Effect of Meropenem on Disposition Kinetics of Valproate and Its Metabolites in Rabbits

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Purpose. We investigated the effect of meropenem (MEPM) on the disposition kinetics of valproate (VPA) and its metabolites in rabbits.
Methods. Rabbits were given 75 mg/kg VPA intravenously with or without 300 mg/kg MEPM.
Results. The plamsa total clearance of VPA was significantly increased to about 1.5 times the control (6.09 mL/min/kg vs. 4.28 mL/min/kg) by MEPM ($P < 0.05$). The values of the area under the plasma concentration-time curve (AUC) of 2-en-VPA, a product of β-oxidation, and VPA-glucuronide (VPA-G) were significantly decreased to about 55% and 78% of the control, respectively ($P < 0.05$). The cumulative urinary excretions of VPA in the control and MEPM-treated groups were 0.54% and 0.62% of the dose, respectively, whereas those of VPA-G were 45.6% and 62.5%, respectively. The urinary excretion of VPA-G was significantly increased by MEPM ($P < 0.05$). Further, in the case of 33.8 mg/kg VPA-G administered intravenously the AUC value of VPA-G was unchanged by MEPM, whereas that of the generated VPA was significantly decreased to about half of the control.
Conclusions. The increase of the total clearance of VPA caused by MEPM appears to be a consequence of increased renal clearance of VPA-G, as well as suppression of VPA-G hydrolysis in the liver.

KEY WORDS: meropenem; valproate; drug interaction; glucuronidation; pharmacokinetics.

INTRODUCTION

The major metabolic pathway of sodium valproate (VPA), a widely used antiepileptic drug, is glucuronidation, and its minor pathways are β-oxidation and ω-hydroxylation, followed by glucuronidation and excretion into urine (1). It is well known that VPA tends to show pharmacokinetic interactions with other antiepileptic drugs (2).

Recently, it has been reported that the serum level of VPA was decreased by combined treatment with new carbapenem antibiotics, panipenem/betamipron (3) and meropenem (MEPM) (4). Kojima *et al.* (5) suggested that panipenem decreases the plasma VPA concentration by suppressing its enterohepatic recirculation, probably due to a panipenem-induced decrease in the numbers of enteric bacteria in rats. Yamamura *et al.* (6) indicated that the enhancement of VPA elimination is specific for carbapenem antibiotics, and metabolic activation of hepatic UDP-glucuronosyltransferase may contribute to this pharmacokinetic interaction in dogs. They further reported that the increase in total clearance (CL_{tot}) of VPA by panipenem is due mainly to increased hepatic intrinsic clearance, especially the enhanced glucuronidation of VPA, in rats (7). However, it is well known that there are species differences in drug pharmacokinetics. We have presented a physiologically based pharmacokinetic model from rabbit data to predict the pharmacokinetics of several drugs in humans (8–10). In this study, to clarify the mechanism of decrease in the serum concentration of VPA by carbapenem antibiotics, we examined, using rabbits, the effects of treatment with MEPM on the disposition kinetics of VPA and its metabolites, 2-en-VPA, 3-keto-VPA and their glucuronides, which are major metabolic products in humans (1).

MATERIALS AND METHODS

Materials

VPA was purchased from Sigma Chemical Co. (St. Louis, MO). Meropen® (meropenem, MEPM) was purchased from Sumitomo Pharmaceuticals Co., Osaka, Japan. 2-Propyl-2-pentanoic acid (2-en-VPA) and 3-oxo-2-propylpentanoic acid (3-keto-VPA) were gifts from Kyowa Hakko Kogyo Co. (Tokyo, Japan). 3,3-Dimethylglutaric acid (DMGA) and 3-methylglutaric acid (MGA) were from Wako Pure Chemical Industries Co., Osaka, Japan. *N*-(*tert*-Butyldimethylsilyl)-*N*-methyl-trifluoracetamide (MTBSTFA), *N*-trimethylsilyl-*N*-methyl-trifluoracetamide (MSTFA), and 2-undecenoic acid were purchased from Tokyo Kasei Organic Chemicals Co. (Tokyo, Japan). β-Glucuronidase/arylsulfatase was obtained from Boehringer Ingelheim Co. (Ingelheim, Germany). All other chemicals, which were purchased from Wako Pure Chemical and Sigma Chemical, were of reagent grade and were used without further purification.

