Characterization of Inhibitory Effect of Carbapenem Antibiotics on the Deconjugation of Valproic Acid Glucuronide

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ABSTRACT:

Serum concentrations of valproic acid (VPA) are markedly decreased by coadministration of carbapenem antibiotics (CBPMs). Although inhibition of deconjugation of VPA-glucuronide (VPA-G) to VPA by CBPMs has been proposed as one of the mechanisms to account for this drug-drug interaction, little information is available on the mode of inhibition. In the present study, we characterized the enzyme involved in the deconjugation of VPA-G by using human and rat liver cytosol. It is suggested that 1) deconjugation activity inhibited by CBPMs may be selective for VPA-G, 2) deconjugation of VPA-G may be mediated by enzyme(s) other than β-glucuronidase, and 3) the irreversible inactivation may be responsible for the inhibition of deconjugation of VPA-G by CBPMs. Finally, the kinetic parameters for inactivation (K_{inact} and k_{inact}) were determined for four CBPMs of diverse structure from in vitro experiments. Based on the results of simulation analyses with these parameters and the degradation rate constant of the putative VPA-G deconjugation enzyme obtained from experiments using rats, it is probable that the deconjugation enzyme for VPA-G in the liver is rapidly and mostly inactivated by these CBPMs under clinical situations.

Introduction

Valproic acid (VPA) is a widely used drug with antiepileptic activity. By inhibiting GABA transaminase, VPA elevates the level of GABA and suppresses epileptic seizures (Peterson and Naunton, 2005). Orally administered VPA is almost completely absorbed in the small intestine and predominantly metabolized in the liver by glucuronidation, oxidation, and hydroxylation and then finally excreted into urine and bile. The glucuronidation reaction is mediated by UDP-glucuronosyl transferase (UGT), which requires UDP-glucuronic acid (UDP-GA) as a cofactor. It is believed that the glucuronidation of VPA is reversible and VPA-G is deconjugated to VPA by β-glucuronidase expressed in the whole body and enterobacteria (Williams et al., 1992; Slattum et al., 1995). Because the effective and toxic concentrations of VPA are relatively close and there is a large interindividual difference in the relation between dose and serum concentration of VPA (Beydoun et al., 1997), its serum concentrations need to be monitored.

Concerning the disposition of VPA, the presence of drug-drug interactions between VPA and carbapenem antibiotics (CBPMs) is well established and was initially reported by Nagai et al. (1997). Coadministration of CBPMs causes a rapid decrease in serum concentrations of VPA and increases the risk of epileptic seizures (Yamamura et al., 1999). This drug-drug interaction occurs rapidly and continues for as long as several days after withdrawal of CBPMs, although the half-life of CBPMs is quite short.

Concerning the interaction between VPA and CBPMs, the following mechanisms have been proposed: 1) inhibition of intestinal VPA absorption by CBPMs (Torii et al., 2001, 2002), 2) interruption of enterohepatic circulation of VPA by CBPMs (Kojima et al., 1998), 3) increased partition of VPA into erythrocytes by CBPMs (Omodea et al., 2005; Ogawa et al., 2006), 4) elevation of UDP-GA levels by CBPMs (Yamamura et al., 1999, 2000), 5) induction of UGT by CBPMs (Mori and Mizutani, 2007), and 6) inhibition of deconjugation of VPA-glucuronide (VPA-G) by CBPMs (Nakajima et al., 2004; Nakamura et al., 2008). However, most of the previously proposed mechanisms (mechanisms 1–5) may not solely explain the interaction observed under clinical conditions for the following reasons: with mechanism 1, the interaction observed after intravenous administration of VPA (Clause et al., 2005; Coves-Orts et al., 2005; Spriet et al., 2007) cannot be accounted for. For mechanism 2, the interaction in bile duct-cannulated rats, in which enterohepatic circulation of VPA was negligible (Yamamura et al., 1999), cannot be accounted for. For mechanism 3, the mass balance calculation indicates that the increase in partition of VPA into erythrocytes cannot sufficiently account for the decrease in serum VPA concentration in clinical situations. For mechanisms 4 and 5, it is difficult to explain the selectivity of the interaction between VPA and CBPMs and also the rapid effect of CBPMs on the plasma concentrations of VPA. In addition to the
Concentrations of MEPM and SL were selected to completely inhibit the Sigma-Aldrich (St. Louis, MO). 4-methylumbelliferyl-D-glucuronide (4-MUG) was obtained from Research Chemicals (North York, ON, Canada). Bile samples after intravenous infusion of mefenamic acid into rats was used as mefenamic acid glucuronide (MFA-G). Only MFA-G was detected, but mefenamic acid was not detected in infusion of mefenamic acid into rats was used as mefenamic acid glucuronide (MFA-G). VPA-G deconjugation in human liver after administration of CBPMs.

