MECHANISM OF THE DRUG INTERACTION BETWEEN VALPROIC ACID AND CARBAPENEM ANTIBIOTICS IN MONKEYS AND RATS

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ABSTRACT:
The Ministry of Health and Welfare, Japan banned coadministration of carbapenems, such as panipenem/betamipron (PAPM), meropenem (MEPM), and valproic acid (VPA) because clinical reports have indicated that the coadministration caused seizures in epileptic patients due to lowered plasma levels of VPA. In this study, we have clarified the mechanism of the drug-drug interaction using PAPM, MEPM, and doripenem (S-4661; (+)-4RS,5S,6S)-6-[(1R,1S)-1-hydroxyethyl]-4-methyl-7-oxo-3-[[3S,5S]-5-[[sulfamoylamino]methyl]-3-pyrrolidinyl][thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate], a newly synthesized carbapenem. In vitro experiments using monkey liver slices suggested that the apparent synthetic rate of VPA glucuronide (VPA-G) increased in the presence of carbapenems. However, no such increase was observed in the experiment using monkey liver microsomes. Although no increase of uridine 5'-diphosphate α-glucuronic acid was found in monkey liver slices in the presence of carbapenems, potent inhibitory activity of carbapenems for the hydrolysis of VPA-G was found in monkey and rat liver homogenate. In vivo hydrolysis of VPA-G was clearly shown by the existence of VPA in plasma after dosing of VPA-G to rats, and its inhibition by carbapenems was also clearly shown by the negligible levels of VPA in rat plasma after coadministration of carbapenems and VPA-G. These results clearly indicate one of the important causes of drug interaction as follows: carbapenems would inhibit the hydrolytic enzyme, which is involved in the hydrolysis of VPA-G to VPA, resulting in a decrease of plasma concentration of VPA.

The increased incidence of convulsions or epileptic fits due to interaction between carbapenem antibiotics, such as panipenem/betamipron (PAPM) and meropenem (MEPM), and the antiepileptic valproic acid (VPA) was first reported clinically in 1997 (Nagai et al., 1997). Both PAPM and MEPM reduced the plasma concentration of concomitantly administered VPA, which resulted in an insufficient concentration to prevent epileptic fit. This led to prohibition of the concomitant use of carbapenem antibiotics and VPA by the Information on Adverse Reactions to Drugs from the Ministry of Welfare in Japan in 1996. To clarify the mechanism of this drug interaction, many studies have been conducted, but the mechanism is still not clear. Doripenem (S-4661), (+)-(4R,5S,6S)-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-3-[[3S,5S]-5-[[sulfamoylamino]methyl]-3-pyrrolidinyl][thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate), a newly synthesized carbapenem. In vitro experiments using monkey liver slices suggested that the apparent synthetic rate of VPA glucuronide (VPA-G) increased in the presence of carbapenems. However, no such increase was observed in the experiment using monkey liver microsomes. Although no increase of uridine 5'-diphosphate α-glucuronic acid was found in monkey liver slices in the presence of carbapenems, potent inhibitory activity of carbapenems for the hydrolysis of VPA-G was found in monkey and rat liver homogenate. In vivo hydrolysis of VPA-G was clearly shown by the existence of VPA in plasma after dosing of VPA-G to rats, and its inhibition by carbapenems was also clearly shown by the negligible levels of VPA in rat plasma after coadministration of carbapenems and VPA-G. These results clearly indicate one of the important causes of drug interaction as follows: carbapenems would inhibit the hydrolytic enzyme, which is involved in the hydrolysis of VPA-G to VPA, resulting in a decrease of plasma concentration of VPA.

Materials and Methods
Chemicals. VPA (sodium valproate) was supplied by Sigma-Aldrich (St. Louis, MO). [Carbonyl-14C]-VPA (55 mCi/mmol, 99% pure by thin-layer chromatography) was supplied by Muromachi Chemical Co. (Tokyo, Japan). Doripenem (S-4661) was synthesized by Shionogi Research Laboratories. PAPM was supplied by Sankyo Co. Ltd. (Tokyo, Japan). MEPM was supplied by Sumitomo Pharmaceutical Co. (Osaka, Japan). All other chemicals were of analytical grade. 14C-VPA-G was extracted with methanol from SEP-PAK C18 in which urine and bile, obtained after intravenous administration of 14C-VPA to rats, were applied, and the methanol extract was condensed and applied to a TLC plate. The TLC plate was developed with the solvent mixture of ethyl acetate/acetonic acid/water (4:1:1). The 14C-VPA-G fraction on the TLC plate, visualized by radioluminography using BAS 2000 (Fuji Photo Film Co., Ltd., Tokyo, Japan), was scrapped and extracted with methanol, and then used as substrate.

