources and were of the highest purity available.

Animals. Male Sprague-Dawley rats (Hilltop Laboratory Animals, Scottsdale, PA) were used in all experiments. Animals were allowed to acclimate for at least 1 week before experimentation. Rats were housed individually in hanging stainless steel cages and were maintained on a 12-h light/dark cycle with food and water available ad libitum. All procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

In Vivo Disposition and ex Vivo Binding Studies. Rats (310–370 g) were anesthetized with urethane (1 g/kg, i.p.), and silicone rubber cannulae were implanted in the right jugular and right femoral veins for blood sampling and drug administration, respectively. A polyethylene (PE-10; Clay-Adams, Parsippany, NJ) cannula was implanted in the bile duct to interrupt enterohepatic recirculation and collect bile. Rats remained anesthetized for the duration of the study; body core temperature was maintained at 37°C with a rectal probe and heating pad (Selectronic, Danvers, MA). Each Amicon device was centrifuged (3500 g, 15 min) in a fixed angle rotor at 37°C for 2 to 3 min (the time required to produce 10% of the initial volume as filtrate).

In Vitro Metabolism. Metabolism of VPA was studied in the 9000g supernatant (S9) fraction of rat liver homogenates, which was prepared by a modified version of a published method (Pang et al., 1985). The in vitro VPA glucuronidation rate was assessed by quantitating VG formation according to a published procedure (Chen et al., 1996). Preliminary experiments were conducted to optimize protein, surfactant, and cofactor concentrations. Reaction mixtures (total volume of 260 μl of 1 mM Tris-HCl buffer, pH 7.4) contained final concentrations of 1 mg/ml Brij 58, 9.6 mM MgCl₂, 2.3 mM uridine 5'-diphosphoglucuronic acid, and 12 mg/ml hepatic S9 protein. Reaction mixtures were preincubated in an orbital shaking water bath (AquaTherm Water Bath Shaker, New Brunswick Scientific, Edison, NJ) at 37°C for 3 min; VPA (7.2–721 μg/ml, dissolved in 20 mM Tris-HCl buffer, pH 7.4) and either PRB (0–2850 μg/ml, dissolved in excess NaOH (neutralized with HCl) and 20 mM Tris-HCl buffer) or PRB vehicle (NaOH, HCl, and Tris-HCl buffer) were added to the preincubated mixture to initiate the reaction. The reaction was terminated by the addition of 20 μl of 3.5 N HCl, and samples were placed immediately on dry ice. In preliminary experiments, the reaction mixtures were incubated for various time periods up to 60 min to determine the linear range for the metabolic reaction; a single time within that range was chosen for subsequent experiments. VG formation rate was expressed as the total amount of VG formed per milligram of protein per minute. Protein concentrations were determined by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Quantitation of VPA and VG. Concentrations of VPA and VG were determined by a modified gas chromatographic method (Liu et al., 1992). Standard curves were linear in the relevant concentration range, with an intraday coefficient of variation of <5%. The lower limit of quantitation of the assay was 2 μg/ml. Alkaline hydrolysis was used to hydrolyze β-glucuronidase-sensitive and -resistant VG. Concentrations of conjugated VPA were determined as the difference between total (sample aliquot subjected to alkaline hydrolysis before extraction) and unconjugated (sample aliquot extracted directly) VPA concentrations.

Data Analysis. In vivo disposition data were analyzed by nonlinear least-squares regression (PCNONLIN version 3.0) to determine an apparent Vₐx and Kₔ for VPA elimination. For comparison, a linear kinetic model also was fit to the data. Goodness of fit and the ability of a given model to describe the data were assessed with standard model selection criteria (Akaike’s information criterion [AIC]; Yamaoka et al., 1978), residual sums of squared error, and condition number of the model). The relationship between steady-state elimination rate (equal to the infusion rate) and VPA serum concentration was described with a Michaelis-Menten function. For the ex vivo binding experiments, the relationship between bound and unbound substrate concentrations (Cₜₜbound and Cₜₜunbound) in serum or cytosol was described with three models (representing specific binding, nonspecific binding, and a combination of the two) defined, respectively, by eqs. 1 through 3:

\[ C_{\text{bound}} = \frac{nP_v \cdot K_v \cdot C_{\text{unbound}}}{1 + K_v \cdot C_{\text{unbound}}} \]  
\[ C_{\text{bound}} = n \cdot C_{\text{unbound}} \]  
\[ C_{\text{bound}} = \frac{nP_v \cdot K_v \cdot C_{\text{unbound}} + m \cdot C_{\text{unbound}}}{1 + K_v \cdot C_{\text{unbound}}} \]
where \( nP_i \) is the binding capacity, \( K_i \) is the association constant for the specific binding site, and \( m \) is the slope of the nonspecific binding profile. The binding equations each were fit to the data, and the model selection criteria described above were used to determine the optimal model.

In vitro metabolism data also were analyzed by nonlinear least-squares regression to determine \( V_{\text{max}} \) and \( K_m \) for VG formation, the mechanism of inhibition [competitive, noncompetitive, or uncompetitive (Segel, 1976)], and the \( K_i \) for inhibition by PRB. The model selection criteria discussed above were used to identify the optimal inhibition model.

**Statistical Analysis.** To assess the significance of differences in \( V_{\text{max}} \) and \( K_m \) for VG formation between different PRB treatment groups, one-way ANOVA was used; comparison of PRB treatment groups to controls was performed by a multiple comparison test with a Bonferroni correction. Differences between group means in the in vivo and ex vivo studies were assessed with an unpaired Student’s \( t \) test where appropriate. To determine the significance of treatment and infusion rate on the apparent clearance of VPA, \( F \) tests were performed by a multiple comparison ANOVA; a Tukey’s Studentized range test was used to determine which groups were statistically different from each other. To determine the significance of treatment and infusion rate on the apparent clearance of VPA, \( t \) tests were performed by a multiple comparison ANOVA; a Tukey’s Studentized range test was used to determine which groups were statistically different from each other.

**Results**

PRB consistently increased VPA serum concentrations in vivo compared with control rats receiving comparable VPA infusion rates, resulting in a decrease in the apparent clearance of VPA (Table 1). In the presence and absence of PRB, apparent VPA clearance decreased with increasing VPA infusion rate. Two-way ANOVA indicated that both PRB treatment (\( P = .012 \)) and VPA infusion rate (\( P = .028 \)) were significant determinants of VPA clearance.

The model selection criteria indicated that a saturable elimination model provided a better description of the elimination rate versus steady-state VPA concentration data (Fig. 1A) than a simple linear model for both the control and PRB-treated data. Estimates for \( V_{\text{max}} \) in control and PRB-treated rats were not significantly different. However, the model-generated estimate of \( K_m \) was nearly 3-fold higher in PRB-treated animals. To increase the accuracy of the model-generated estimate of \( K_m \), a Michaelis-Menten model with a single

![Fig. 1. VPA elimination rate profiles.](image)

\( V_{\text{max}} \) and either a control \( K_m \) or a PRB-treated \( K_m \) was fit simultaneously to the combined data (serum VPA concentration range of 7.5–1000 \( \mu \)g/ml) from control and PRB-treated animals. The parameter estimates generated for this model were \( V_{\text{max}} = 1914 \pm 215 \mu \)g/min, control \( K_m = 442 \pm 110 \mu \)g/ml, and PRB-treated \( K_m = 1186 \pm 244 \mu \)g/ml.

In contrast to the relationship between elimination rate and serum VPA concentrations, the rate of VPA elimination at steady-state appeared to increase proportionately with VPA concentrations in hepatic cytosol (Fig. 1B); a linear model provided the optimal fit to the observed data, based on standard model selection criteria (AIC, residual sums of squared error, and condition number of the model). Linear least-squares regression of elimination rate versus cytosolic VPA concentrations provided apparent intrinsic clearance estimates for control (1.81 \( \pm \) 0.58 ml/min) and PRB-treated (1.23 \( \pm \) 0.23 ml/min) rats that were significantly different (\( P = .025 \), two-tailed unpaired Student’s \( t \) test).