Animal Experiments

Animal studies were performed according to the regulations approved by the institutional animal committee. Adult male albino rabbits weighing 2.2–2.8 kg (Nippon SLC, Inc., Hamamatsu, Japan) were fasted for 16 h prior to the experiment with free access to water. The procedure was performed by essentially the same method as described previously (11). Briefly, under light ether anesthesia, the femoral vein, the urethra, and the bile duct were cannulated with polyethylene tubing. VPA (75 mg/kg) in saline was injected into the rabbit *via* the marginal auricular vein. MEPM (300 mg/kg) was similarly injected at 5 min prior to the administration of VPA. Blood samples were withdrawn from the femoral vein through the cannula at designated time intervals and collected in heparinized tubes. Plasma was separated by centrifugation at 3000 × *g* for 10 min and stored at –30°C. Samples of urine and bile were withdrawn through the cannulas at designated time intervals and stored at –30°C.

Plasma Unbound Fraction (f_p) of VPA

To determine the f_p of VPA in the *in vivo* study, plasma samples were collected following 75 mg/kg VPA administered intravenously (i.v.) with or without 300 mg/kg MEPM injection. After incubation at 37°C for 15 min, centrifugal ultrafil-

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tration of the plasma was performed through an MPS-3 membrane (Amicon Division, W.R. Grace & Co., Beverly, MA) at 1000 × *g* for 10 min.

Assay for VPA and Its Metabolites

Concentrations of VPA and its metabolites in plasma, bile, and urine were determined by gas chromatography–mass spectrometry (GC-MS, Model GC-17 system Class 5000, Shimadzu, Kyoto, Japan). The assays for VPA, 2-en-VPA, and 3-keto-VPA were performed according to Darius and Meyer (12) for VPA and Tatsuhara and Muro (13) for 2-en-VPA and 3-keto-VPA, respectively. The extraction solvent for VPA and 2-en-VPA was ethyl acetate containing 2.5 μg/mL of DMGA for plasma or 6.25 μg/mL of MGA for bile and urine as an internal standard. The extraction solvent of 3-keto-VPA was ethyl acetate containing 0.15 μg/mL of 2-undecenoic acid as an internal standard.

Aliquots of 100 μL of plasma, urine, and bile were each mixed with 100 μL of 1 M NaH_2PO_4 buffer (adjusted to pH 5) and 1000 μL of ethyl acetate containing the internal standard. Then the mixture was shaken for 20 min and centrifuged for 2 min at 2000 × *g*. The supernatant organic phase (VPA, 2-en-VPA) was transferred to another glass tube and preconcentrated to approximately 100 μL under a stream of nitrogen gas at 37°C in a heating block. Then 40 μL of MTBSTFA was added to the residue, and the mixture was shaken vigorously. The sample was transferred to an automated sampler microvial and incubated for 3 h at room temperature. An aliquot (1 μL) of the sample was injected into the GC–MS system. The supernatant organic phase (3-keto-VPA) was transferred to another glass tube, 300 mg of Na_2SO_4 was added, and the mixture was shaken vigorously for 5 min. After centrifugation, the sample was preconcentrated to approximately 60 μL under a stream of nitrogen gas at 37°C in a heating block. The residue was shaken vigorously with 60 μL of MTBSTFA. The sample was transferred to an automated sampler microvial and incubated for 2 h at room temperature. An aliquot (1 μL) of the sample was injected into the GC–MS system.