In the present study, we characterized the deconjugation activity involving VPA-G in human and rat liver cytosol in a series of in vitro and ex vivo experiments. First, we examined substrate specificity and inhibitor sensitivity. Then, the mode of inhibition by CBPMs was examined. Finally, we simulated time profiles of the activity of VPA-G deconjugation in human liver after administration of CBPMs.

**Materials and Methods**

**Chemicals.** Human liver pooled cytosol prepared from 150 donors was purchased from BD Biosciences (San Jose, CA). Panipenem (PAPM), meropenem (MEPM), DRPM, and biapenem (BIPM) were purchased from Daiichi Sankyo (Tokyo, Japan), Dainippon Sumitomo Pharma (Osaka, Japan), Shionogi (Osaka, Japan), and Meiji-Seika Kaisha (Tokyo, Japan), respectively (Fig. 1). VPA, hexanoic acid, and mefenamic acid were purchased from Wako Pure Chemical Industries (Osaka, Japan). VPA-G was obtained from Toronto Research Chemicals (North York, ON, Canada). Bile samples after intravenous infusion of mefenamic acid into rats was used as mefenamic acid glucuronide (MFA-G). Only MFA-G was detected, but mefenamic acid was not detected in the collected bile. SL and 4-methylumbelliferone (4-MU) were obtained from Sigma-Aldrich (St. Louis, MO), 4-methylumbelliferol-β-D-glucuronide (4-MUG) was purchased from Nacalai Tesque (Kyoto, Japan).

**Animals.** Male Sprague-Dawley rats were obtained from SLC Inc. (Shizuoka, Japan). All animals used in this study were housed in temperature- and humidity-controlled animal cages with a 12-h dark/light cycle and with free access to water and standard animal chow (MF; Oriental Yeast, Tokyo, Japan). Experiments involving rats were conducted using protocols approved by the Animal Studies Committee of the University of Tokyo (approval number P08-088).

**Glucuronide Deconjugation Experiment In Vitro.** After a 15-min preincubation of a reaction mixture (72 µl) consisting of 1.6 mg/ml human or rat liver cytosol and inhibitors (50 µM MEPM or 2 mM SL in 100 mM potassium phosphate buffer, pH 7.4, at 37°C), enzyme reactions were initiated by adding 8 µl of substrates: final 50 µM VPA-G, 100 µM 4-MUG, or 200 µM MFA-G. Concentrations of MEPM and SL were selected to completely inhibit the deconjugation reaction of MEPM (see Results) and β-glucuronidase (Oleson and Court, 2008), respectively. After incubation at 37°C in a shaking water bath for 40 min, the reaction was terminated by transferring the 20 µl aliquot to another tube containing 70 µl of water, 10 µl of HClO4, and 10 µl of hexanoic acid (100 µM) (internal standard) and then 10 µl of KHCO3 (2 M) was added for neutralization. Deconjugated compounds from glucuronides were quantified by LC-MS/MS as described in the next section. Data are presented as means ± S.D. of triplicate experiments unless otherwise indicated.