The effect of PRB on ex vivo VPA protein binding in serum also was evaluated. A model incorporating a single specific binding site provided the best description of the data in both control and PRB-treated rats. The unbound VPA fraction in serum was 0.722 \( \pm \) 0.129
VPA elimination in bile accounted for only ~1% of the total dose administered, and PRB did not appear to affect this process (0.94 ± 0.57 versus 1.16 ± 0.49% in control versus PRB-treated rats, respectively). In individual rats, the standard deviation of the VG biliary excretion rate was relatively small throughout the 60-min sampling period (Fig. 4A): PRB did not appear to alter the VG biliary excretion rate when similar VPA infusion rates were administered. The VG biliary excretion rate increased as unbound VPA concentrations in cytosol increased (Fig. 4B). Surprisingly, the VG biliary excretion rate at similar unbound VPA concentrations in hepatic cytosol was not lower in PRB-treated rats, even though PRB impaired VG formation.

Bile flow was increased significantly by the excretion of VG into bile (Fig. 5). A direct relationship between bile flow and biliary VG excretion was demonstrated with an intercept of ~10 μl/min. Bile flow increased proportionately with the mass of VG excreted into bile; the slope of the relationship between bile flow and the excretion of VG in bile was 29.0 μl/μmol.

PRB did not appear to alter the hepatic subcellular distribution of VPA (Table 2). Although significant differences in the distribution of VPA were observed across fractions in both control and PRB-treated groups, PRB treatment did not alter VPA distribution within a given fraction. VPA was localized predominantly in hepatic cytosol.

The rate of conversion of VPA to VG in male Sprague-Dawley rat hepatic S9 fractions was linear through at least 10 min (data not shown); this incubation time was selected for termination of subsequent incubations. The rate of VG formation in the absence and presence of PRB was analyzed according to the Michaelis-Menten equation to obtain estimates of $V_{\text{max}}$ and $K_m$ at each PRB concentration (Table 3). Addition of increasing PRB to the reaction mixture significantly increased the $K_m$ for VG formation while $V_{\text{max}}$ remained relatively constant. Mathematical modeling of the data was used to determine the mechanism of inhibition. All models were of full rank, indicating that none were overparameterized, and both the AIC and the residual sums of squared error indicated that the competitive model best described the data. To estimate the relevant metabolic parameters, the data from experimental conditions (control and all PRB concentrations) were combined and fit with a single competitive inhibition model. The model-generated $K_i$ for inhibition of VG formation by PRB in vitro was 876 ± 559 μg/ml. In addition, mathematical modeling of the data gave Michaelis-Menten parameter estimates that indicated a significant increase in $K_m$ (173 ± 28.8 versus...
584 ± 278 μg/ml) and no change in $V_{\text{max}}$ 0.80 ± 0.06 versus 0.76 ± 0.37 μg/min/mg of protein) at PRB concentrations of 0 versus 0.2850 μg/ml (Table 3).

Discussion

In the in vivo disposition studies performed in this investigation, PRB decreased the apparent clearance of VPA due to a significant decrease in intrinsic clearance. These findings are consistent with the in vitro study. PRB significantly increased the $K_m$, but not the $V_{\text{max}}$, for VPA glucuronidation in vitro. These data support the hypothesis that PRB competitively inhibits the elimination of VPA and are consistent with previously published observations in which PRB treatment increased the $K_m$ for VPA elimination in rats (Golden et al., 1993).

