Identification of Valproic Acid Glucuronide Hydrolase As a Key Enzyme for the Interaction of Valproic Acid with Carbapenem Antibiotics

Eiko Suzuki, Naotoshi Yamamura, Yuji Ogura, Daisuke Nakai, Kazuishi Kubota, Nobuhiro Kobayashi, Shin-ichi Miura, and Osamu Okazaki

Drug Metabolism and Pharmacokinetics Research Laboratories (E.S., N.Y., D.N., N.K., O.O.) and Translational Medicine and Clinical Pharmacology Department (Y.O., K.K., S.M.), Daiichi Sankyo Co., Ltd., Tokyo, Japan

ABSTRACT:

Plasma levels of valproic acid (VPA) are decreased by concomitant use with carbapenem antibiotics, such as panipenem (PAPM). One of the plausible mechanisms of this interaction is the inhibition of VPA glucuronide (VPA-G) hydrolysis by carbapenems in the liver. To elucidate this interaction mechanism, we purified VPA-G hydrolase from human liver cytosol, in which the hydrolytic activity was mainly located. After chromatographic purification, the VPA-G hydrolase was identified as acylpeptide hydrolase (APEH). APEH-depleted cytosol, prepared by an immunodepletion method, completely lacked the hydrolytic activity. These results demonstrate that APEH is a single enzyme involved in PAPM-sensitive VPA-G hydrolysis in cytosol. In addition, the hydrolytic activity of recombinant human APEH was inhibited by PAPM and the inhibition profile by typical esterase inhibitors (diisopropyl fluorophosphate, 5,5′-dithiobis(2-nitrobenzoic acid), p-chloromercuribenzoic acid, and 1-saccharic acid 1,4-lactone) was similar to that of human liver cytosol. Cytosolic VPA-G hydroxylase activity was slightly inhibited by cholinesterase and carboxylesterase inhibitors. β-Glucuronidase activity remained in APEH-depleted cytosol, whereas VPA-G hydroxylase activity was completely abolished. Thus, either cholinesterase, carboxylesterase, or β-glucuronidase in cytosol would not be involved in VPA-G hydrolysis. Taken together, APEH plays a major role in the PAPM-sensitive VPA-G hydrolysis in the liver. These findings suggest that APEH could be a key enzyme for the drug interaction of VPA with carbapenems via VPA-G hydrolysis.

Introduction

Valproic acid (VPA) is widely used as an antiepileptic drug with a narrow therapeutic window (50–100 µg/ml) (Rimmer and Richens, 1985). The plasma levels of VPA decrease below the window while patients are receiving carbapenem antibiotics, resulting in the recurrence of epileptic seizures (Nagai et al., 1997; De Turck et al., 1998). In 1996, the prohibition of the concomitant use of these drugs was added to the Information on Adverse Reactions to Drugs from the Ministry of Welfare in Japan, whereas in other countries careful concomitant use is allowed, and clinical interactions have still been reported (Fudio et al., 2006; Lunde et al., 2007; Gu and Huang, 2009).

The mechanism of this interaction has been studied by a number of researchers (Mori et al., 2007). Some mechanisms related to absorption, distribution, and metabolism of VPA have been proposed (Kojima et al., 1998; Yamamura et al., 1999; Torii et al., 2001; Nakajima et al., 2004; Omoda et al., 2005). Among them, metabolism in the liver has been considered to be the most important for interaction, based on the following findings. VPA is mainly metabolized to the acylglucuronide (VPA-G) in the liver and subsequently excreted into bile in rats. In hepatectomized rats, the interaction of VPA with carbapenems was not observed (Yamamura et al., 1999). After treatment with panipenem (PAPM) (Fig. 1) in rats, the biliary excretion of VPA-G increased and the biliary excretion clearance of VPA-G did not change, suggesting that the formation of VPA-G was apparently accelerated. Although UDP-glucuronosyltransferases were not induced or activated by PAPM in rat liver microsomes, the UDP-glucuronic acid level was increased by 1.7 times, consistent with the 1.8 times increase in the apparent glucuronidation clearance of VPA (Yamamura et al., 2000). However, VPA-G formation in monkey liver slices was increased 10 to 20 times by doripenem (DRPM), whereas the UDP-glucuronic acid level in the slice was increased only 1.4 times (Nakajima et al., 2004), suggesting that other processes contribute to the apparent acceleration in VPA-G formation. As one of the processes, the inhibition of the hydrolysis of VPA-G to VPA apparently accelerates the VPA-G formation. The hydrolysis of VPA-G was observed in rat and monkey liver homogenates (Nakajima et al., 2004). The hydrolysis in rat liver cytosol was strongly inhibited by DRPM (Nakajima et al., 2004). After intravenous admin-