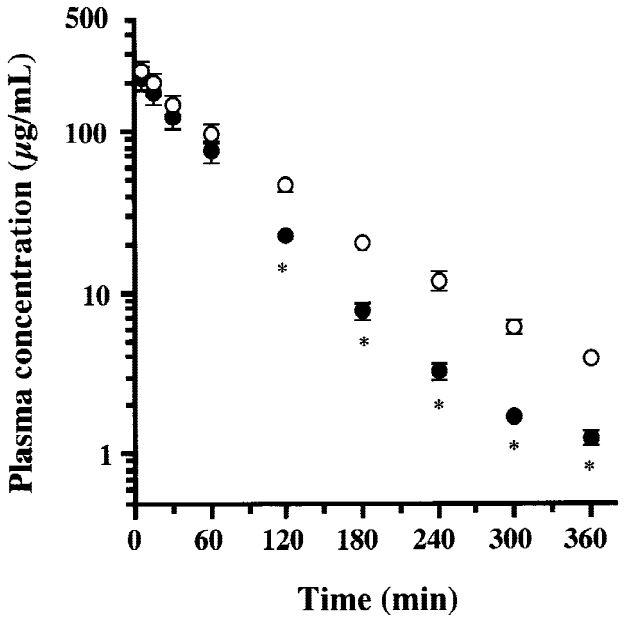

Fig. 1. Plasma valproate (VPA) concentration–time profiles after intravenous 75 mg/kg VPA administration with (●) or without (○) 300 mg/kg meropenem in rabbits. Each symbol with vertical bar represents the mean ± SE of six rabbits. *Significantly different from VPA alone at $P < 0.01$.

Analyses were performed in the selected-ion monitoring mode, monitoring ions at m/z 199, m/z 201, m/z 241, m/z 287, m/z 317, and m/z 331 for 2-en-VPA, VPA, undecenoic acid, 3-keto-VPA, MGA, and DMGA, respectively. Chromatographic separation of VPA and its metabolites was achieved with a methyl siloxane-crosslinked capillary column (HP-1; 25 m × 0.2 mm I.D.; Hewlett-Packard) in a gas chromatograph equipped with a splitless injector. The oven temperature was set at 60°C for 1 min and then programmed up to 90°C at 30°C/min, up to 150°C at 5°C/min, and up to 250°C at 40°C/min. The final temperature was maintained for 10 min.

In the case of estimation of glucuronides, plasma, urine, and bile, samples were incubated with β-glucuronidase/arylsulfatase at 37°C for 70 min, then VPA, 2-en-VPA, and 3-keto-VPA were extracted and analyzed, as mentioned above. The concentration of glucuronide of each metabolite was obtained by subtracting the value for the unconjugated form from that for the hydrolysate.

Preparation of Valproate Glucuronide (VPA-G)

According to Williams *et al.* (14), VPA-G was biosynthesized by i.v. infusion of VPA into rabbits. Ten milliliters of urine, collected after the i.v. injection of VPA, was adjusted to pH 2.0 with 1 N HCl. The sample was shaken vigorously three times with 30 mL of 1-chlorobutane to remove VPA, followed by extraction three times with 30 mL of diethyl ether. The ether solutions were combined and evaporated to dryness under a stream of nitrogen gas, affording VPA-G of over 95% purity, as checked by GC–MS analysis. The pale syrupy residue was taken up in water and carefully adjusted to pH 5 with 0.5 N NaOH. The solution was stored at –80°C and used as VPA-G solution.

Data Analysis

The data were analyzed using Student's *t* test to compare the unpaired means of two sets of data. The number of determinations (N) is noted in each table and figure. A *P* value of 0.05 or less was taken to indicate a significant difference between sets of data.

RESULTS

Effect of MEPM on the Plamsa Concentration–Time Profile of VPA

Figure 1 shows the plasma VPA concentration–time profiles after 75 mg/kg VPA i.v. administration with or without

Table I. Pharmacokinetic Parameters of Valproate (VPA) Estimated by Noncompartment Analysis

Parameter	VPA alone	Meropenem treatment
Area Under the Curve (mg · min/mL)	17.5 ± 1.7	12.3 ± 1.5*
Vd_{ss} (mL/kg)	361 ± 75	352 ± 94
CL_{tot} (mL/min/kg)	4.28 ± 0.41	6.09 ± 0.76*

Each value represents the mean ± SD of six rabbits.
The Area Under the Curve was determined by using the data up to 360 min.
* Significantly different from VPA alone at $P < 0.01$.