Animals. Male and female cynomolgus monkeys were purchased from Keary Japan Co., (Wakayama, Japan), CLEA Japan, Inc., (Osaka, Japan), and Japan SLC, Inc. (Shizuoka, Japan) and raised at Shionogi Laboratories. Male monkeys used were 4 to 5 years old (weight, 5.4–6.6 kg) and female monkeys used were 10 to 17 years old (weight, 3.0–4.2 kg). Male Sprague-Dawley rats (6 weeks old) were purchased from CLEA Japan, Inc. and raised at Shionogi Laboratories, and then were used at 8 to 9 weeks of age (weight, 275–366 g).

ABBREVIATIONS: PAPM, panipenem/betamipron; MEPM, meropenem; VPA, valproic acid; S-4661, doripenem ((+)-(4R,5S,6S)-6-[(1R)-1-hydroxyethyl]-4-methyl-7-oxo-3-[[3S,5S]-5-[[sulfamoylamino]methyl]-3-pyrrolidinyl][thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid monohydrate); VPA-G, valproic acid glucuronide; TLC, thin-layer chromatography; GLC, gas-liquid chromatography; KRB, Krebs-Ringer bicarbonate buffer; UDPGA, uridine 5'-diphosphate α-glucuronic acid; MRT, mean residence time.
Experiments in Monkeys.

Plasma levels of VPA after oral administration of VPA with carbapenems. The monkey oral dosing study was referred to the Information on Adverse Reactions to Drugs (137-1) from the Ministry of Health and Welfare in Japan, 1996. Namely, three male monkeys were orally administered at 25 mg/kg VPA. After 30 min of VPA dosing, PAPM, MEPM, S-4661, and physiological saline were intravenously administered at 20 mg/kg each to three monkeys. Blood was collected at 15 and 30 min, and 1, 2, 4, and 6 h after dosing, and centrifuged to obtain the plasma sample. Plasma concentration of VPA was measured with an immunoassay kit (EMIT, Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan; Rubenstein et al., 1972).

Experiments using monkey liver and kidney slices. Under pentobarbital anesthesia (25 mg/kg), monkeys were killed by bleeding, and the liver and kidneys were removed and stored in chilled Krebs-Ringer-bicarbonate buffer (KRB). After the connective tissues and fat had been removed, the liver and kidneys were cut into slices about 0.2 mm thick with a tissue slicer, KN-822 (Natsume Co., Ltd., Tokyo, Japan). One gram of liver and kidney slice was added to a 30-ml flask containing 4 ml of KRB that had been bubbled with an O2/CO2 (95:5) gas mixture for more than 15 min. Next, 103 μCi of 14C-VPA (10 μCi/mg) and various concentrations of carbapenems (PAPM, MEPM, and S-4661) or flomoxef sodium (an analog compound of the cephem group; Shionogi Co., Ltd.) were added. The mixtures were finally bubbled again with the same gas mixture for 30 s and then incubated for 1 h at 37°C with constant shaking. After incubation, the mixtures were homogenized in a glass homogenizer. Portions of 3 ml of each homogenate were acidified by adding 0.3 ml of 6 N HCl, and then extracted with ethyl acetate containing 1-methyl-1-cyclohexanecarboxylic acid as an internal standard after acidification with an equal volume of 0.5 N HCl. The mixture was shaken with a mixer for 1 min and centrifuged at 14,000 rpm for 1 min. Next, 2 μl of the ethyl acetate layer was injected into a gas chromatograph (GLC). The gas chromatograph (model GC-9A; Shimadzu, Kyoto, Japan) was equipped with a flame ionization detector and a 30 m x 0.53 mm wide-bore fused silica capillary column with free fatty acid phase (Supelco Wax 10). The temperature of the injection port was 120°C. The column oven temperature was 110°C. The carrier gas (He) flow rate was 50 ml/min. For the determination of free VPA concentration in plasma and urine, 50 μl of sample was extracted with twice the volume of ethyl acetate containing 1-methyl-1-cyclohexanecarboxylic acid as an internal standard after acidification with an equal volume of 0.5 N HCl. The mixture was shaken with a mixer for 1 min and centrifuged at 14,000 rpm for 1 min. Next, 2 μl of the ethyl acetate layer was injected into a gas chromatograph (GLC). The gas chromatograph (model GC-9A; Shimadzu, Kyoto, Japan) was equipped with a flame ionization detector and a 30 m x 0.53 mm wide-bore fused silica capillary column with free fatty acid phase (Supelco Wax 10). The temperature of the injection port was 120°C. The column oven temperature was 110°C. The carrier gas (He) flow rate was 50 ml/min. For the determination of total VPA concentration in plasma and urine, 50 μl of sample was heated in a hot water bath at 90°C for 1 h after addition of an equal volume of 2.5 N NaOH. After hydrolysis, the sample was acidified with 50 μl of 4 N HCl, and then extracted and subjected to GLC analysis. The peak area ratio to the internal standard was determined by a reporting integration. Linearity for the concentration of VPA (r = 0.999) was observed up to 100 μg/ml. The limit of determination was 2.5 μg/ml with a coefficient of variation <20%.