ABBREVIATIONS: VPA, valproic acid; VPA-G, VPA glucuronide; PAPM, panipenem; DRPM, doripenem; DFP, diisopropyl fluorophosphate; pNP, p-nitrophenyl β-glucuronide; BNPP, bis-p-nitrophenyl phosphate; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); PCMB, p-chloromercuribenzoic acid; sodium salt; AANA, acetyl-alanine p-nitroanilide; APEH, acylpeptide hydrolase; IS, internal standard; LC, liquid chromatography; LDH, lactate dehydrogenase; PAGE, polyacrylamide gel electrophoresis; MS/MS, tandem mass spectrometry.
Materials and Methods

Materials. VPA-G was synthesized enzymatically in Chemtech Labs, Inc. (Tokyo, Japan). PAPM was synthesized at Sankyo Co., Ltd. (Tokyo, Japan). VPA sodium salt and EDTA were purchased from Sigma-Aldrich (St. Louis, MO). Disisopropyl fluorophosphate (DFP), eserine, p-nitroaniline, p-nitrophe-nyl β-glucuronide (pNPG), and p-nitrophenol were purchased from Wako Pure Chemicals (Osaka, Japan). Bis-p-nitrophenyl phosphate (BNPP) and 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). p-Chloromercuribenzoic acid sodium salt (PCMB) and d-saccharic acid 1,4-lactone monohydrate (saccharolactone) were purchased from Research Organics (Cleveland, OH) and MP Biomedicals, Inc. (Solon, OH), respectively. Acetyl-alanine p-nitroanilide (AANA) was purchased from Bachem AG (Bubendorf, Switzerland). The other reagents were all commercially available and of guaranteed grade. Pooled human liver subcellular fractions and human liver tissue used for the purification of VPA-G hydrolase were obtained from XenoTech, LLC (Lenexa, KS) and the Human and Animal Bridging Research Organization (Chiba, Japan). Bovine liver β-glucuronidase was purchased from ProZyme, Inc. (San Leandro, CA).

VPA-G Hydrolysis and Inhibition. Either an human liver subcellular fraction (cytosol, microsomes, mitochondria, and lysosomes; 1 mg/ml each) or recombinant human APEH (0.1 mg/ml) was preincubated in 50 mM Tris-HCl buffer (pH 7.4) at 37°C. Then VPA-G (30 μM) was added to make a final volume of 0.05 to 0.1 ml, and further incubation was performed at 37°C. The reaction was stopped by adding the same volume of acetonitrile containing chlorozoxazone [0.5-5 μM, internal standard (IS)] as the reaction mixture and was centrifuged. An aliquot of 10 μl of each supernatant was injected into an LC-mass spectrometry system. Human liver lysosomes were also incubated in 50 mM sodium acetate buffer (pH 5). In the inhibition studies, either PAPM, β-glucuronidase inhibitor (saccharolactone), or esterase inhibitor (DFP, eserine, BNPP, DTNB, EDTA, and PCMB) was added to the reaction mixture before incuba-tion. In the β-glucuronidase study, bovine liver β-glucuronidase (200 Fishman units/ml) was preincubated in 50 mM sodium acetate buffer (pH 5), and further incubation was performed after the addition of VPA-G.

Measurement of VPA Concentration. The concentrations of VPA in the incubation samples were analyzed using an LC-mass spectrometry system consisting of an LC-10A pump (Shimadzu Corporation, Kyoto, Japan) coupled to an API3000 mass spectrometer (Applied Biosystems/MDS SCIEX; Foster City, CA). Chromatographic separation was achieved on a Capcell Pak C18 MG column (5 μm, 2.0 × 150 mm; Shiseido Co., Ltd., Tokyo, Japan) at a column temperature of 40°C. A mixture of acetoni-trite-water (5:95, v/v) containing 5 mM ammonium acetate (mobile phase A) and acetoni-trite-water (95:5, v/v) containing 5 mM ammonium acetate (mobile phase B) was used at a flow rate of 0.3 ml/min. The following gradient elution program was used: a linear increase of mobile phase B from 40 to 60% for 4 min followed by elution with 60% mobile phase B for 0.45 min. The eluent from the column was introduced directly to the API3000 using the electrospray ionization interface in negative ion mode. The ions of m/z 143 for VPA and m/z 168 for IS were monitored using the selected ion monitoring mode. For both VPA and IS, the declustering potential, curtain gas, ion spray voltage, and ion source temperature were −31 V, 6 psi, −3500 V, and 550°C, respectively.