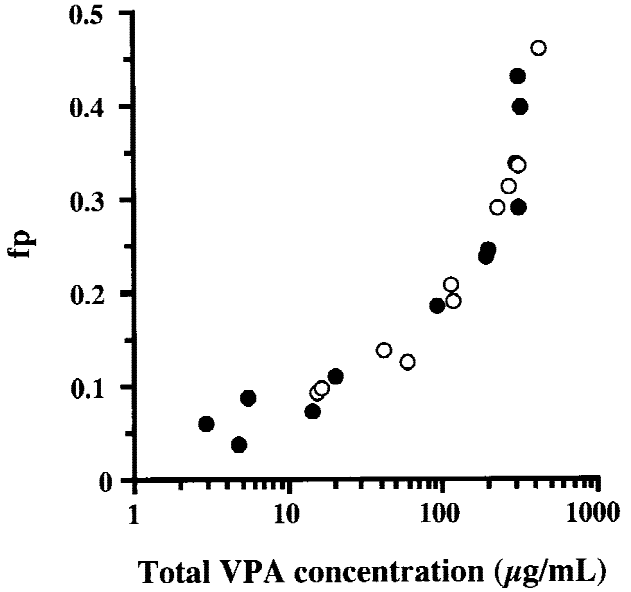

Fig. 2. Relationship between total valproate (VPA) concentration and the unbound fraction of VPA (f_p) after intravenous 75 mg/kg VPA administration with (●) or without (○) 300 mg/kg meropenem in rabbits.

300 mg/kg MEPM in rabbits. Although there was no difference between the plasma concentrations of VPA with and without MEPM until 60 min after VPA administration, thereafter the concentration of VPA in the MEPM-treated group fell significantly below that in the control group. The kinetic parameters of VPA for the control and the MEPM-treated groups are listed in Table I. The value of the area under the plasma concentration-time curve (AUC) from 0 to 360 min for the MEPM combined group was significantly smaller than that for the control group. The plasma CL_{tot} of VPA was increased about 1.5 times compared with the control by MEPM treatment, whereas the distribution volume at the steady state (Vd_{ss}) showed no clear difference.

Plasma Unbound Fraction of VPA

Figure 2 shows the relationship between total plasma concentration and the fp value of VPA with or without MEPM treatment in the *in vivo* experiment. Although the fp value of VPA depended on the total plasma concentration of VPA, there was no significant difference between the control and the MEPM-treated groups.

Effect of MEPM on the Plamsa Concentration–Time Profiles of VPA Metabolites after VPA Administration

The disposition kinetics of the major metabolites of VPA after i.v. administration of 75 mg/kg VPA with or without 300

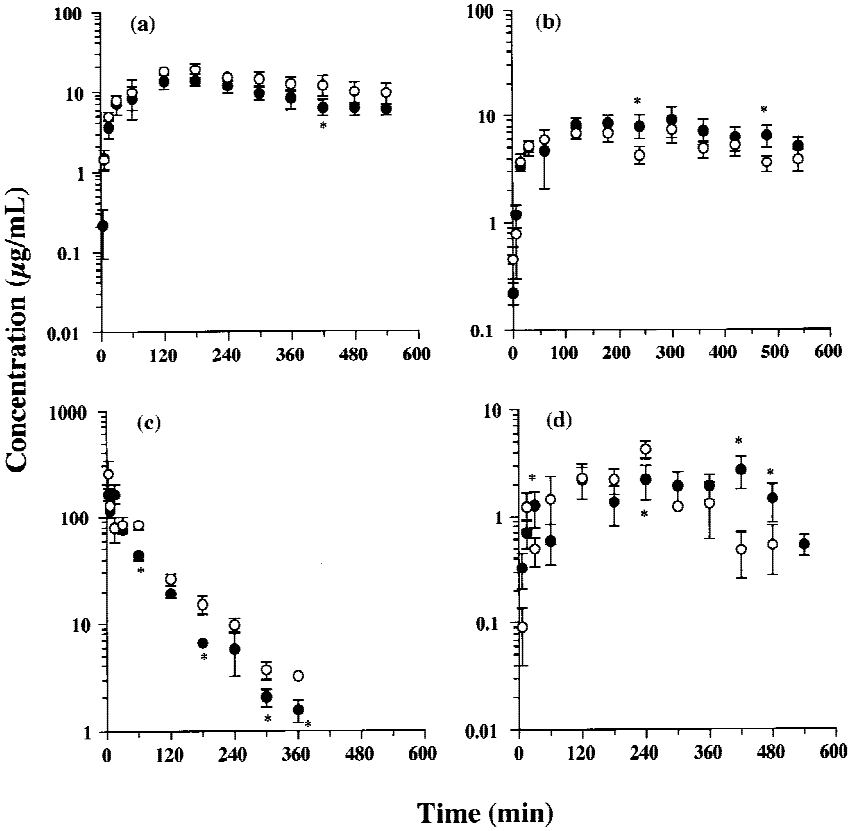

Fig. 3. Plasma concentration-time profiles of 2-en-valproate (VPA) (a), 3-keto-VPA (b), VPA-G (c), and 3-keto-VPA-G (d) after intravenous 75 mg/kg VPA administration with (●) or without (○) 300 mg/kg meropenem in rabbits. Each symbol with vertical bar represents the mean ± SE of 3–4 rabbits. *Significantly different from VPA alone at $P < 0.05$.