PRB treatment did not alter VPA protein binding in serum or hepatic cytosol or the binding of VG in serum. VPA binding parameters in serum were similar to previously published ex vivo results ($nP_{\text{T}} = 0.229 ± 0.079 \text{ mg/ml}; K_m = 3.25 ± 1.86 \text{ ml/mg}$ (Brouwer et al., 1993)]. VPA, PRB, and the associated glucuronide conjugates are moderately to highly protein-bound in serum (Vree et al., 1992; Brouwer et al., 1993). The apparent lack of any binding interactions in the present study suggests that these compounds are bound to different sites.

The relationship between unbound VPA concentrations in serum and hepatic cytosol appeared to be nonlinear. These results are in agreement with previously published in vivo data (Brouwer et al., 1993) suggesting that a regulatory process is involved in the uptake of VPA into hepatocytes. Although the mechanism underlying this nonlinear distribution is unclear, PRB did not alter the distribution of VPA between cytosol and serum.

In general, the VG biliary excretion rate increased as unbound VPA concentrations increased (Fig. 4B). Modest variability in this relationship presumably was due to differences in the formation rate of VG in individual rats, and due to the fact that cytosolic VG, and not cytosolic VPA, is the driving force for biliary VG excretion. Due to the absence of complete mass balance data for the glucuronide

![Graph A](image1)

**Fig. 4.** VG biliary excretion rates.

A, mean (± S.D.; $n = 4$/rat) VG biliary excretion rates (μg/min) over the 60-min collection period in individual rats. Open bars represent control rats; solid bars represent PRB-treated rats. B, relationship between VG biliary excretion rate (45–60 min) and unbound cytosolic VPA concentrations at 60 min. ○, control animals; ●, PRB-treated animals (each data point represents one rat per infusion rate).

![Graph B](image2)

**TABLE 2**

<table>
<thead>
<tr>
<th>Subcellular Fraction</th>
<th>Vehicle ($n = 7^a$)</th>
<th>Probenecid ($n = 6^b$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear</td>
<td>0.523 ± 0.110</td>
<td>0.621 ± 0.137</td>
</tr>
<tr>
<td>Mitochondrial</td>
<td>0.212 ± 0.052</td>
<td>0.175 ± 0.032</td>
</tr>
<tr>
<td>Lysosomal</td>
<td>0.211 ± 0.092</td>
<td>0.210 ± 0.042</td>
</tr>
<tr>
<td>Microsomal</td>
<td>0.248 ± 0.178</td>
<td>0.194 ± 0.070</td>
</tr>
<tr>
<td>Cytosolic$^c$</td>
<td>2.82 ± 0.90</td>
<td>3.37 ± 0.77</td>
</tr>
</tbody>
</table>

$^a$ Data are expressed as mean ± S.D.; concentrations of VPA in each fraction were normalized for the respective concentration in whole liver homogenate.

$^b$ Significantly different across fractions; $P < .001$.

$^c$ Significantly different from all other subcellular fractions (Tukey’s Studentized range test); $P < .05$.

![Graph C](image3)

**TABLE 3**

<table>
<thead>
<tr>
<th>PRB</th>
<th>$V_{\text{max}}$</th>
<th>$K_m^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 μg/ml</td>
<td>μg/min/mg of protein</td>
<td>μg/ml</td>
</tr>
<tr>
<td>0</td>
<td>0.80 ± 0.06</td>
<td>173 ± 29</td>
</tr>
<tr>
<td>7.14</td>
<td>0.98 ± 0.09</td>
<td>273 ± 59</td>
</tr>
<tr>
<td>14.3</td>
<td>1.09 ± 0.13</td>
<td>371 ± 91</td>
</tr>
<tr>
<td>143</td>
<td>0.75 ± 0.12</td>
<td>342 ± 87</td>
</tr>
<tr>
<td>714</td>
<td>0.88 ± 0.15</td>
<td>461 ± 124</td>
</tr>
<tr>
<td>2850</td>
<td>0.76 ± 0.37</td>
<td>584 ± 278</td>
</tr>
</tbody>
</table>