**Quantification by LC-MS/MS.** LC/electrospray ionization-MS/MS multiple reaction monitoring analysis were conducted on a Quattro Premier XE Tandem Quadrupole Mass Spectrometer coupled to an ACQUITY Ultra Performance LC System with an integral autosampler (Waters, Milford, MA). The Premier XE spectrometer was run in electrospray ionization (negative)-MS/MS multiple reaction monitoring mode at a source temperature of 120°C and a desolvation temperature of 350°C by monitoring the following mass transitions (parent to daughter ion): m/z 142.9 to 142.9 for VPA, m/z 174.77 to 132.69 for 4-MU, m/z 240.23 to 195.50 for mefenamic acid, and m/z 114.76 to 114.76 for hexanoic acid. Because VPA is known not to be further fragmented (Ji et al., 2003), VPA and hexanoic acid were quantified by nonreactive transition to reduce background interference. The cone voltage was set at 30 V for VPA, 47 V for 4-MU, 30 V for mefenamic acid, and 25 V for hexanoic acid. Collision energy was set at 7 V for VPA, 26 V for 4-MU, and 30 V for mefenamic acid. Incubation product mixtures were separated on an ACQUITY Ultra Performance LC bridged ethyl hybrid 1.7 µm, 2.1 x 100-mm C18 high-performance liquid chromatography column (Waters) by using 2.5 µl of each sample with a flow rate of 0.3 ml/min and a binary solvent system of water containing 5 mM ammonium acetate (solvent A) and liquid chromatography-grade methanol (Nacalai Tesque) containing 5 mM ammonium acetate (solvent B). Chromatography of VPA and hexanoic acid (internal standard) was performed using a method as follows: t = 0 min, 20% solvent B; t = 1 min, 20% solvent B; t = 1.5 min, 65% solvent B; t = 2.5 min, 65% solvent B; t = 2.6 min, 95% solvent B; t = 4.5 min, 95% solvent B; and t = 4.6 min, 20% solvent B (total run time was 6 min). The retention times for VPA and hexanoic acid were 3.1 and 2.6 min, respectively. Chromatography of 4-MU and hexanoic acid (internal standard) was performed using a method as follows: t = 0 min, 20% solvent B; t = 1 min, 20% solvent B; t = 1.5 min, 70% solvent B; t = 2.5 min, 70% solvent B; t = 2.6 min, 90% solvent B; t = 4.5 min, 90% solvent B; and t = 4.6 min, 20% solvent B (total run time was 6 min). The retention times for VPA and hexanoic acid were 3.1 and 2.6 min, respectively. Chromatography of mefenamic acid and hexanoic acid (internal standard) was performed using a method as follows: t = 0 min, 20% solvent B; t = 1 min, 20% solvent B; t = 1.5 min, 70% solvent B; t = 2.5 min, 70% solvent B; t = 2.6 min, 90% solvent B; t = 4.5 min, 90% solvent B; and t = 4.6 min, 20% solvent B (total run time was 6 min). The retention times for mefenamic acid and hexanoic acid were 3.6 and 2.6 min, respectively. Data analyses were performed using MassLynxNT software (version 4.1) and quantified using sample peak area.

**Determination of K_{int} for VPA-G Deconjugation Activity.** VPA-G deconjugation experiments were performed by the method described previously using 12.5, 25, 50, and 100 µM VPA-G. Incubation was performed...
for 5, 10, or 20 min to confirm the linearity of the activity versus the incubation time. Experiments were also performed in the presence of 50 μM MEPM or 5 mM SL.

**Examination of Irreversible Inactivation In Vitro.** The preincubation time and CBPM concentration dependence of VPA-G deconjugation inhibition was then examined. Preincubation was initiated by mixing 6.4 μl of 20 mg/ml human liver cytosol with different concentrations of CBPMs (PAPM, MEPM, DRPM, or BIPM). After preincubation, the enzyme reaction was initiated by adding 72 μl of VPA-G to produce a final concentration of 50 μM. After incubation at 37°C in a shaking water bath for 40 min, the reaction was terminated and deconjugated VPA was quantified by the method described above. Conditions involving the CBPM concentrations and preincubation times are summarized in Table 1. Data are presented as the means ± S.D. of triplicate experiments.

Kinetic parameters for irreversible inactivation were obtained as reported elsewhere (Ito et al., 1998). The logarithm of the remaining enzyme activity, which was determined as the formation rate of VPA, was plotted against the preincubation time, and the apparent inactivation rate constant \( k_{\text{obs}} \) was determined from the slope of the initial three points. Then, the value of \( k_{\text{obs}} \) was plotted against CBPM concentrations ([I]), and the parameters \( k_{\text{max}} \) and \( K_{\text{app}} \) were obtained by the nonlinear least-squares regression method (MULTI) (Yamaoka et al., 1981) according to eq. 1:

\[
 k_{\text{obs}} = \frac{k_{\text{max}} \cdot I}{K_{\text{app}} + I} \tag{1}
\]

where \( k_{\text{max}} \) and \( K_{\text{app}} \) represent the maximum inactivation rate constant and the apparent dissociation constant between VPA-G deconjugation enzyme and CBPM, respectively.

