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Note

Carbapenem antibiotics inhibit valproic acid transport in Caco-2 cell monolayers

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Abstract

The concomitant use of carbapenem antibiotics with valproic acid has been prohibited because carbapenems induced a decrease in plasma concentration of valproic acid in epileptic patients during valproic acid therapy. Our previous in vivo study in rats proposed that inhibition by carbapenem of the intestinal absorption of valproic acid might be a possible mechanism for the drug–drug interaction. To demonstrate the hypothesis, we examined the effects of imipenem and panipenem on intestinal transepithelial transport of valproic acid using Caco-2 cell monolayers. Imipenem and panipenem inhibited the transport of [^{14}C]–valproic acid across the Caco-2 cell monolayers from apical-to-basolateral side in a concentration-dependent manner, although they had no effect on the uptake of [^{14}C]–valproic acid by Caco-2 cells. The inhibition by the carbapenems of the valproic acid transport was found even when they were added to only the basolateral side. From these results, the carbapenems may inhibit the absorption of valproic acid at the basolateral membrane of intestinal epithelial cells, which contributes to the decrease in plasma concentration of valproic acid after oral administration.

Keywords: Valproic acid; Imipenem; Panipenem; Caco-2 cell monolayer

Valproic acid, an anticonvulsant drug, is widely used for treatment of various forms of epilepsy. In pharmacotherapy with valproic acid it is important to maintain its plasma concentration in the therapeutic range of 50–150 μg/ml (Covain et al., 1982). Recently it was reported that coadministration of a carbapenem antibiotic, panipenem or meropenem, induced a reduction of plasma concentration of valproic acid in epileptic patients undergoing valproic acid therapy, resulting in the recurrence of epileptic seizures (Nagai et al., 1997; De Turck et al., 1998). Therefore, the concomitant use of carbapenems with valproic acid has

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been prohibited (Ministry of Health and Welfare, Japan, 1996). However, the mechanism of the pharmacokinetic interaction between them has not been clarified.

Our previous study demonstrated that pretreatment with intravenous injection of imipenem or panipenem induced a decrease in the plasma concentrations of valproic acid when valproic acid was orally, but not intravenously, administered in rats (Torii et al., 2001). These findings lead to the hypothesis that carbapenems may inhibit the intestinal absorption of valproic acid, which contributes to the decrease in plasma concentration of valproic acid after oral administration. The in situ intestinal absorption study provided us with supporting evidence that the absorption of valproic acid from the luminal to the vascular perfusate was decreased when imipenem was perfused in the vasculature. To confirm the hypothesis, this study was undertaken to examine the effects of imipenem and panipenem on valproic acid transport across intestinal epithelial cells using monolayers of human adenocarcinoma cell line, Caco-2 cells. Since Caco-2 cells have been shown to have the function of the small intestine, this is a useful model for studying intestinal transepithelial transport (Hidalgo et al., 1989).

Caco-2 cells (American Type Culture Collection, Rockville, MD) were cultured at 37 °C in Eagle's minimum essential medium containing $NaHCO_3$, 1% glutamin, 1% peniciline G-streptomycin, 1% amphotericine B, 1% non-essential amino acids and 10% fetal bovine serum in an atmosphere of 5% CO_2 and 95% humidity. Cells grown were passaged every 5 days and confluency was reached within 7 days after passage. For experiments, cells between passages 13 and 15 were seeded in transwell inserts coated with fibrillar collagen and cultured for 5 days using a BIOCOAT™ HTS Caco-2 Assay System (Becton Dickinson, Franklin Lakes, NJ).

On the day of experiment, the culture medium was replaced with 0.4 ml Hank's balanced salts solution containing 25 mM glucose and 10 mM 2-morpholinoethanesulfonic acid (pH 6.0) in the apical side and 1.2 ml Hank's balanced salts solution containing 25 mM glucose and 10 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (pH 7.4) in the basolateral side, respectively. Imipenem, panipenem or 2,4-dinitrophenol was added to both sides to give a designated final concentration and incubated for 15 min at 37 °C. Then 10 μM [^{14}C]–valproic acid and 1 nM [^{3}H]-mannitol were added to the apical side. After an incubation at 37 °C for 3 min (uptake experiment) or 60 min (transport experiment), the amounts of [^{14}C]–valproic acid accumulated in Caco-2 cells and transported to the basolateral side were determined using an Aloka LAS-3500 liquid scintillation counter. [^{14}C]–Valproic acid (2035 GBq/mol) and [^{3}H]-mannitol (740 GBq/mmol) were purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO). Imipenem/cilastatin (Banyu Pharmaceutical Co., Ltd., Tokyo, Japan) and panipenem/betamipron (Sankyo Co., Ltd., Tokyo, Japan) were used in the form of commercial preparations. Sodium valproate and 2,4-dinitrophenol were purchased from Sigma Chemicals (St. Louis, MO). All results are shown as mean ± S.D. Data were statistically analyzed by Student's t-test.

The levels of enterocyte-specific enzymes in Caco-2 cells increase during differentiation. In this study Caco-2 cells showed the activity of alkaline phosphatase over 15 mU/mg-protein, which is similar to that reported by others (Trotter et al., 1991). Monolayers in which the [^{3}H]-mannitol leakage was less than 1% of the dose were used for the experiment. [^{14}C]–Valproic acid permeated across the Caco-2 cell monolayers from apical to basolateral side. Apparent permeability coefficient (P_{app}) of valproic acid was $22.6 \pm 6.6 \times 10^{-6}$ cm/s ($n = 3$), which was calculated according to the following equation: $P_{app} = 1/A\mathrm{Ci}\cdot \mathrm{d}Q/\mathrm{d}T$, where A is the surface area of the insert, Ci is the initial concentration of valproic acid and $\mathrm{d}Q/\mathrm{d}T$ is the flux across the cell monolayers (Karlsson and Arturson, 1992).