mg/kg MEPM in rabbits were examined. Figure 3 shows the plasma concentration–time profiles of the β-oxidation products (2-en-VPA, 3-keto-VPA) and the glucuronides (VPA-G, 3-keto-VPA-G). After i.v. administration of VPA alone, the plasma concentrations of 2-en-VPA and 3-keto-VPA reached about 20 and 8 μ/mL at 120 min, respectively. The concentration of 2-en-VPA tended to be lower and that of 3-keto-VPA became slightly higher in the case of co-administration of MEPM (Fig. 3a and b). The plasma concentration of 3-keto-VPA-G was also little affected by MEPM (Fig. 3d). On the other hand, VPA-G rapidly appeared and decreased in plasma, and the elimination rate of VPA-G was significantly accelerated by MEPM (Fig. 3c), as in the case of free VPA. The AUC value of VPA-G (55.7 ± 4.9 μmol min/mL, mean ± SD) from zero to 360 min in the case of MEPM coadministration was significantly smaller than that of the control (71.1 ± 7.8 μmol min/mL), $P < 0.05$. Other metabolites were hardly detected in plasma. The 2-en-VPA was detected only as (E)-2-en-VPA but not (Z)-2-en-VPA, as in the case of humans (13).

Urinary Excretion of VPA and Its Metabolites

Figure 4 shows the cumulative urinary excretion of VPA and its metabolites up to 540 min following 75 mg/kg VPA i.v. administration with or without 300 mg/kg MEPM. The cumulative amounts of VPA and VPA-G in the MEPM-treated group tended to increase with time, compared with those of the control group (Fig. 4a and b). MEPM treatment significantly altered the urinary excretion of VPA-G ($P < 0.05$). At 540 min after administration, the cumulative amounts of VPA in the control and MEPM-treated groups were 0.54 ± 0.05 and 0.62 ± 0.13% of the dose, respectively, whereas those of VPA-G were 45.6 ± 5.6 and 62.5 ± 9.3%, respectively. The