**Calculation of Rat \( k_{\text{obs}} \) In Vivo.** The enzyme is continuously synthesized and degraded. The change in enzyme amount can be expressed by eq. 2:

\[
 \frac{dE}{dt} = v_{\text{syn}} - k_{\text{deg}} \cdot E
\]

where \( E, v_{\text{syn}} \), and \( k_{\text{deg}} \) are the enzyme amount, the rate of enzyme synthesis, and the degradation rate constant, respectively. At steady-state, \( v_{\text{syn}} \) equals the degradation rate \( (k_{\text{deg}} \cdot E_s) \), where \( E_s \) is the enzyme amount at steady state.

**TABLE 1**

<table>
<thead>
<tr>
<th>Conditions involving CBPM concentrations and preincubation times</th>
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<tbody>
<tr>
<td>CBPM</td>
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<tr>
<td>------</td>
</tr>
<tr>
<td>PAPM</td>
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<td>BIPM</td>
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Moreover, when the rate of enzyme synthesis is constant, the change in the enzyme amount can be also described by eq. 3:

\[
 \frac{dE}{dt} = k_{\text{deg}} \cdot E_s - k_{\text{obs}} \cdot E
\]

By solving differential eq. 3, the active enzyme amount \((E(t))\) can be explained by eq. 4:

\[
 E(t) = E_s - (E_s - E_0) \cdot \exp(-k_{\text{deg}} \cdot t)
\]

where \( t \) and \( E_0 \) are the time after administration of inhibitor and the trough amount of active VPA-G deconjugation enzyme after being inactivated by MEPM in vivo.

From eq. 4, the \( k_{\text{deg}} \) of rat VPAGase was calculated by monitoring the recovery of VPA-G deconjugating activity after inactivation by MEPM under in vivo conditions. Rats were lightly anesthetized by diethyl ether and a bolus dose of MEPM (100 mg/kg) was injected through the jugular vein to inactivate VPAGase. Rats were housed under the above-mentioned conditions after recovery from anesthesia. Then, 3, 12, 24, 48 and 72 h after dosing, rats \((n = 2)\) were sacrificed by bleeding via a central artery under anesthesia with diethyl ether, and blood was removed completely by a chilled saline perfusion. The liver was quickly removed and homogenized in 4 volumes of 100 mM potassium phosphate buffer (pH 7.4). Liver cytosol was prepared by centrifugation of the liver homogenate at 9000g for 10 min at 4°C followed by ultracentrifugation at 100,000g for 60 min at 4°C. The supernatant was kept frozen at −80°C until use. The protein concentration of each cytosol sample was determined using a BCA protein assay kit (Pierce Chemical, Rockford, IL) with bovine serum albumin as a standard.

VPA-G deconjugation activity of each cytosol \((E(t))\) and control cytosol \((E_s)\) was evaluated by the method mentioned previously and the parameters \((k_{\text{deg}} \) and \( E_s/E_0)\) were obtained by the nonlinear least-squares regression method (MULTI) according to eq. 5:

\[
 E(t) = \frac{E_s}{1 - \left(1 - \frac{E_t}{E_s}\right) \cdot \exp (-k_{\text{deg}} \cdot t)}
\]

**Determination of the Amount of Active VPAGase.** Without irreversible inhibitors, the change in enzyme amount can be accounted for by eq. 3. In contrast, in the presence of irreversible inhibitors, the change in enzyme amount can be described by eq. 6:

\[
 \frac{dE}{dt} = k_{\text{deg}} \cdot E_s - (k_{\text{obs}} + k_{\text{deg}}) \cdot E
\]

The time profile of enzyme amount after CBPM administration to humans can be calculated from eq. 6 with kinetic parameters for irreversible inactivation of human enzyme \((k_{\text{max}} \) and \( K_{\text{app}})\) and rat \( k_{\text{deg}}\). After intravenous infusion of CBPMs for Time\(_{\text{inf}}\) hours, the relative enzyme amount can be given by eq. 7.

\[
 \frac{dE(t)}{dt} = k_{\text{deg}} \cdot E_s - \left(\frac{k_{\text{max}} \cdot K_{\text{app}} + k_{\text{deg}} \cdot I(t)}{K_{\text{app}} + k_{\text{deg}} \cdot I(t)} + k_{\text{deg}}\right) \cdot E(t)
\]

where

1) \( 0 < t < \text{Time}_{\text{inf}} \)

\[
 I(t) = \frac{\text{Dose}}{\text{MW} \cdot \text{Time}_{\text{inf}} \cdot k_{\text{obs}} \cdot V_d} \cdot (1 - \exp (-k_{\text{deg}} \cdot t))
\]

