PANIPENUM, A CARBAPENEM ANTIBIOTIC, INCREASES THE LEVEL OF HEPATIC UDP-GLUCURONIC ACID IN RATS

NAOTOSHI YAMAMURA, KAORU IMURA-MIYOSHI, AND HIDEO NAGANUMA

Drug Metabolism and Pharmacokinetics Research Laboratories, Sankyo Co., Ltd., Tokyo, Japan

(Received June 1, 2000; accepted Sept. 14, 2000)

ABSTRACT:

To investigate the mechanism for the enhanced glucuronidation of valproic acid (VPA) by panipenem (PAPM), a carbapenem antibiotic, in rat liver, we carried out studies to investigate whether PAPM increases the activity of UDP-glucuronosyltransferase or the level of hepatic UDP-glucuronic acid (UDPGA) in rats. PAPM had no effect on the UDP-glucuronosyltransferase activity toward VPA both in vivo and in vitro. On the other hand, in vivo treatment with PAPM significantly increased the hepatic UDPGA level by about 1.7-fold (control: 434.5 ± 65.5 nmol/g of liver; PAPM-treated: 755.2 ± 92.3 nmol/g of liver). The in vitro formation of VPA glucuronide increased proportionally as a function of the UDPGA concentration up to 0.8 mM. Therefore, the increase in the level of hepatic UDPGA by PAPM is likely to be one of the causal factors for enhancing VPA glucuronidation in vivo.

In 1997, the first clinical report appeared showing that concomitant use of panipenem/betamipron (PAPM/BP1), a carbapenem antibiotic, unexpectedly caused the recurrence of an epileptic seizure in patients who had until then been successfully treated with valproic acid (VPA) (Nagai et al., 1997). The seizure was caused by the rapid and significant reduction of VPA in plasma that occurred after the start of coadministration of PAPM/BP. We previously reported that 1) this VPA-PAPM interaction also occurs in beagle dogs with increased glucuronidation of VPA; 2) the major pathway of VPA clearance is via glucuronidation (Yamamura et al., 1998); 3) PAPM also enhances the hepatic glucuronidation of VPA in rats (Yamamura et al., 1999a); and 4) BP is not involved in this drug interaction in both dogs and rats.

Glucuronidation reactions in liver cells are subject to various rate-limiting factors such as the substrate availability in the liver, UDP-glucuronic acid (UDPGA) levels, NAD/NADH redox state, carbohydrate reserve, and the activity of UDP-glucuronosyltransferases (UGTs) (Shipley and Weiner, 1987). Of these factors, the NAD/NADH redox state and carbohydrate reserve are related to the UDPGA levels. Therefore, the rate-limiting factors can roughly be classified as the hepatic availability of substrate, the UDPGA level, and the UGT activity. However, increased hepatic availability of the substrate VPA by PAPM can be disregarded as the causal factor for the VPA/PAPM interaction because the uptake of VPA by hepatocytes is mediated by passive diffusion (Booth et al., 1996) and is unaffected by PAPM (Yamamura et al., 1999a). Thus, the present study was designed to examine the effects of PAPM on hepatic UDPGA levels and UGT activity as the causal factor for this drug interaction.

Experimental Procedures

Materials. Male, Sprague-Dawley rats (Charles River Japan Inc., Ibaragi, Japan) were kept under controlled conditions of temperature, humidity, and ventilation and allowed free access to laboratory food and water. The body weight of the rats used for the experiments ranged from 200 to 350 g.

Sodium valproate, p-nitrophenol (pNP), and p-nitrophenyl glucuronide (pNP-Glu) were purchased from Sigma Chemical Co. (St. Louis, MO). 14C-Labeled sodium valproate (specific activity: 55 mCi/mmol; chemical purity: 99%) was obtained from American Radiolabeled Chemicals Inc. (St. Louis, MO). PAPM was synthesized in our laboratories (Miyadera et al., 1991). Silica gel plates (Art. 5715) for thin-layer chromatography (TLC) were purchased from E. Merck (Darmstadt, Germany). Other chemicals used were of commercially available, reagent grade.