LDH Activity in Human Liver Subcellular Fractions. The LDH activity in human liver subcellular fractions was measured using an LDH assay kit (L-type Wako LDH; Wako Pure Chemicals) according to the manufacturer’s instructions.

Purification of VPA-G Hydrolase from Human Liver Cytosol. Human liver tissue was homogenized in a buffer [250 mM sucrose, 3 mM Tris, and 1 mM EDTA (pH 7.4)], and the cytosol fraction was prepared by a conventional method. In brief, the cytosol was fractionated by ammonium sulfate and 20 to 70% (w/v) precipitate was obtained. The precipitate was dissolved in 20 mM HEPES (pH 7) and dialyzed against the same buffer. The dialysate was applied to a HiPrep 16/10 Q FF column (20 ml; GE Healthcare, Uppsala, Sweden) and equilibrated with 20 mM HEPES (pH 7), and then a linear gradient of 0 to 0.5 M NaCl was applied to elute the enzyme. The active fractions were combined and dialyzed against 10 mM potassium phosphate buffer (pH 6.8) containing 0.3 mM CaCl2. The dialysate was applied to a Bio-Scale CHT2-I column (2 ml; Bio-Rad Laboratories, Hercules, CA) and then a linear gradient of 0 to 50% potassium phosphate buffer (500 mM, pH 6.8) containing 0.01 mM CaCl2 was applied to elute the enzyme. After each active fraction was analyzed by SDS-PAGE, a 75-kDa protein band visualized by silver staining (EzStain Silver; ATTO Corporation, Tokyo, Japan) was excised and digested with trypsin within the gel. The resulting peptides were extracted and sequenced by LC-MS/MS, which consisted of a DIna-nano-flow liquid chromatography system (KYA Technologies, Tokyo, Japan) and an LTQ Orbitrap mass spectrometry (Thermo Fisher Scientific, Waltham, MA). The MS/MS spectra data were searched against a composite target/decoy International Protein Index (IP) human database (version 3.72; 172,784 forward and reversed protein sequences) using Mascot 2.2 (Matrix Science, London, UK). Parent mass and MS/MS tolerances were set at 50 ppm and 0.8 Da, respectively. We required strict enzyme specificity and allowed for up to two missed cleavage sites. Carbamidomethylation of cysteine was set as fixed modification, and oxidation of methionine and N-acetylation of protein were searched as variable modifications. The search results were filtered and summarized using in-house developed software. In the software, the estimated false discovery rate of all peptide identifications was fixed at less than 1% by automatically filtering on mass error and peptide score of all forward and reverse peptide identifications.

Preparation of Recombinant Human APEH with FLAG Tag. The expression vector, pcDNA-NFLAG-GW, was prepared by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Ultimate ORF Clone (IOH3679, matching nucleotide accession number: NM_001640.3; Invitrogen, Carlsbad, CA), which has an...
open reading frame of human APEH, was recombined with pcDNA-NFLAG-GW by a Gateway LR reaction. This vector was transfected into FreeStyle 293F cells (Invitrogen) using 293fectin (Invitrogen) according to the manufacturer’s instructions. The transfected cells were cultured for 96 h, and the harvest was centrifuged. Recombinant APEH in the supernatant was applied to anti-FLAG M2 affinity gel (Sigma-Aldrich) and was eluted with FLAG peptide (Sigma-Aldrich). The eluate was purified using the same procedure as described above.