$^a$ Significantly different across PRB treatment; $P = .0194$, one-way ANOVA.
conjugate, the VG formation rate could not be calculated in the present study. PRB is a potent inhibitor of the MRP efflux pump (Laupeze et al., 1999). In previous studies, PRB significantly enhanced fluorescein accumulation in primary cultured bovine microvessels endothelial cells (Hua-Yun et al., 1998), indicating that inhibition occurs at the MRP transport site. Additionally, PRB was reported to increase MRP2-ATPase activity and to inhibit ATPase activity of MRP1 (Bakos et al., 2000). Because the mechanism(s) of VG canalicular transport have not been characterized, a clear hypothesis regarding the effects of PRB on the biliary excretion of VG could not be formulated. In the present study, PRB decreased the elimination rate of VPA due to inhibition of metabolism. Because PRB decreased the phase II metabolism of VPA to VG, and because PRB is a known inhibitor of organic anion transport, it was anticipated that the rate of VG appearance in bile would also decrease in PRB-treated rats. However, this effect was not observed (Fig. 4). Two explanations for these results are possible. PRB may inhibit oxidative VPA metabolism in addition to inhibiting VG formation. This could result in increased amounts of VPA available for glucuronidation and, therefore, no apparent change in cytosolic VG concentrations (Ward et al., 2001). Previous work has indicated that PRB inhibits the oxidative metabolism of other compounds (Damm and Erttmann, 1975). It also is possible that PRB inhibits VG translocation from the hepatocyte into blood (via MRPs (Konig et al., 1999)) or some other transporter located on the basolateral hepatocyte membrane (Muller et al., 1996), thereby increasing the driving force for VG biliary excretion. The mechanism(s) responsible for maintenance of VG biliary excretion rates at similar unbound cytosolic VPA concentrations despite inhibition of VG formation by PRB require further investigation.

Canalicul bile formation is attributed to the mechanisms generating bulk movement of water into bile canaliculi; possible mechanisms include filtration, vesicular transport, and active transport of certain solutes leading to passive water flow (Klaassen and Watkins, 1984). According to Fig. 5, there is a linear relationship between bile volume and the amount of VG transported into bile. The intercept of the line (or the baseline bile formation) was ~10 µl/min (similar to published reports; Shaw and Heath, 1975; Watkins and Klaassen, 1982; Erlinger, 1988) and represents both bile acid-dependent and -independent flow. VG clearly was choleretic; this effect has been noted previously for VG and a number of bile acids, including taurocholic acid (Klaassen, 1974; Watkins and Klaassen, 1981; Liu et al., 1992).

Because VPA binds significantly to proteins and is metabolized by both mitochondrial and microsomal enzyme systems, a differential distribution of the drug within the hepatocyte was anticipated. In general, VPA appeared to localize preferentially in cytosol, in agreement with previously reported data (Brouwer et al., 1993). PRB did not alter the subcellular distribution of VPA. Lack of measurable VG concentrations in cytosol was not surprising because VG is excreted readily into bile and translocated rapidly into blood. In vitro metabolism studies often are proposed as a surrogate for, or at least a supplement to, more costly and time-consuming in vivo disposition studies. The results of this study demonstrate an inherent difficulty in predicting in vivo interactions from in vitro data. Typically, a particular metabolic pathway is of interest, and in vitro conditions are adjusted to optimize formation of the resulting metabolite. This approach allows assessment of metabolic interactions only for the optimized pathway. In the case of competing metabolic reactions, such as the competition between VPA oxidation and glucuronidation in the present study, in vitro data may not predict the scope of metabolic interactions in vivo. Although perturbations in the hepatobiliary disposition of VPA by PRB may be attributed, at least in part, to competitive inhibition of VPA glucuronidation, other PRB-associated alterations in VPA metabolism or hepatobiliary translocation must be operative in the intact organ. Accurate predictions of alterations in vivo disposition based upon in vitro data require a complete understanding of potential sites of interaction in the hepatobiliary system.

References