Preparation of Liver Microsomes. Rats (n = 4) were lightly anesthetized with diethyl ether, and the femoral vein was cannulated with polyethylene tubing (PE-50, Becton Dickinson and Company, Sparks, MD). An initial bolus loading dose of 10 mg/kg PAPM followed by a constant infusion of 1.43 mg/min/kg PAPM was given to the rats, after recovery from the anesthesia, through the femoral cannula via an infusion pump (Pump 11, Harvard Apparatus, Inc., South Natick, MA). The maintenance dose of PAPM was expected to produce a steady-state plasma level of about 50 μg/ml (Yamamura et al., 1999a). Control rats (n = 6) were cannulated in the same manner as described above and were given 0.9% NaCl solution at an infusion rate of 1.2 ml/h. Thirty minutes after starting the PAPM treatment, the liver was excised rapidly after decapitating the animals and homogenized in four volumes of 10 mM phosphate buffer (pH 7.4) containing 1.15% KCl. Heparin was added to prevent clotting.

Effect of PAPM on UGT Activity toward VPA in Vivo and in Vitro;
Preparation of Liver Microsomes. Rats (n = 4) were lightly anesthetized with diethyl ether, and the femoral vein was cannulated with polyethylene tubing (PE-50, Becton Dickinson and Company, Sparks, MD). An initial bolus loading dose of 10 mg/kg PAPM followed by a constant infusion of 1.43 mg/min/kg PAPM was given to the rats, after recovery from the anesthesia, through the femoral cannula via an infusion pump (Pump 11, Harvard Apparatus, Inc., South Natick, MA). The maintenance dose of PAPM was expected to produce a steady-state plasma level of about 50 μg/ml (Yamamura et al., 1999a). Control rats (n = 6) were cannulated in the same manner as described above and were given 0.9% NaCl solution at an infusion rate of 1.2 ml/h. Thirty minutes after starting the PAPM treatment, the liver was excised rapidly after decapitating the animals and homogenized in four volumes of 10 mM phosphate buffer (pH 7.4) containing 1.15% KCl. Heparin was added to prevent clotting.

Measurement of UGT Activity toward VPA. The incubation mixture (final volume of 0.5 ml) contained microsomes (1 mg of protein), 0.05% Triton X-100, 0.25% pNP-Glu, 1 μM sodium valproate, 2 mM UDP-glucuronic acid, 2 mM NAD, 2 mM NADP, 2 μM α-naphthoflavone, 2 μM p-nitrophenol, 0.5 mM 3-methylcholanthrene, 10 μM progesterone, and 100 μM testosterone. The reaction was initiated by the addition of the microsomal protein and proceeded for 0.5 h. The reaction was terminated by the addition of 0.5 ml of ZnSO4 (final concentration: 10 mM). The reaction mixture was cooled to 0°C and the extent of glucuronidation was determined by high-performance liquid chromatography (Kawamura et al., 1998). Glucuronides were detected at 214 nm.

Acknowledgments

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

Send reprint requests to: Naotoshi Yamamura, Drug Metabolism and Pharmacokinetics Research Laboratories, Sankyo Co., Ltd., 2-58, Hiromachi 1-chome Shinagawa-ku, Tokyo 140-8710, Japan. E-mail: yamamu@shina.sankyo.co.jp
To investigate the in vitro effect of PAPM on UGT activity, PAPM was added at a range of concentrations (2, 20, and 200 µM/ml) to the incubation mixture (n = 4). This mixture was preincubated for 10 min at 37°C in a shaking water bath, and the reaction was started by the addition of [14C]VPA (37 kBq, 0.1, 0.25, 0.40, 0.67, 5, and 10 nM). The reaction rates were linear up to an incubation time of 45 min and at a microsomal protein content of at least 2 mg/ml. Thirty minutes after the addition of [14C]VPA, the reaction was stopped by adding an equal volume of ethanol to the incubation mixture. The mixture was centrifuged, and about 20 µl of the supernatant was subjected to silica gel TLC (developing solvent: n-butanol/acidic acid/distilled water = 4:1:1). Valproate glucuronide (VPA-Glu), as an authentic standard, was synthesized according to a previous report (Yamamura et al., 1999b). The radioactivity of VPA-Glu was quantified using a Bio-Imaging Analyzer (BAS-2000, Fuji Photo Film Co., Ltd., Tokyo, Japan). The UGT activity was represented as the amount of VPA-Glu produced per minute per milligram of microsomal protein (nmol/min/mg of protein).