Antiserum and Immunodepletion. A rabbit was immunized 8 times over a period of 3 months by subcutaneous injection of a mixture of recombinant APEH (100 μg) and oil adjuvant [complete Freund’s adjuvant (first immunization only) or incomplete Freund’s adjuvant; Difco, Detroit, MI]. In 7 days after the final immunization, whole blood was collected, and anti-APEH antiserum was obtained by centrifugation. Aliquots of 100 μl of Protein G Sepharose 4 Fast Flow beads (GE Healthcare) were washed with 500 μl of TNN.15 buffer [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 0.1% Nonidet P-40] and centrifuged. After the supernatant was discarded, the precipitate was suspended in 400 μl (pH 7.4), 150 mM NaCl, and 0.1% Nonidet P-40]. The beads were washed with 500 μl of TNN.1. Aliquots of 250 μl of the antiserum binding bead suspension were centrifuged, and the supernatant was discarded. To the beads, 40 μl of human liver cytosol and 360 μl of TNN.1 buffer were added, and the suspension was mixed gently for 2 h at 4°C. After centrifugation, the supernatant was treated with 250 μl of the bead suspension again. The resulting supernatant obtained after centrifugation was used as APEH-depleted cytosol, and Western blot analysis was performed to check the depletion of APEH. The hydrolytic activity in the cytosol was also measured. As a negative control, cytosol was applied to beads treated with either preimmune serum or TNN.15 buffer.

Western Blotting. APEH-depleted cytosol (10 μg) was separated by SDS-PAGE and was transferred electrophoretically onto a polyvinylidene difluoride membrane (Immun-Blot PVDF Membrane, 0.2 μm; Bio-Rad Laboratories). APEH protein was detected with anti-APEH antiserum as a primary antibody and ECL anti-rabbit IgG horseradish peroxidase-linked, from donkey (1: 1,000,000 dilution; GE Healthcare) as a secondary antibody. The immunoblots were visualized by chemiluminescence with an ECL Advance Western Blotting Detection Kit (GE Healthcare).

APEH Activity. APEH activity in immunodepleted cytosol (0.1 mg/ml) was assayed as described below. Each cytosol was incubated with AANA as a substrate in 100 mM Tris-HCl buffer (pH 7.4) at 37°C. The formation of p-nitroaniline was measured spectrophotometrically by monitoring the increase in absorbance at 405 nm.

β-Glucuronidase Activity. β-Glucuronidase activity in immunodepleted cytosol (0.1 mg/ml) was measured as described previously with a slight modification (Akao et al., 1987). Each cytosol was incubated with pNPG (5 mM) as a substrate in 100 mM sodium acetate buffer (pH 5) or 100 mM Tris-HCl buffer (pH 7.4) at 37°C. The formation of p-nitrophenol was measured spectrophotometrically by monitoring the increase in absorbance at 405 nm.

Results

VPA-G Hydrolysis in Human Liver Subcellular Fraction and Inhibition by PAPM. VPA-G hydrolysis activity in human liver cytosol, microsomes, mitochondria, and lysosomes at pH 7.4 is shown in Fig. 2. All of the subcellular fractions showed the hydrolysis activity, and cytosol had the highest activity. Lysosomes also showed VPA-G hydrolysis activity at pH 5. PAPM (0.03 mM) inhibited VPA-G hydrolysis activity in all of the subcellular fractions, except for lysosomes at pH 5. The subcellular distribution of LDH activity, a marker enzyme of cytosol, was almost consistent with that of VPA-G hydrolysis activity (Fig. 3).

VPA-G Hydrolysis by β-Glucuronidase. Because VPA-G hydrolysis is a cleavage reaction of the carboxyl ester bond, we examined the possibility of hydrolysis by typical esterases in cytosol. As shown in Table 1, DFP, a strong serine esterase inhibitor, completely inhibited the hydrolytic reaction at more than 0.1 mM. Eserine and BNPP, a specific inhibitor for cholinesterase and carboxylesterase, showed little or no inhibition of the hydrolysis, respectively. DTNB and PCMB, known as inhibitors of esterase containing –SH groups, completely inhibited the hydrolytic reaction at more than 0.1 mM. Dithiothreitol (DTT), a thiol protecting agent, and bromoacetamide (BAA), a thiol inhibitor, completely inhibited the hydrolytic reaction at more than 0.1 mM. Therefore, VPA-G hydrolysis is a cleavage reaction of the carboxyl ester bond, and typical esterases in cytosol do not contribute to the hydrolysis.

