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

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

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

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/BP), 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. Hepatic microsomes were prepared by centrifugation of the liver homogenate at 9,000 rpm for 20 min at 4°C followed by ultracentrifugation at 105,000 rpm for 60 min at 4°C. The microsomal pellet was washed and then suspended by homogenization in the above buffer containing 30% glycerol and stored frozen at −80°C until use. The protein concentration of the microsomal preparations was determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

**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, NADP (10 µM), glucose-6-phosphate (3.0 mM), fructose-6-phosphate (3.0 mM), phosphoenolpyruvate (10 mM), pyruvate kinase (1.0 U/ml), glyceraldehyde-3-phosphate dehydrogenase (1.0 U/ml), NADPH (0.5 mM), and sodium valproate (1.0 mM) in a total volume of 125 µl. After incubation at 37°C for 1 h, the reaction was stopped by the addition of 1 ml of ice-cold ether, and the upper phase was used for TLC or liquid chromatography.
X-100, 50 mM Tris-HCl buffer (pH 7.4), 10 mM UDPGA, and 50 mM MgCl₂. 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 [5-¹⁴C]UDP (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 [5-¹⁴C]UDP, the reaction was stopped by adding an equal volume of ethanol to the incubation mixture. The reaction mixture was centrifuged, and about 20 μl of the supernatant was subjected to silica gel TLC (developing solvent: n-butanol/acetic 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 [5-¹⁴C]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.** 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 UDPGA-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 pNP-Glu, a 50-ml aliquot of the supernatant was subjected to TLC (developing solvent: n-butanol/acetic 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 [5-¹⁴C]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.** In vivo UGT activity toward VPA (10 mM) did not change at all after treatment of rats with PAPM compared with the control (12.0 ± 1.7 nmol/min/mg of protein in PAPM-treated rats, n = 4 versus 12.4 ± 6.3 nmol/min/mg of protein in control rats, n = 6). Also, in vitro, the kinetic parameters (Vₘₐₓ and Kₘ) of VPA glucuronidation were not affected by adding PAPM at increasing concentrations from 2 to 200 μM/ml as shown in Table 1. Therefore, it is clear that PAPM causes neither enzyme induction nor allosteric activation of UGT.

**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 (755.2 ± 92.3 nmol/g of liver in PAPM-treated rats, n = 6 versus 434.5 ± 65.5 nmol 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.

**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 and in Vitro.** In vivo UGT activity toward VPA (10 mM) did not change at all after treatment of rats with PAPM compared with the control (12.0 ± 1.7 nmol/min/mg of protein in PAPM-treated rats, n = 4 versus 12.4 ± 6.3 nmol/min/mg of protein in control rats, n = 6). Also, in vitro, the kinetic parameters (Vₘₐₓ and Kₘ) of VPA glucuronidation were not affected by adding PAPM at increasing concentrations from 2 to 200 μM/ml as shown in Table 1. Therefore, it is clear that PAPM causes neither enzyme induction nor allosteric activation of UGT.

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**TABLE 1**

<table>
<thead>
<tr>
<th>UDPGA formation rate</th>
<th>Values represent the mean ± S.D. of four samples of liver microsomes.</th>
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</thead>
<tbody>
<tr>
<td>nM/nm/mg protein</td>
<td>nM/nm/mg protein</td>
</tr>
<tr>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>26.36 ± 3.23</td>
<td>1.90 ± 0.31</td>
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<tr>
<td>1.39 ± 0.7</td>
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<tr>
<td>The PAPM added</td>
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<tr>
<td>27.39 ± 1.56</td>
<td>2.15 ± 0.40</td>
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<tr>
<td>13.1 ± 2.3</td>
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<tr>
<td>200 μg/ml</td>
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<tr>
<td>30.53 ± 3.37</td>
<td>2.56 ± 0.38</td>
</tr>
<tr>
<td>12.0 ± 0.9</td>
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<tr>
<td>200 μg/ml</td>
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<tr>
<td>26.69 ± 3.53</td>
<td>2.17 ± 0.65</td>
</tr>
<tr>
<td>12.7 ± 2.1</td>
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</tbody>
</table>

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