Effect of PAPM on Hepatic UDPGA Levels in Vivo. Rats were treated with 0.9% NaCl (n = 5) or PAPM (n = 6) in the same manner as described in the preceding section, and the livers were collected after 30 min. Liver sample preparation and measurement of UDPGA were performed according to the method of Watkins et al. with a slight modification (Watkins and Klaassen, 1982). p-Nitrophenol was used as the UDG-glucuronide acceptor substrate, and the liver microsomes were used as the enzyme source. Extracts of liver with water were used to measure the concentration of UDPGA by an enzymatic assay based on the formation of pNP-Glu from pNP and UDPGA present in the liver extracts. The incubation mixture for this assay (final volume of 0.5 ml) contained 1 mM pNP, 0.5 mg/ml of washed rat liver microsomes (as a source of UGT), 0.05% Triton X-100, 50 mM Tris-HCl buffer (pH 7.4), 50 mM MgCl₂, and 0.2 ml of liver extract. The calibration curve for UDPGA was prepared by adding known amounts of UDPGA instead of the liver extract to the incubation mixture. Blanks had the same composition except that the liver extract was replaced with water. The reaction was started by the addition of the liver extract or known amounts of UDPGA. Incubation was carried out at 37°C for 30 min with gentle shaking and stopped by the addition of 0.5 ml of methanol. To measure the UDP-Glu, a 50-µl aliquot of the supernatant was subjected to TLC under the following conditions: TLC plate: silica gel TLC (developing solvent: n-butanol/acidic acid/distilled water = 4:1:1). Valproate glucuronide (VPA-Glu), as an authentic standard, was synthesized according to a previous report (Yamamura et al., 1999b). The radioactivity of [14C]VPA-Glu was quantified using a Bio-Imaging Analyzer (BAS-2000, Fuji Photo Film Co., Ltd., Tokyo, Japan). The GGT activity was represented as the amount of VPA-Glu produced per minute per milligram of microsomal protein (nmol/min/mg of protein).

Affinity of UDPGA for UDP-Glucuronosyltransferase Using VPA as Substrate. To estimate the Km value of UDPGA in VPA glucuronidation, the reaction was carried out at a constant concentration of VPA (0.5 mM) and different concentrations of UDPGA. The components of the incubation mixture were the same as those described above. The liver microsomes were obtained from three rats. [14C]VPA was added at a final concentration of 0.5 mM (37 kBq). The reaction was started by the addition of UDPGA at concentrations of 0.1, 0.2, 0.4, 0.8, 1.6, and 3.2 mM and stopped by the addition of 0.5 ml of methanol after incubation at 37°C for 15 min. After centrifugation, a 20-µl aliquot of the supernatant was subjected to TLC under the same conditions as described in the preceding section. The Km of UGT for UDPGA using VPA as the substrate was calculated by fitting the assay data to the Michaelis-Menten equation.

Statistical Methods. All results are shown as the mean ± standard deviation (S.D.). The enzyme activity or VPA-Glu formation rate was fitted to the Michaelis-Menten equation using the nonlinear least-squares program WinNonlin Professional (version 1.5, Scientific Consulting, Inc., Lexington, KY) to calculate the kinetic parameters. Statistical differences in the enzyme kinetic parameters between the control groups and PAPM-treated groups were tested using Student’s t test, and the level of significance was taken as P < .05.