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Purification of VPA-G Hydrolyase from Human Liver Cytosol. We tried to purify VPA-G hydrolyase(s) from human liver cytosol by anion exchange chromatography and hydroxyapatite affinity chromatography. The active fractions were subjected to SDS-PAGE, and a thick band was found at 75 kDa (Fig. 4). The band was identified as pH 7.4 by 53.8% at 5 mM and abolished the activity at 25 mM (Table 1). However, purified β-glucuronidase hardly hydrolyzed VPA-G at the same pH (0.710 pmol/Fishman unit/h). Because β-glucuronidase is mainly located in lysosomes, the activity of this enzyme at lysosomal pH was also tested. This enzyme showed greater activity at pH 5 (110 pmol/Fishman unit/h) than at pH 7.4. However, the hydrolysis activity at pH 5 was not inhibited by PAPM at 0.3 mM (10 times higher than the inhibitory concentration in cytosol) (Table 2).

Inhibition of VPA-G Hydrolyase Activity by Typical Esterase Inhibitors. Because VPA-G hydrolysis is a cleavage reaction of the carboxyl ester bond, we examined the possibility of hydrolysis by typical esterases in cytosol. As shown in Table 1, DFP, a strong serine esterase inhibitor, completely inhibited the hydrolytic reaction at more than 0.1 mM. Eserine and BNPP, a specific inhibitor for cholinesterase and carboxylesterase, showed little or no inhibition of the hydrolysis, respectively. Dithiothreitol (DTT) and phenylmethylsulfonfyl fluoride (PMSF), known as inhibitors of esterase containing –SH groups, completely inhibited the hydrolysis at 5 and 1 mM, respectively. The inhibitory effect of EDTA, a chelating agent, was very weak.

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FIG. 2. VPA-G hydrolysis activity in human liver subcellular fractions. Subcellular fractions without indication of pH were tested at pH 7.4. The data are expressed as the mean of two experiments.

FIG. 3. VPA-G hydrolysis activity and LDH activity in human liver subcellular fractions at pH 7.4. Each activity is shown as a percentage of cytosol. The data are expressed as the mean of two experiments.
APEH (IPI accession number: IPI00337741) with a sequence coverage of 71% by LC-MS/MS (Table 3).

**Inhibitory Effect of PAPM and Esterase Inhibitors on the VPA-G Hydrolytic Activity of Recombinant APEH.** The construct of human APEH (derived from IOH3679, matching nucleotide accession number: NM_001640.3) with FLAG tag was transfected in FreeStyle 293F cells, and the resulting protein was purified using the affinity tag and two-step column chromatography. Then, the inhibitory effect of PAPM and esterase inhibitors on the VPA-G hydrolytic activity of the recombinant human APEH was examined. As shown in Table 1, PAPM significantly inhibited the hydrolase activity of the recombinant APEH at 0.03 mM. The effect of DFP, DTNB, PCMB, and saccharolactone on the recombinant APEH was similar to that on human liver cytosol as well.

**Immunodepletion of APEH by Rabbit Anti-Human APEH Antiserum.** To examine the contribution ratio of APEH to VPA-G hydrolysis in human liver cytosol, rabbit anti-human APEH antibody was raised by an injection of recombinant human APEH. The resultant antiserum showed ability to bind to APEH protein, but it could not inhibit the APEH activity (data not shown); therefore, we used an immunodepletion method. Human liver cytosol was treated with the antiserum to deplete APEH. Western blot analysis revealed that APEH was successfully depleted in the resulting cytosol (Fig. 5a). The APEH-depleted cytosol completely lacked VPA-G hydrolase activity, whereas cytosol treated with preimmune serum kept 97% of the control activity (Fig. 5b). Likewise, AANA, a typical substrate of APEH, was hardly hydrolyzed in APEH-depleted cytosol (Fig. 5b). In contrast, cytosolic β-glucuronidase activity, measured as pNPG activity, was not affected by depletion of APEH at pH 5 (Fig. 5b).

**TABLE 1**

Residual VPA-G hydrolase activity in human liver cytosol and recombinant human APEH at pH 7.4 after treatment with various inhibitors

The data are expressed as the mean of two experiments.

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Conc.</th>
<th>Residual Activity</th>
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<tr>
<td></td>
<td>mM</td>
<td>%control</td>
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<tr>
<td>β-Glucuronidase</td>
<td>Saccharolactone 5</td>
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</tr>
<tr>
<td></td>
<td>25</td>
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<tr>
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<td></td>
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<td>0.0</td>
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<td>Eserine 0.1</td>
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<td>1</td>
<td>102.5</td>
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<tr>
<td>Carboxylesterase</td>
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<td>Carbapenem-sensitive VPA-G hydrolase</td>
<td>PAPM 0.03</td>
<td>14.0</td>
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HLC, human liver cytosol; rhAPEH, recombinant human APEH; N.D., not determined.