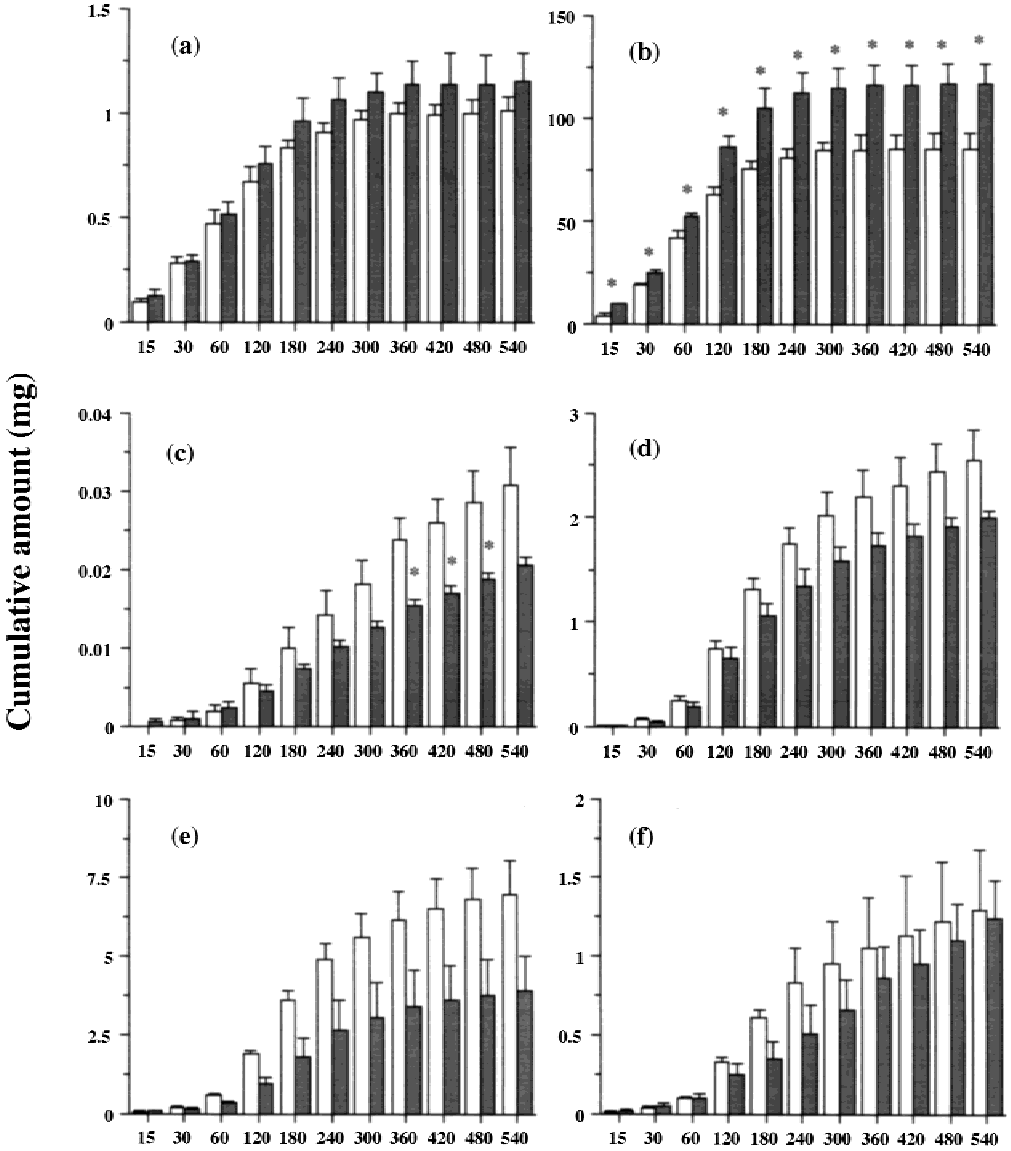

Fig. 4. Urinary excretion of valproate (VPA) (a), VPA-G (b), 2-en-VPA (c), 2-en-VPA-G (d), 3-keto-VPA (e), and 3-keto-VPA-G (f) for 540 min after intravenous 75 mg/kg VPA administration with (□) or without (■) 300 mg/kg meropenem in rabbits. Each column with vertical bar represents the mean ± SE of three rabbits.

urinary excretion of VPA-G was significantly increased by the MEPM treatment ($P < 0.05$). The cumulative amount of 2-en-VPA in the MEPM-treated group was significantly lower than in the control group (Fig. 4c and d). The cumulative amounts of 2-en-VPA and 2-en-VPA-G were about 0.15 and 1.5% of the dose of VPA, respectively. The cumulative amounts of 3-keto-VPA and 3-keto-VPA-G in the MEPM-treated group were also lower than those in the control, and were about 3 and 0.7% of the dose of VPA, respectively (Fig. 4e and f).

Biliary Excretion of VPA and Its Metabolites

The total biliary excretion of VPA and its metabolites was very small, below 1.2% up to 8 h, and there was no clear difference between the control and the MEPM-treated groups.

Effect of MEPM on the Plasma Concentration–Time Profile of VPA-G and the Amount of VPA Generated after VPA-G Administration

After i.v. administration of VPA-G (33.7 mg equivalent VPA/kg) with or without MEPM, the plasma concentration–time profiles of VPA-G and the generated VPA were determined. As shown in Fig. 5, no significant difference in the plasma concentration of VPA-G between the control and the MEPM-treated groups was observed. The AUC and CL_{tot} value of VPA-G were about 3.5 mg min/mL and about 10 mL/min/kg, respectively. On the other hand, VPA rapidly appeared in serum, reached a peak at 20 min after VPA-G administration, and then decreased. The plasma concentrations of VPA in the MEPM-treated group were significantly lower than those in the control group from 60 min after VPA-G administration. The AUC value of VPA (2.05 ± 0.35 mg min/mL) for the MEPM-treated group was about half that for the control group (4.7 ± 1.8 mg min/mL) ($P < 0.05$).