2) \( \text{Time}_{\text{inf}} < t \)

\[
 I(t) = \frac{\text{Dose}}{\text{MW} \cdot \text{Time}_{\text{inf}} \cdot k_{\text{obs}} \cdot V_d} \cdot (1 - \exp (-k_{\text{deg}} \cdot \text{Time}_{\text{inf}})) \cdot \exp (-k_{\text{deg}} \cdot (t - \text{Time}_{\text{inf}}))
\]
In the calculation, all parameters except for $K_{v}$ were obtained from the package inserts of each CBPM and are summarized in Table 3. Pharmacokinetic parameters of VPA (Depakene), PAPM (Carbenin), MEPM (Meropen), DRPM (Finibax), and BIPM (Omegacin) were obtained from the section on package inserts on the Japanese Pharmaceuticals and Medical Devices Agency website (http://www.info.pmda.go.jp/psearch/html/menu_tenpu_base.html). The $K_{v}$ value was changed from 0.01 to 1. Simulations were performed by using the Scientist program (MicroMath Inc., Salt Lake City, UT). The repeated administration condition (12-h intervals) was examined for some CBPMs to predict the prolonged effect.

### Results

**Substrate Specificity and Inhibitor Sensitivity of VPA-G Deconjugation Activity.** In human and rat liver cytosol, the extent of VPA-G deconjugation activity was similar. These activities were inhibited by MEPM (Fig. 2, A and B) as reported previously (Nakajima et al., 2004; Nakamura et al., 2008). 4-MUG and MFA-G were used as authentic substrates to examine whether MEPM inhibits the deconjugation of other glucuronides. Although these glucuronides were deconjugated in liver cytosol, no inhibition by MEPM was observed (Fig. 2, C–F). Consequently, inhibition by MEPM may be selective for the VPA-G deconjugation reaction.

Because glucuronides are deconjugated by $\beta$-glucuronidase expressed ubiquitously in the body and enterobacteria in general, the involvement of $\beta$-glucuronidase in VPA-G deconjugation, which was inhibited by MEPM, was examined using SL, a well-established $\beta$-glucuronidase inhibitor. It was found that 5 mM SL inhibited deconjugation of 4-MUG completely and MFA-G by 75% in both human and rat liver cytosol but only partially inhibited deconjugation of VPA-G in human and rat liver cytosol by 30 and 25%, respectively (Fig. 2). These results suggest the involvement of an unidentified enzyme other than $\beta$-glucuronidase for the deconjugation of VPA-G. Hereinafter, this unidentified enzyme is referred to as VPAGase.

Although we tried to determine $K_{v}$ and $v_{\text{max}}$ values of VPAGase, it was found that the VPA-G deconjugation reaction was already saturated at the concentration range from 12.5 to 100 $\mu$M. Unfortunately, experiments using a lower concentration of VPA-G were difficult, because of the poor sensitivity of VPA detection under the present experimental conditions. Assuming that the VPAGase activity was saturated under these experimental conditions, the $v_{\text{max}}$ values of VPAGase for VPA-G deconjugation in human and rat liver cytosol were determined to be 0.15 and 0.10 nmol/min/mg protein, respectively.

**Inhibition Mode of CBPMs against VPAGase.** Although the half-life of CBPMs in humans is approximately 1 h, the decrease in serum VPA concentration continues for several days after administration of CBPM is stopped (Haroutunian et al., 2009). It is difficult to explain such a prolonged effect of CBPMs by the time lag to reach another steady state of VPA, because the half-life of VPA is as short as 10 h (Addison et al., 2000). In consideration of these facts, it is possible that the prolongation of this interaction may be caused by an irreversible process.

As shown in Fig. 3, preincubation time- and CBPM concentration-dependent inactivation of deconjugation activity for VPA-G was observed in human and rat liver cytosol for all of the CBPMs examined (PAPM, MEPM, DRPM, and BIPM). Parameters for irreversible inactivation ($k_{\text{obs}}$ and $K_{\text{app}}$) calculated from the results shown in Fig. 3 are summarized in Table 2. Because saturation of $k_{\text{obs}}$ was not observed for BIPM up to 256 $\mu$M (Fig. 4), the inhibition potential was calculated from the relationship between $k_{\text{obs}}$ and BIPM concentration. Comparison of the inhibition potentials ($k_{\text{obs}}/K_{\text{app}}$) among CBPMs in human liver cytosol revealed that PAPM had the strongest inhibition potential followed by MEPM, DRPM, and BIPM (Table 2).
There was little species difference in these parameters between humans and rats.