Plasma levels of VPA and urinary excretion of total VPA after intravenous administration of VPA with carbapenems. Four female monkeys subjected to bladder cannulation were intravenously coadministered 25 mg/kg VPA and 20 mg/kg carbapenems (PAPM, MEPM, and S-4661), or physiological saline as the control. Blood was collected at 15 and 30 min, and 1, 2, 4, and 6 h after dosing, and centrifuged to obtain the plasma sample. Urine was collected for the periods of 0 to 1, 1 to 2, 2 to 4, and 4 to 6 h after dosing. The analytical method used was basically according to the GLC method (Liu et al., 1992), with a little modification. For the determination of free VPA concentration in plasma and urine, 50 μl of sample was extracted with twice the volume of ethyl acetate containing 1-methyl-1-cyclohexanecarboxylic acid as an internal standard after acidification with an equal volume of 0.5 N HCl. The mixture was shaken with a mixer for 1 min and centrifuged at 14,000 rpm for 1 min. Next, 2 μl of the ethyl acetate layer was injected into a gas chromatograph (GLC). The gas chromatograph (model GC-9A; Shimadzu, Kyoto, Japan) was equipped with a flame ionization detector and a 30 m x 0.53 mm wide-bore fused silica capillary column with free fatty acid phase (Supelco Wax 10). The temperature of the injection port was 120°C. The column oven temperature was 110°C. The carrier gas (He) flow rate was 50 ml/min. For the determination of total VPA concentration in plasma and urine, 50 μl of sample was heated in a hot water bath at 90°C for 1 h after addition of an equal volume of 2.5 N NaOH. After hydrolysis, the sample was acidified with 50 μl of 4 N HCl, and then extracted and subjected to GLC analysis. The peak area ratio to the internal standard was determined by a reporting integration. Linearity for the concentration of VPA (r = 0.999) was observed up to 100 μg/ml. The limit of determination was 2.5 μg/ml with a coefficient of variation <20%.

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of 1 N HCl and centrifuged, and the resultant supernatant was used for TLC analysis. The supernatant obtained from the incubation with 14C-VPA was extracted with a solvent mixture of ethyl acetate/acetic acid/water tetrahydrofuran aqueous solution, and then subjected to TLC using silica gel. The plate was brought into contact with X-ray film (Fuji Photo Germany) with a developing solvent system of ethyl acetate/acetic acid/water. The supernatant obtained from the incubation with 14C-VPA was washed with 3 ml of 0.1 N HCl two times and then eluted with 6 ml of ethyl acetate.

Each value represents the mean ± S.D. of three monkeys.

### TABLE 1

**Pharmacokinetic parameters of VPA after administration of VPA (25 mg/kg i.v.) with carbapenems (20 mg/kg i.v.) or saline to monkeys**