Urinary Excretion of VPA-G and Generated VPA after VPA-G Administration

Figure 6 shows the cumulative urinary excretion of VPA-G and generated VPA up to 360 min after an i.v. administration of VPA-G (33.7 mg equivalent VPA/kg) with or without 300 mg/kg MEPM. The urinary excretion of VPA-G in the MEPM-treated group was significantly higher than that in the control group from 120 min after VPA-G administration. The excretion of generated VPA in the MEPM-treated group was rather lower than that in the control group. The rates of urinary recovery of VPA-G for the control and MEPM-treated groups were about 72 and about 88% of the dose, respectively, whereas those of generated VPA were only 0.33 and 0.27%, respectively.

DISCUSSION

In this study, we found that the plasma concentration–time profile of VPA was significantly lowered by co-administration with the carbapenem antibiotic MEPM in rabbits, as reported in humans (3,4). Our data suggest that the change is due to altered metabolism and/or excretion rather than distribution because MEPM significantly increased the total clearance of VPA but not the distribution volume. Kojima *et al.* (5) and Yamamura *et al.* (7) reported that the plasma

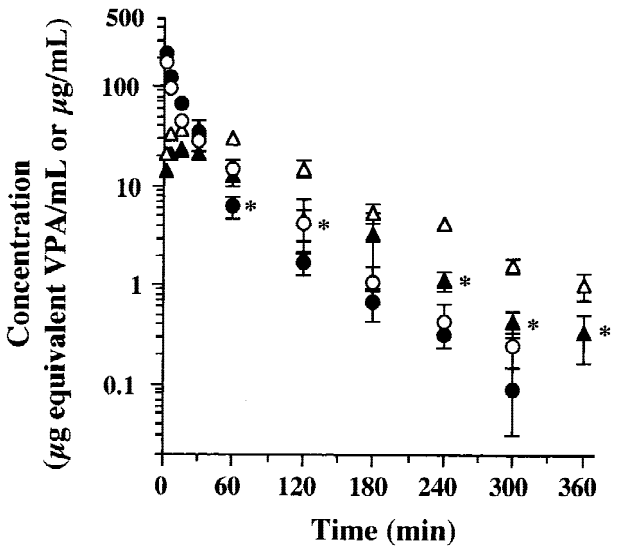

Fig. 5. Plasma concentration-time profiles of valproate (VPA)-G (○) and VPA (△) following a 33.7 mg equivalent VPA/kg VPA-G intravenous administration with (closed symbol) and without (open symbol) 300 mg/kg meropenem in rabbits. Each symbol with vertical bar represents the mean ± SE of three rabbits. *Significantly different from VPA-G alone at $P < 0.05$.

binding of VPA is unchanged by carbapenem antibiotics in rats. In contrast, Hobara *et al.* (15) reported that carbapenem antibiotics increase the unbound fraction of VPA in rats and humans. However, our results showed that the plasma binding of VPA was unchanged by MEPM in rabbits (Fig. 2). Therefore, it was suggested that the change of the total clearance is caused by alteration of the intrinsic hepatic metabolism.

It has been reported that the most important metabolic pathway of VPA in human is glucuronization; however, other routes of VPA metabolism include β-oxidation and ω-oxidations and further minor P-450-catalyzed metabolism (1). Riva *et al.* (2) reported that VPA is almost completely eliminated by metabolism, with less than 4% of the dose being excreted in unchanged form into the urine in humans. Furthermore, direct glucuronic conjugation alone accounts for about 40% of the total clearance, and about 30–35% of the dose is eliminated via the mitochondrial β-oxidation pathway in mitochondria to form 2-en-VPA and 3-oxo-VPA, with the remaining 10% undergoing cytochrome P450 dependent oxidation to form minor derivatives. The results of this and our previous study (8) indicate that the kinetic behavior of VPA, appearance of metabolic products in serum, and the excretion pattern in rabbits are similar to those in human. We investigated the effect of MEPM on the disposition kinetics of VPA metabolites in rabbits. MEPM increased not only the clearance of VPA but also the clearance of VPA-G produced after VPA administration (Figs. 1 and 3) and significantly increased the urinary excretion of VPA-G (Fig. 4). The serum concentration–time profiles of other metabolites, such as 2-en-VPA, 3-keto-VPA, and their glucuronides were hardly influenced by MEPM (Fig. 3), but their urinary excretion was decreased (Fig. 4). On the other hand, because biliary excretion of VPA and its metabolites is minor in rabbits, the influence of