**Calculation of \( k_{\text{deg}} \) of VPAGase in Rats.** In addition to the parameters for the irreversible inactivation by CBPMs, the degradation rate constant of the enzyme itself is required for the purpose of predicting the in vivo effect of CBPMs on the disposition of VPA. By assuming that the velocity of enzyme synthesis is constant, the \( k_{\text{deg}} \) was calculated from the recovery profile of VPA-G deconjugation activity in rat liver cytosol, prepared at different time points after intravenous bolus administration of MEPM. Soon after MEPM administration, VPA-G deconjugation activity was significantly decreased, and the relative activities at 3, 12, 24, 48, and 72 h after MEPM administration were 19, 34, 44, 61, and 72% of the control, respectively (Fig. 5). Finally, the \( k_{\text{deg}} \) of rat VPAGase was calculated to be 0.39 day\(^{-1}\) and \( E_{\text{eq}}/E_{\text{ss}} \), the relative activity soon after MEPM administration, was 0.16.

**Prediction of the Amount of Active VPAGase.** Finally, we simulated VPAGase activity in human liver after CBPM administration (500 mg/body) using the pharmacokinetic parameters of CBPMs (Table 3), the parameters for irreversible inactivation obtained for human liver cytosol (Table 2), and the \( k_{\text{deg}} \) obtained in rats (Fig. 5). Because there is little information about the \( K_p \) values of CBPMs, these values were changed from 0.01 to 1. When the \( K_p \) value was set at 1 or 0.1, complete inactivation of VPAGase was predicted within less than 2 h after administration of all the CBPMs examined (Fig. 6). Complete and rapid inactivation was predicted for PAPM when the \( K_p \) value was set at 0.01. Although significant activity still remained at 2 h after a single administration of MEPM, DRPM, and BIPM (20, 6, 2,

<table>
<thead>
<tr>
<th>CBPM</th>
<th>Human Liver Cytosol</th>
<th>Rat Liver Cytosol</th>
<th>Human Liver Cytosol</th>
<th>Rat Liver Cytosol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( k_{\text{inact}} )</td>
<td>( K_{\text{app}} )</td>
<td>( k_{\text{inact}}/K_{\text{app}} )</td>
<td>( k_{\text{inact}} )</td>
</tr>
<tr>
<td>PAPM</td>
<td>9.66</td>
<td>51</td>
<td>0.189</td>
<td>5.65</td>
</tr>
<tr>
<td>MEPM</td>
<td>7.34</td>
<td>166</td>
<td>0.044</td>
<td>5.03</td>
</tr>
<tr>
<td>DRPM</td>
<td>6.92</td>
<td>142</td>
<td>0.049</td>
<td>4.92</td>
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<tr>
<td>BIPM</td>
<td>0.013</td>
<td>0.013</td>
<td>0.013</td>
<td>0.013</td>
</tr>
</tbody>
</table>

**TABLE 2**

Calculated parameters for irreversible inactivation

The \( k_{\text{inact}} \) and \( K_{\text{app}} \) values for each CBPM were obtained by nonlinear curve fitting of Fig. 3 into eq. 1.

Fig. 4. Concentration-dependent inactivation of VPA-G deconjugation activity by CBPMs. Each \( k_{\text{obs}} \) value was calculated from the data shown in Fig. 3. The \( k_{\text{obs}} \) values were plotted against the CBPM concentration for human (A) and rat (B) liver cytosol. Fitted curves are shown as solid lines. Each value represents the mean ± S.D. of triplicate experiments.
and 44%, respectively), almost complete inactivation is expected after repeated administration of these CBPMs even under such stringent condition ($K_p = 0.01$) (Fig. 7).

**Discussion**

Although inhibition of VPA-G deconjugation has been proposed as one of the mechanisms of the drug-drug interaction between VPA and CBPMs, the identity and characteristics of the deconjugation activity have not been fully established. In the present study, we demonstrated that the deconjugation of VPA is mediated by enzyme(s) other than β-glucuronidase. This enzyme selectively accepts VPA-G as a substrate and is also selectively inhibited by CBPMs. Moreover, we demonstrated that CBPMs extensively inactivate this putative enzyme (VPAGase) in an irreversible manner.