<table>
<thead>
<tr>
<th>Group</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (µg/ml)</th>
<th>AUC&lt;sub&gt;0-6h&lt;/sub&gt; (µg·h/ml)</th>
<th>MRT (h)</th>
<th>CL (ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (VPA + Saline)</td>
<td>0.7 ± 0.3</td>
<td>39.9 ± 9.8</td>
<td>160 ± 11</td>
<td>2.51 ± 0.49</td>
<td>157 ± 10</td>
</tr>
<tr>
<td>PAPM (VPA + PAPM)</td>
<td>1.0 ± 0.9</td>
<td>37.8 ± 14.7</td>
<td>93 ± 50*</td>
<td>1.40 ± 0.34*</td>
<td>374 ± 291</td>
</tr>
<tr>
<td>MEPMP (VPA + MEPMP)</td>
<td>1.0 ± 0.9</td>
<td>40.8 ± 2.9</td>
<td>98 ± 22*</td>
<td>1.46 ± 0.33</td>
<td>265 ± 64</td>
</tr>
<tr>
<td>S-4661 (VPA + S-4661)</td>
<td>0.7 ± 0.3</td>
<td>50.9 ± 16.1</td>
<td>118 ± 24</td>
<td>1.58 ± 0.34</td>
<td>218 ± 40</td>
</tr>
</tbody>
</table>

*p < 0.05 (Dunnett’s t test) vs control (VPA + Saline).

### TABLE 2

**Pharmacokinetic parameters of free VPA after administration of VPA (25 mg/kg i.v.) with carbapenems (20 mg/kg i.v.) or saline to monkeys**

<table>
<thead>
<tr>
<th>Group</th>
<th>V&lt;sub&gt;d&lt;/sub&gt; (ml/kg)</th>
<th>AUC&lt;sub&gt;0-6h&lt;/sub&gt; (µg·h/ml)</th>
<th>CL (ml/kg)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>MRT (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (VPA + Saline)</td>
<td>177 ± 23</td>
<td>251 ± 37</td>
<td>102 ± 15</td>
<td>1.3 ± 0.2</td>
<td>1.8 ± 0.4</td>
</tr>
<tr>
<td>PAPM (VPA + PAPM)</td>
<td>173 ± 23</td>
<td>172 ± 28*</td>
<td>149 ± 28*</td>
<td>0.8 ± 0.2*</td>
<td>1.2 ± 0.3*</td>
</tr>
<tr>
<td>MEPMP (VPA + MEPMP)</td>
<td>161 ± 13</td>
<td>191 ± 34*</td>
<td>134 ± 26*</td>
<td>0.9 ± 0.1*</td>
<td>1.2 ± 0.3*</td>
</tr>
<tr>
<td>S-4661 (VPA + S-4661)</td>
<td>164 ± 22</td>
<td>201 ± 32*</td>
<td>127 ± 20*</td>
<td>0.9 ± 0.3*</td>
<td>1.3 ± 0.3*</td>
</tr>
</tbody>
</table>

*p < 0.05 (paired t test) vs control (VPA + Saline).
of supernatant was subjected to TLC analysis as described above. The TLC plate was placed in contact with an imaging plate (Fuji Photo Film Co., Ltd.). The percentages of VPA and VPA-G to all the radioactivity on the developing area of the TLC plate, which were obtained by radioluminography using BAS 2000 (Fuji Photo Film Co., Ltd.), were validated using the mixture of known amounts of 14C-VPA and 14C-VPA-G.

Urinary and biliary excretion of radioactivity after intravenous dosing of 14C-VPA or 14C-VPA-G with carbapenem. The rats were anesthetized with ether, the femoral vein and bile duct were cannulated with polyethylene tubing, and rats were held in Bollman cages. Thirty minutes after starting the S-4661 infusion as described above, a bolus dose of 14C-VPA (30 mg/kg) or 14C-VPA-G (6.83 mg Eq of VPA/kg) was injected through the tail vein. Urine and bile were collected at 30-min intervals for 120 min. Urine and bile samples were directly subjected to the same TLC analysis described for rat plasma.

**Excretion of total VPA in urine after administration of VPA (25 mg/kg i.v.) with carbapenems (20 mg/kg i.v.) or saline to monkeys**

Each value represents the mean ± S.D. of four monkeys.