APEH (IPI accession number: IPI00337741) with a sequence coverage of 71% by LC-MS/MS (Table 3).

**Discussion**

We examined the localization of VPA-G hydrolyase in the liver to determine a target fraction for hydrolase identification (Fig. 2). The highest hydrolase activity was found in cytosol at neutral pH. The other fractions, such as microsomes, mitochondria, and lysosomes also showed the hydrolytic activity. However, the activity of these fractions was almost in parallel with that of the contaminated fraction of cytosol. Lysosomes showed the hydrolytic activity at acidic pH. This lysosomal activity was not changed by PAPM, whereas the cytosolic activity at neutral pH was inhibited. Thus, VPA-G hydrolyase, subjected to inhibition by PAPM, is mainly located in cytosol.

From human liver cytosol, we purified a single enzyme with a molecular mass of 75 kDa as a VPA-G hydrolase (Fig. 4). This enzyme was identified as APEH, a serine peptidase. Then, we prepared the recombinant human APEH and found that the inhibition profiles of the recombinant enzyme by PAPM and enzyme inhibitors (DFP, DTNB, PCMB, and saccharolactone) was similar to that of human liver cytosol (Table 1). APEH-depleted cytosol prepared by anti-APEH antiserum completely lacked the VPA-G hydrolytic activity (Fig. 5b). These results demonstrate that APEH is a single enzyme involved in PAPM-sensitive VPA-G hydrolysis in human liver cytosol.

We examined VPA-G hydrolysis by other cytosolic enzymes (Table 1). Cholinesterase inhibitor (eserine) and carboxylesterase inhibitor (BNPP) slightly inhibited VPA-G hydrolyase activity. EDTA showed a weak inhibitory effect on the hydrolytic activity. Therefore, the contribution of cholinesterase, carboxylesterase, and metalloen-
zyme to VPA-G hydrolysis was minor in cytosol. β-Glucuronidase is known as a lysosomal enzyme, but localization in cytosol has been suggested (Beem et al., 1987). Purified bovine liver β-glucuronidase hardly hydrolyzed VPA-G at cytosolic pH (Table 2). In addition, APEH-depleted cytosol lacked VPA-G hydrolase activity but showed hardly hydrolyzed VPA-G at cytosolic pH (Table 2). In addition, suggested (Beem et al., 1987). Purified bovine liver/1542 SUZUKI ET AL./H9252zyme to VPA-G hydrolysis was minor in cytosol.

APEH has been found in various tissues including the kidney, intestine, and erythrocytes as well as the liver (Giardina et al., 1999). Therefore, we cannot rule out the possibility that APEH in these tissues contributes to the interaction of VPA with carbapenems. However, we have considered the major contribution of the liver to this interaction based on the following published data. In rats, the liver is the most important tissue because interaction did not occur after hepatectomy (Yamamura et al., 1999), although it has not been clarified whether hepatic hydrolysis was mainly responsible for the interaction. Nakajima et al. (2004) reported that urinary excretion of VPA-G after intravenous administration of VPA-G in rats failed to increase for concomitant administration of DRPM. No significant change in VPA-G in plasma and urine after treatment of VPA with carbenapens was observed in monkeys (Nakajima et al., 2004), suggesting that the biliary excretion of VPA-G would increase by the inhibition of hepatic APEH. The above results indicate that the main tissue of hydrolysis of VPA-G is the liver. In rabbits, the urinary excretion of VPA-G was increased from 72 to 88% by the treatment of VPA-G with meropenem, although the plasma level of VPA-G changed only slightly (Yokogawa et al., 2001). In this case, renal APEH seems to contribute to the interaction via VPA-G hydrolysis; however, the extent of the impact is unknown because the change in biliary excretion has not been examined. In the case of humans, information on VPA-G disposition in plasma, urine, and bile in patients who are concomitantly receiving VPA and carbapenems is not available. The VPA glucuronidation activities in the human liver and kidney microsomes are very similar (Soars et al., 2001). Moreover, we observed that VPA-G hydrolytic activities in both tissues are also comparable (data not shown). However, the total activity in the liver is greater than that in the kidney because of the difference in the blood flow and total protein between both tissues. Taken together, these results indicate that the APEH in the liver is considered to be most important for the interaction via VPA-G hydrolysis in humans.