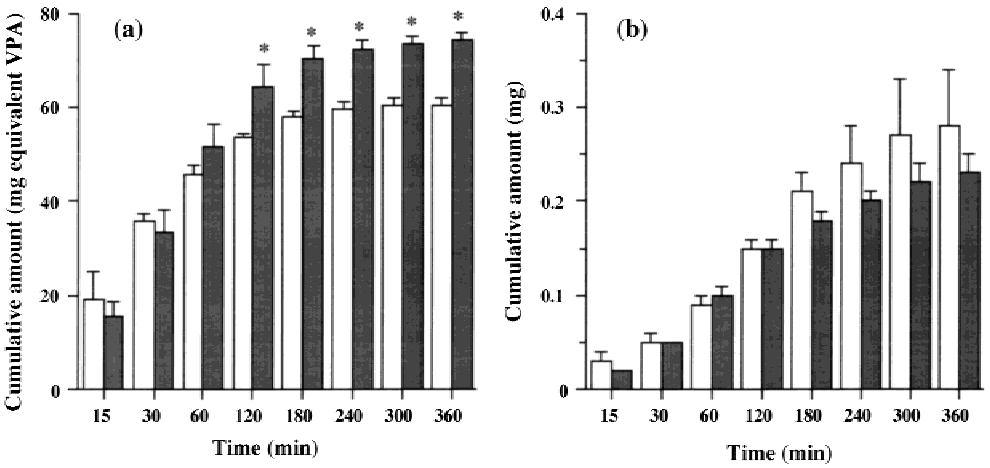

Fig. 6. Urinary excretion of valproate (VPA)-G (a) and produced VPA (b) for 360 min after a 33.7 mg equivalent VPA/kg VPA-G intravenous administration with (□) or without (■) 300 mg/kg meropenem in rabbits. Each column with vertical bar represents the mean ± SE of three rabbits. *Significantly different from VPA-G at $P < 0.05$.

MEPM could not be clearly observed. Previously, Kojima *et al.* (5) suggested that the drug interaction between VPA and panipenem is caused by suppression of the enterohepatic recirculation of VPA-G in rats. Because VPA-G is excreted into bile in rats but is excreted into urine in rabbits, there should be little opportunity for enterohepatic recirculation to occur in rabbits. In this study, the renal clearance of VPA-G in the MEPM-treated group (5.1 ± 0.9 mL/min/kg) was about 1.7 times higher than that of the control group (2.9 ± 0.5 mL/min/kg). Thus, MEPM appeared to promote the glucuronization of VPA, rather than β-oxidative metabolism, and to accelerate the urinary excretion of VPA-G.

Furthermore, we calculated the clearance of VPA-G production (metabolite formation clearance) by dividing the urinary cumulative amount by the AUC value of VPA according to Rudy *et al.* (16). This value in the MEPM-treated group (3.80 ± 0.74 mL/min/kg) was significantly higher than that in the control group (1.95 ± 0.31 mL/min/kg) ($P < 0.05$). We suggest that the increased total clearance of VPA in the MEPM-treated group may be caused by the clearance of VPA-G production.

Next, we examined the influence of MEPM on the disposition kinetics of VPA-G. After VPA-G administration, the plasma concentration–time profile of VPA-G itself was unchanged by MEPM treatment, whereas that of generated VPA was significantly decreased by MEPM (Fig. 5), and its AUC value was decreased to about half that of the control roup. Urinary excretion of VPA tended to be decreased, but that of VPA-G was significantly increased by MEPM (Fig. 6). These results indicate that MEPM may suppress the hydrolysis of VPA-G to VPA and increase the excretion of VPA-G into urine, although further *in vitro* study is needed to confirm this.

In summary, we conclude that the increase of the total clearance of VPA in the case of combined treatment with MEPM is closely associated with the increase of renal clearance of VPA-G. Although in this study we did not examine the direct effects of MEPM on metabolic enzymes for VPA, glucuronization of VPA is an important factor in the total clearance of VPA and it is possible that MEPM suppresses the hydrolysis of VPA-G to VPA. Further studies of the effects of carbapenem antibiotics on the renal excretion of VPA and its metabolites are needed to clarify fully the mechanism of the drug interaction.

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