<table>
<thead>
<tr>
<th>Group</th>
<th>0–1 h</th>
<th>1–2 h</th>
<th>2–4 h</th>
<th>4–6 h</th>
<th>0–6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (VPA + Saline)</td>
<td>22.50 ± 8.05</td>
<td>18.01 ± 3.04</td>
<td>14.87 ± 2.87</td>
<td>3.14 ± 0.39</td>
<td>58.52 ± 12.23</td>
</tr>
<tr>
<td>PAPM (VPA + PAPM)</td>
<td>27.87 ± 6.46</td>
<td>20.16 ± 3.14*</td>
<td>12.74 ± 2.64</td>
<td>1.88 ± 0.78*</td>
<td>62.65 ± 6.80</td>
</tr>
<tr>
<td>MEPM (VPA + MEPM)</td>
<td>28.06 ± 5.34</td>
<td>20.78 ± 3.12*</td>
<td>14.68 ± 2.35</td>
<td>2.01 ± 1.01</td>
<td>65.52 ± 6.28</td>
</tr>
<tr>
<td>S-4661 (VPA + S-4661)</td>
<td>27.02 ± 6.11</td>
<td>17.98 ± 3.40</td>
<td>13.21 ± 1.57</td>
<td>2.65 ± 1.20</td>
<td>60.85 ± 9.48</td>
</tr>
</tbody>
</table>

*p < 0.05 (paired t test) vs control (VPA + Saline).

**Fig. 4.** Effect of carbapenems on VPA glucuronidation by monkey liver and kidney slice.

**Fig. 5.** Effect of UDPGA and carbapenems on VPA glucuronidation by monkey liver microsome.
mixture was incubated as in the case of the monkey liver slices. After incubation, the mixture was homogenized and centrifuged, and the resulting supernatant was subjected to TLC analysis as described for the monkey liver slices.

VPA-G hydrolysis by rat liver homogenate and subcellular fraction.

Rat liver was homogenized (22.5%, w/v) in KRB buffer, and further fractionation was done by a usual method (Vincent, 1959) to obtain mitochondria, lysosome, microsome, and cytosol fractions. To a 10-ml test tube, $^{14}$C-VPA-G (52–56 μg Eq of VPA), S-4661 (100 μg), and homogenate or a subcellular fraction were added to a final volume of 1 ml with KRB buffer and then incubated for 1 h at 37°C. After incubation, the mixtures were centrifuged, and the resulting supernatant was subjected to TLC analysis as described above.

Data Analysis. For plasma concentration of individual rats, model-independent pharmacokinetic parameters, such as area under the plasma concentration-time curve (AUC), biological half-life ($t_{1/2}$), and total body clearance (CLtot), were calculated using WinNonlin (Pharsight, Mountain View, CA) software.

Statistical Analysis. The results are expressed as the mean ± standard deviation for the indicated numbers of experiments. Statistical differences in pharmacokinetic parameters between control and carbapenem-treated groups were tested by the paired t test, the Dunnett method, and Student’s t test. The criterion for statistical significance was $p < 0.05$.

Results

Confirmation of Drug Interaction between Carbapenems and Valproic Acid in Monkeys in Vivo. Plasma concentrations of VPA after oral administration of VPA at 25 mg/kg to male monkeys that had been intravenously coadministered carbapenems after 30 min of VPA dosing are shown in Fig. 2. The plasma concentration of VPA at 2 h after administration of VPA decreased rapidly with coadministration of carbapenems, and the resulting AUCs and MRTs were smaller than those of the control (Table 1).

After intravenous administration of VPA and carbapenems to female monkeys, the plasma concentration of VPA decreased more rapidly than that of the control (Fig. 3), and the resulting AUCs and MRTs were smaller than those of the control (Table 2). Urinary excretion rates of total VPA, which was mostly as VPA-G, showed a tendency of increase at 2 h after dosing of VPA (Table 3). These results clearly indicated that drug interaction between carbapenems and VPA was observed in monkeys as in humans.

In Vitro Experiment using Monkey Liver. The effect of carbapenems on VPA glucuronidation in monkey liver and kidney slices is shown in Fig. 4. Carbapenems increased VPA-G synthesis up to 10 times that of the control in liver slices. The potency of this effect increased as the concentration of carbapenems increased, and the maximum effect was observed at 2 μg/ml.

The same effect of glucuronidation was observed in kidney slices; however, the response was lower than that of liver slices; the maximum effect on VPA-G synthesis in kidney slices (5–6 μg Eq of VPA/g of slice) was observed at 6 μg/ml carbapenems, whereas the maximum effect of liver slices (10–15 μg Eq of VPA/g of slice) was observed at about 2 μg/ml. Flomoxef sodium, which is an analog compound of the cepham group, showed no effect on VPA glucuronidation.