Inhibition of VPA-G hydrolysis by carbenapens is the most plausible mechanism for the interaction in humans as well as in animals. A significant increase in the formation of VPA-G by PAPM was observed in human liver slices (Yamamura et al., 2001). Likewise, DRPM accelerated VPA-G formation in rat and monkey liver slices and inhibited VPA-G hydrolysis in rat and monkey liver homogenates (Nakajima et al., 2004). No induction or activation of UDP glucuronosyltransferases by PAPM was observed in rats (Yamamura et al., 2000). Moreover, we found that the VPA-G hydrolytic activity was highly localized in cytosol in human liver and was inhibited by PAPM, consistent with the fact that DRPM-sensitive VPA-G hydrolytic activity was highly abundant in rat liver cytosol (Nakajima et al., 2004). These findings suggest that the inhibition of VPA-G hydrolysis in the liver is observed as the accelerated VPA-G formation, leading to the rapid decrease of VPA in plasma. In contrast, the other three proposed mechanisms, namely, 1) interaction at intestinal absorption process, 2) interaction at enterohepatic circulation process, and 3) interaction at blood cell distribution process (Mori et al., 2007), are less likely in humans than the interaction at the VPA-G hydrolysis process. Torii et al. (2001) reported that the 1542 SUZUKI ET AL./H9252Cmax and area under the curve of VPA were decreased by 50 to 60% after oral administration of VPA with imipenem and PAPM treatment in rats, suggesting that the intestinal absorption of VPA might be inhibited by carbenapens. However, the plasma level of VPA was also decreased at later time points even after intravenous administration of VPA with the carbenapens. In a case
report of a Chinese patient who received 800 mg p.o. q.d. of VPA, the plasma concentration of VPA was decreased from 39 to 10 μg/ml after treatment with meropenem treatment (1 g i.v. b.i.d.), and it was not improved by a change in the dose regimens of VPA (400 mg i.v. and 1200 mg p.o. q.d.) and meropenem (0.5 g i.v. b.i.d.) (Gu and Huang, 2009). These findings indicate that the interaction of VPA and carbapenems occurs after intravenous administration of VPA as well as after oral administration, and the inhibition of intestinal absorption of VPA would not be significant in rat and human. Kojima et al. (1998) reported that the decrease in VPA in plasma was not observed after intravenous administration of VPA and PAPM to bile duct-cannulated rats, suggesting that carbapenems excreted into bile may suppress the enterohepatic recirculation of VPA by killing intestinal flora, which deconjugate VPA-G in gut. However, this result has been controversial because another report showed that the interaction occurred in bile duct-cannulated rats (Yamamura et al., 1999). In addition, the biliary concentrations of carbapenems in human are less than those of other β-lactam antibiotics, which have been reported not to interact with VPA (Thomas et al., 1981; Shiramatsu et al., 1988; Morimoto et al., 1991; Granai et al., 1992; De Turck et al., 1998; Yamagata et al., 1998), supporting the fact that the interaction at the enterohepatic circulation process would be improbable in humans. As to the blood cell distribution process, Omoda et al. (2005) reported that the blood levels of VPA were not changed when the plasma level was decreased after intravenous administration of VPA with carbapenems in rats. However, both the blood and plasma levels of VPA were decreased in patients (Omoda et al., 2005); therefore, this mechanism does not contribute to the interaction in humans. Thus, the inhibition of VPA-G hydrolysis in the liver is the most plausible mechanism of the interaction of VPA with carbapenems in humans at present.

In conclusion, VPA-G hydrolase activity is mainly located in cytosol in human liver. In addition, VPA-G hydrolysis in cytosol is inhibited by PAPM and caused by APEH only, suggesting that APEH could play an important role in the drug interaction of VPA and carbapenems via VPA-G hydrolysis.

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Address correspondence to: Eiko Suzuki, Drug Metabolism & Pharmacokinetics Research Laboratories, Daiichi Sankyo Co., Ltd., 1-2-58, Hiromachi, Shinagawa-Ku, Tokyo 140-8710, Japan. E-mail: tsukada.eiko.x7@daiichisankyo.co.jp