Concerning the characterization of the putative VPAGase, we found that the activity to deconjugate VPA-G in liver cytosol was only minimally inhibited by 5 mM SL, a β-glucuronidase inhibitor (Fig. 2). This finding was in marked contrast to a previous report in which Nakamura et al. (2008) showed complete inhibition of VPA-G deconjugation by 10 mM SL in human liver cytosol. One of the possible reasons to account for such a discrepancy is the difference in the experimental conditions. Nakamura et al. (2008) used water as an incubation medium for the inhibition study with SL, whereas we used buffered solution (pH 7.4) throughout the experiments. Because SL is an acidic compound, the pH of the incubation medium may be decreased by the addition of 10 mM SL to water, and this may result in nonspecific inhibition (Oleson and Court, 2008). In accordance with this hypothesis, we found complete inhibition of VPA-G deconjugation activity by 10 mM SL in water and confirmed that the pH of the incubation medium was as low as pH 2 under this condition. Moreover, VPA-G deconjugation activity was also completely inhibited by acidifying the incubation solution to pH 2 in the absence of SL. It can also be concluded that the putative enzyme is pH-sensitive but insensitive to SL. Concerning the subcellular localization of VPA-G deconjugation activity, it is reported that the activity is higher in the

**TABLE 3**

Parameters for simulation of CBPM concentration and VPAGase remaining activity after CBPM administration

<table>
<thead>
<tr>
<th>CBPM</th>
<th>$t_{max}$</th>
<th>Dose</th>
<th>MW</th>
<th>$k_d$</th>
<th>$V_d$</th>
<th>MW, molecular weight.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPM</td>
<td>1</td>
<td>500</td>
<td>339</td>
<td>1.07</td>
<td>21.0</td>
<td></td>
</tr>
<tr>
<td>MEPM</td>
<td>1</td>
<td>500</td>
<td>438</td>
<td>0.684</td>
<td>22.0</td>
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<tr>
<td>DRPM</td>
<td>1</td>
<td>500</td>
<td>350</td>
<td>1.01</td>
<td>16.4</td>
<td></td>
</tr>
<tr>
<td>BIPM</td>
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<td>500</td>
<td>439</td>
<td>0.81</td>
<td>12.4</td>
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Fig. 5. Recovery of VPA-G deconjugation activity after MEPM administration to rats. After intravenous bolus administration of MEPM (100 mg/kg), liver cytosol was prepared at the indicated times, and VPA-G deconjugation activity was examined. The control value corresponds to the activity of untreated rat liver cytosol. The fitted curve is shown as a solid line. Each value represents the average of duplicate experiments.

Fig. 6. Simulated time profiles of CBPM concentration and VPAGase remaining activity after administration of CBPMs. Five hundred milligrams of each CBPM (A, PAPM; B, MEPM; C, DRPM; and D, BIPM) were assumed to be administered to humans by intravenous infusion for 1 h. Time profiles for the remaining VPAGase activity in the liver (dotted line) and those for serum concentrations of CBPMs (solid line) were simulated. The $K_p$ values were set at 1, 0.1, and 0.01 for each CBPM.
is not likely that the inhibitor sensitivity and subcellular localization of the activity, it is enriched (Medda and Swank, 1985; Shipley et al., 1993). Considering et al., 2004; Nakamura et al., 2008), where liver cytosol fraction than in the liver microsome fraction (Nakajima concentrations of CBPM (solid line) were simulated. The activity after repeated administration of CBPMs. Five hundred milligrams of each FIG. 7. Simulated time profiles of CBPM concentration and VPAGase remaining activity after repeated administration of CBPMs. Five hundred milligrams of each CBPM (A, MEPM; B, DRPM; and C, BIPM) were assumed to be repeatedly administered to humans by intravenous infusion at 12-h intervals. Time profiles for the remaining VPAGase activity in the liver (dotted line) and those for serum concentrations of CBPM (solid line) were simulated. The $K_p$ values were set at 0.01 for each CBPM.

liver cytosol fraction than in the liver microsome fraction (Nakajima et al., 2004; Nakamura et al., 2008), where $\beta$-glucuronidase is enriched (Medda and Swank, 1985; Shipley et al., 1993). Considering the inhibitor sensitivity and subcellular localization of the activity, it is not likely that $\beta$-glucuronidase is the predominant enzyme involved in the deconjugation of VPA-G.