Results and Discussion

Effect of PAPM on UGT Activity toward VPA in Vivo. p-Nitrophenol was used as the UDG-glucuronide acceptor substrate, and the liver microsomes were used as the enzyme source. Extracts of liver with water were used to measure the concentration of UDPGA by an enzymatic assay based on the formation of pNP-Glu from pNP and UDPGA present in the liver extracts. The incubation mixture for this assay (final volume of 0.5 ml) contained 1 mM pNP, 0.5 mg/ml of washed rat liver microsomes (as a source of UGT), 0.05% Triton X-100, 50 mM Tris-HCl buffer (pH 7.4), 50 mM MgCl₂, and 0.2 ml of liver extract. The calibration curve for UDPGA was prepared by adding known amounts of UDPGA instead of the liver extract to the incubation mixture. Blanks had the same composition except that the liver extract was replaced with water. The reaction was started by the addition of the liver extract or known amounts of UDPGA. Incubation was carried out at 37°C for 30 min with gentle shaking and stopped by the addition of 0.5 ml of methanol. To measure the UDP-Glu, a 50-µl aliquot of the supernatant was subjected to TLC under the following conditions: TLC plate: silica gel TLC (developing solvent: n-butanol/acidic acid/distilled water = 4:1:1). Valproate glucuronide (VPA-Glu), as an authentic standard, was synthesized according to a previous report (Yamamura et al., 1999b). The radioactivity of [14C]VPA-Glu was quantified using a Bio-Imaging Analyzer (BAS-2000, Fuji Photo Film Co., Ltd., Tokyo, Japan). The UGT activity was represented as the amount of VPA-Glu produced per minute per milligram of microsomal protein (nmol/min/mg of protein).

Effect of PAPM on Hepatic UDPGA Levels in Vivo. The mean correlation coefficient of the standard curves for UDPGA was 0.997 ± 0.002 in four separate experiments. The assay was linear from 30 to 500 nmol of UDPGA in the assay mixture. Hepatic UDPGA levels in the rats treated with PAPM by infusion for 30 min were significantly higher (P < .05), about 1.7-fold, than those in the control rats (75.5 ± 92.3 nmol/g of liver in PAPM-treated rats, n = 6 versus 43.4 ± 65.5 nmol/g of liver in control rats, n = 5).

Affinity of UDP-Glucuronosyltransferase for UDPGA Using VPA as Substrate. The VPA-Glu formation rate in vitro seemed to increase with increasing UDPGA levels up to 0.8 mM as shown in Fig. 1. As the hepatic UDPGA level under normal conditions was about 0.4 mM (434.5 nmol/g of liver), the rise in UDPGA of about 1.7-fold (755.2 nmol/g of liver) produced by PAPM treatment directly caused an increase in VPA-Glu formation. This is consistent with our previous results whereby PAPM increased the apparent metabolic clearance of VPA through glucuronidation by 1.8-fold when the plasma VPA level had reached steady state (Yamamura et al., 1999a).

In conclusion, PAPM given intravenously to rats at a clinically relevant dose increased the hepatic UDPGA level by 1.7-fold. PAPM treatment had no effect on the activity of UGT both in vitro and in vivo. Therefore, the rise in UDPGA in the liver due to PAPM directly causes an increase in VPA-Glu formation that leads to the reduced plasma levels of VPA in rats.

The mechanism for the rapid increase in UDPGA in the liver in response to PAPM-treatment is currently unknown. However, our preliminary results indicate that the PAPM treatment increases hepatic glycogenolysis, which might lead to an increased production of UDPGA.

### Table 1

<table>
<thead>
<tr>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Vmax (nmol/min/mg protein)</td>
</tr>
<tr>
<td>Km</td>
<td>Km (mM)</td>
</tr>
<tr>
<td>V&lt;sub&gt;max&lt;/sub&gt;/K&lt;sub&gt;m&lt;/sub&gt;</td>
<td>Vmax/Km (nmol/min/mg protein)</td>
</tr>
</tbody>
</table>

**Fig. 1.** Effects of UDPGA on the VPA-Glu formation rate in vitro.

Downloaded from dmd.aspetjournals.org on June 20, 2017
References