To clarify the mechanism of accelerating glucuronidation for VPA
with carbapenems, more suitable conditions for glucuronidation of VPA were examined using monkey liver microsomes. Addition of detergent (Brij 58, 0.1 mg) increased the glucuronidation activity to about twice without detergent (Fig. 5A). Maximum glucuronidation activity was observed around 5 mM UDPGA with detergent and at about 10 mM without detergent. Under the optimum conditions of glucuronidation with microsomes, carbapenems (S-4661 or PAPM) showed no accelerating effect on glucuronidation. With a lower concentration of UDPGA (1 mM), the carbapenem effect was also not observed (Fig. 5B). These results suggested that acceleration of glucuronidation was not due to activation of glucuronyl transferase or to a detergent effect of carbapenems.

One possibility for glucuronidation acceleration is thought to be an increase in the UDPGA content by carbapenems. However, under the condition of glucuronidation of VPA being enhanced to about 10 to 20 times that of the control on addition of S-4661, the carbapenem effect was also not observed (Fig. 5B). These results suggested that acceleration of glucuronidation was not due to activation of glucuronol transferase or to a detergent effect of carbapenems.

In Vivo Experiment in Rats. To clarify how much hydrolysis of VPA-G actually contributes to drug interaction between carbapenems and VPA in rat in vivo, the plasma concentrations of VPA and VPA-G were measured after intravenous administration of 14C-VPA or 14C-VPA-G with or without carbapenems. The plasma concentrations of VPA-G after coadministration of VPA with carbapenems were lower than those of the control rats (Fig. 10), and their AUCs (26.0 µg · h/ml for S-4661, 23.2 µg · h/ml for PAPM) were significantly lower than that of the control rats (31.2 µg · h/ml) (Table 4).

The plasma concentration of VPA-G after coadministration of VPA-G with carbapenems to rats tended to be lower compared with that of the control rats (Fig. 11), and their AUCs (2.59 µg · h/ml for S-4661, 2.16 µg · h/ml for PAPM) showed a tendency for a lower value compared with control rats (2.89 µg · h/ml) (Table 5).

Plasma levels of VPA decreased dramatically after coadministration of VPA-G with carbapenems compared with control rats (Fig. 11). Their AUCs (0.23 µg · h/ml for S-4661, 0.19 µg · h/ml for PAPM) were significantly lower than that of control rats (1.88 µg · h/ml) (Table 5). These data were applied to the interconversion model (Scheme 1; Ebling and Jusko, 1986), and the resultant clearance parameters were shown in Table 6. VPA-G hydrolysis clearance (CL21) dramatically decreased to one-sixth to one-seventh that of control after coadministration with carbapenems; however, VPA-G compared with the control was observed after 1 h of incubation with carbapenems in rat liver slices (Fig. 8).

In contrast, carbapenems greatly inhibited hydrolysis of VPA-G in the rat liver homogenate. These results indicated that a phenomenon similar to the one in monkey liver occurred in rat liver.

The subcellular localization of VPA-G hydrolase activity was examined using rat liver subcellular fractions obtained by ultracentrifugation method. Each fraction of mitochondria, lysosomes, microsomes, and cytosols showed hydrolyzing ability of VPA-G; however, only hydrolyzing activity in cytosol was greatly inhibited by addition of carbapenems (Fig. 9).

In Vitro Experiments using Rat Liver. To confirm whether or not a similar drug interaction is observed between carbapenems and VPA in rats, in vitro experiments were done using rat liver slices and homogenates. About 10 times accelerated glucuronidation of VPA was observed after 1 h of incubation with carbapenems in rat liver slices (Fig. 8).
synthesis clearance (CL12) increased only 1.4 to 1.7 times that of control. These results clearly indicated that hydrolysis of VPA-G occurs, and carbapenems specifically inhibit hydrolysis of VPA-G in rats in vivo.

To clarify the influence on the excretion process, urinary and biliary excretion was examined after intravenous administration of 14C-VPA or 14C-VPA-G with or without carbapenems to rats. In the case of VPA administration, biliary excretion of VPA-G increased about 1.8 times after coadministration of VPA with S-4661 compared with control rat (Table 7), and total excretion of radioactivity was also significantly increased by coadministration with S-4661. In the case of VPA-G administration, urinary, biliary, and total excretion of radioactivity tended to increase after coadministration with S-4661 compared with the control rat (Table 8). The values of VPA-G excretion into urine after coadministration of VPA-G with S-4661 showed great variation, which originated the unstableness of VPA-G, especially for one rat during storage.