VPAGase may be relatively selective for VPA-G, because it is not involved in the deconjugation of other glucuronide conjugates, including 4-MUG and MFA-G (Fig. 2). From a structural point of view, glucuronide conjugates can be classified by conjugated functional groups; if carboxyl, hydroxyl, and amino groups are conjugated, they are classified as acyl-, $O$-, and $N$-glucuronides, respectively. Based on this classification, both VPA-G and MFA-G are acyl-glucuronides. If VPAGase generally recognizes this bond moiety, inhibition by CBPMs would be also expected for MFA-G. However, this was not the case (Fig. 2, E and F). This result indicates that VPAGase does not necessarily accept acyl-glucuronide in general but is selective for VPA-G. Moreover, 4-MUG, a typical $O$-glucuronide, is not recognized as a good substrate for VPAGase (Fig. 2, C and D). These results suggest that not only glucuronide or the bond moieties but also the structure of the parent compound (VPA) may be important for substrate recognition by VPAGase. Although our in vitro results showing that VPAGase is relatively selective for VPA-G are consistent with the clinical observations that such an interaction has been only reported for VPA, we cannot exclude the effect of CBPMs on other drug conjugates. Because therapeutic dose monitoring is performed for a limited number of drugs, it is possible that there are drugs whose serum concentrations are altered by CBPMs that have not been detected yet.

In addition, we have demonstrated that VPAGase is inactivated by all of the CBPMs examined (PAPM, MEPM, DRPM, and BIPM) in an irreversible manner. Calculated $k_{\text{inact}}$ values were approximately 10 to 100 times higher than the values reported for cytochrome P450 inactivation via mechanism-based inhibition (Chiba et al., 1995). This inactivation is not merely an artifact obtained from in vitro experiments, because CBPM-sensitive deconjugation activity in rat liver cytosol was also reduced to the same extent after in vivo administration of CBPMs (Fig. 5). Consistent with the irreversible inactivation of VPAGase by MEPM, the recovery of the deconjugation activity for VPA was slow enough ($t_{1/2} = 1.8$ day) compared with the relatively rapid elimination of MEPM ($t_{1/2} = 1$ h in humans). If inhibition of VPAGase by CBPMs is the mechanism responsible for the drug-drug interaction between VPA and CBPMs, VPAGase should be significantly inactivated by CBPMs in clinical situations. According to the time profiles for the recovery of CBPM-sensitive VPA-G deconjugation activity in rat liver cytosol, the $k_{\text{deg}}$ value of VPAGase in rat was calculated to be 0.39 day$^{-1}$, and the half-life of VPAGase was approximately 2 days. Such slow turnover rate of VPAGase is also consistent with the observations in humans, in whom the interaction between VPA and CBPMs continues for a week after the final administration of CBPMs.

Finally, we performed simulation analyses to examine the time profiles of the active amounts of VPAGase after CBPM administration in clinical situations. According to the standard regimen of CBPMs in Japan, 0.5 g of CBPM is intravenously administered for 1 h, repeated twice a day. Because the tissue concentration of CBPMs in humans is mostly unknown, the $K_p$ value for the liver was changed from 0.01 to 1 according to the information from mouse experiments; the $K_p$ values for DRPM and imipenem were more than 0.1 at 5 min after administration (Horiiuchi et al., 2006). When the $K_p$ value was set at 0.1 or 1, VPAGase would be almost completely inactivated after a single administration of CBPMs, irrespective of the difference in parameters among these four CBPMs. Even if the $K_p$ value was set to 0.01, almost complete inactivation was expected after repeated administration of these CBPMs. The limitation of this simulation is that the $k_{\text{deg}}$ values of VPAGase determined in rats are used for the prediction of drug-drug interactions in humans. Such substitution is not always suitable; indeed, the $k_{\text{deg}}$ values of most cytochrome P450 enzymes in humans are smaller than those in rats (Venkatakrishnan et al., 2007). However, the results of our simulation indicated that, even if $k_{\text{deg}}$ values of VPAGase in humans are also smaller than those in rats, complete inactivation may still be observed as shown in Figs. 6
and 7. The simulated results are consistent with the fact that the serum VPA is decreased rapidly after initiation of CBPM therapy. Despite the lack of clinical evidence for DRPM and BIPM, coadministration of these CBPMs with VPA are all contraindicated in Japan. Our simulation showed that DRPM and BIPM may be as potent as other CBPMs and may have a severe interaction with VPA.

In conclusion, we have demonstrated for the first time that the predominant deconjugation activities for VPA-G in human and rat liver cytosol are extensively inactivated by CBPMs in an irreversible manner. Such inactivation may take place under clinical situations based on our simulation analyses.

References


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