**Discussion**

Carbapenems (PAPM, MEPM, S-4661) reduced plasma concentration of VPA after coadministration of VPA and carbapenems to monkeys, as observed in humans. There are many possible mechanisms for the drug interaction between VPA and carbapenem, such as the inhibition of plasma protein binding (Hobara et al., 1998), and the suppression of its enterohepatic recirculation (Kojima et al., 1998), but the precise reason remains to be clarified. We carried out in vitro experiments using monkey liver and kidney slices and found that carbapenems increased the production of VPA-G in proportion to the amount added, but did not affect the glucuronidation activity with monkey liver microsomes. This means that the acceleration of VPA glucuronidation with carbapenems is not due to UDP-glucuronosyltransferase activation or to the detergent effect, which will be increased due to use of UDPGA as a cofactor. The most plausible reason for acceleration of VPA glucuronidation is the carbapenems increasing UDPGA in the liver, as suggested by Yamamura et al. (1999, 2000). However, under the in vitro conditions of glucuronidation being enhanced 10 to 20 times by addition of carbapenems, the UDPGA contents increased only 1.4 times the control. On the other hand, we found that carbapenems greatly inhibited the hydrolysis of VPA-G in monkey liver homogenates. The same phenomena, such as accel-

### TABLE 5

<table>
<thead>
<tr>
<th>Administration</th>
<th>VPA-G AUC0–2h</th>
<th>VPA AUC0–2h</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPA-G + Saline</td>
<td>2.89 ± 0.32</td>
<td>1.88 ± 0.32</td>
</tr>
<tr>
<td>VPA-G + S-4661</td>
<td>2.59 ± 0.45</td>
<td>0.23 ± 0.05*</td>
</tr>
<tr>
<td>VPA-G + PAPM</td>
<td>2.16 ± 0.24*</td>
<td>0.19 ± 0.06*</td>
</tr>
</tbody>
</table>

### TABLE 6

<table>
<thead>
<tr>
<th>Administration</th>
<th>Area measured</th>
<th>Dose symbol</th>
<th>Area symbol</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPA</td>
<td>VPA</td>
<td>Dosev</td>
<td>AUCv</td>
</tr>
<tr>
<td>VPA-G</td>
<td>VPA-G</td>
<td>Dosev</td>
<td>AUCv-G</td>
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<tr>
<td>VPA-G</td>
<td>VPA-G</td>
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</tr>
<tr>
<td>VPA</td>
<td>VPA-G</td>
<td>Dosev</td>
<td>AUCv-G</td>
</tr>
</tbody>
</table>

### SCHEME 1

![Scheme 1](https://example.com/scheme1.png)

![Scheme 2](https://example.com/scheme2.png)
erating VPA glucuronidation and inhibiting hydrolysis of VPA-G in rat liver by addition of carbapenems, were also observed in rat in vitro, and this hydrolysis activity was shown to exist only in the liver cytosol.

To clarify how much hydrolysis actually contributes to the drug interaction between VPA and carbapenems in rat in vivo, the plasma concentration of VPA was measured after intravenous administration of VPA-G with or without carbapenems to rats. Significant amounts of deconjugated VPA were observed in the control rat plasma, and these concentrations decreased dramatically in coadministered rats. These results indicate that hydrolysis of VPA-G occurs, and carbapenems specifically inhibit hydrolysis of VPA-G in rat in vivo.

According to Ebling and Jusko’s model (Scheme 1; Ebling and Jusko, 1986), which is applied to the pharmacokinetic study of compounds displaying interconversion reaction, the biggest difference in the pharmacokinetic parameters between coadministered rats and control rats was observed at CL12, the step of the hydrolysis process of VPA-G, although, some small differences were observed at CL12 and CL20 (Table 6). These results suggest that the most critical point on drug interaction between carbapenems and VPA is the hydrolysis process of VPA-G, which carbapenems inhibit specifically. Since the main site of hydrolysis of VPA-G is thought to be liver, VPA-G stabilized by the inhibitory effect of carbapenems would be more rapidly eliminated from hepatocytes, compared with the control. This might be the reason for increased clearance of VPA-G (CL20) with coadministration of carbapenems. This hypothesis is confirmed by the excretion study showing that the more rapid excretions of radioactivity into urine and bile were observed after coadministration of 14C-VPA or 14C-VPA-G with carbapenems, compared with the control rat. Research to identify the kind of enzyme contributing to this reaction is now under way.

**References**


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