The existence of an energy-dependent process has been reported in intestinal valproic acid absorption (Cato et al., 1995), in which proton-coupled monocarboxylic acid specific transport and anion antiport mechanisms are involved at the intestinal brush-border membrane (Tamai et al., 1997). In this study, we also found the inhibition

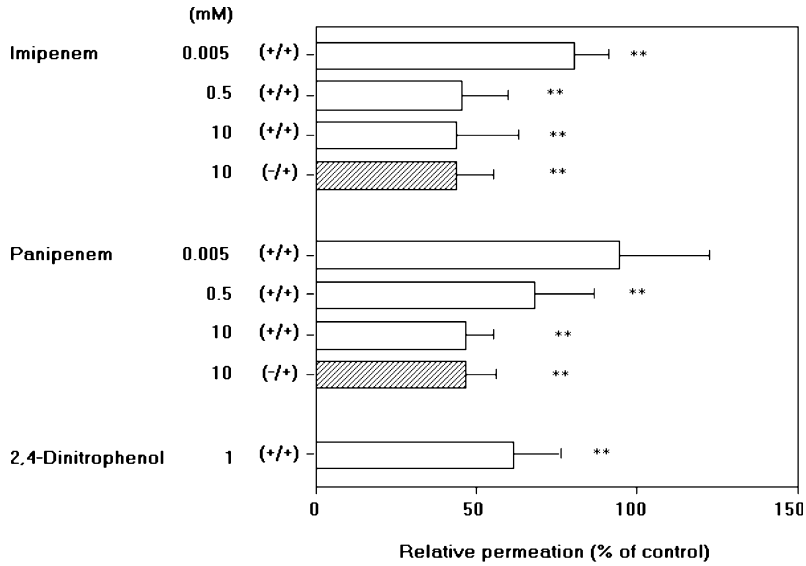

Fig. 1. Effects of imipenem, panipenem or 2,4-dinitrophenol on transport of valproic acid across Caco-2 cell monolayers. The permeation of [^{14}C]–valproic acid from apical-to-basolateral side of the Caco-2 cell monolayers was measured at 37 °C for 60 min. Test drugs were added to the apical and the basolateral sides (+/+) or only to the basolateral side (−/+) 15 min before the experiment. Each column is expressed as percentage of the control and represents the mean ± S.D. of 4–6 experiments. $^{**}P < 0.01$ versus control.

by a metabolic inhibitor 2,4-dinitrophenol (1 mM) of the transport of [^{14}C]–valproic acid across the Caco-2 cell monolayers from apical-to-basolateral side (Fig. 1).

Imipenem and panipenem at a concentration range from 5 μM to 10 mM inhibited the transport of [^{14}C]–valproic acid across the Caco-2 cell monolayers in a concentration-dependent manner (Fig. 1). They inhibited the transport of valproic acid even when they were added to only the basolateral side, to the same extent as the case in which they were added to both sides. The uptake of [^{14}C]–valproic acid by Caco-2 cells was inhibited by 2,4-dinitrophenol, but not by imipenem and panipenem even at 10 mM (Fig. 2). Therefore, considering that the carbapenems are very slightly lipophilic and are hardly absorbed (Drusano and Standiford, 1985), they may inhibit the valproic acid transport at the basolateral side. Since the transport mechanism of valproic acid at the intestinal basolateral membrane is still uncertain in comparison with that at the intestinal brush-border membrane, further studies are needed to clarify the precise mechanism of the inhibitory action of the carbapenems.

The carbapenems are distributed to the small intestine after intravenous injection (Hara et al., 1985; Takahagi et al., 1991). We have reported that in the in situ intestinal perfusion study the

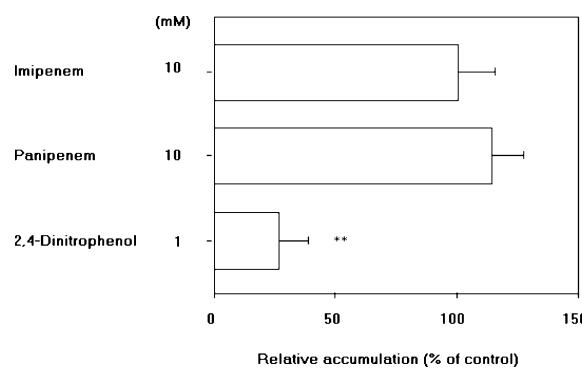

Fig. 2. Effects of imipenem, panipenem or 2,4-dinitrophenol on uptake of valproic acid by Caco-2 cells. The accumulation of [^{14}C]–valproic acid in Caco-2 cells was measured at 37 °C for 3 min. Test drugs were added to the apical and the basolateral sides 15 min before the experiment. Each column is expressed as percentage of the control and represents the mean ± S.D. of 6 experiments. $^{**}P < 0.01$ versus control.

valproic acid absorption from the luminal to the vascular perfusate was inhibited when 0.5 mM imipenem was perfused in the vasculature. However, the study using the everted gut sac showed that the active transport of valproic acid was not affected by imipenem at the same concentration (Torii et al., 2001). Perhaps, the lack of the effect of imipenem in the everted gut sac study may be due to that imipenem could not be delivered to the basolateral membrane of the epithelial cells in such an in vitro condition.

In conclusion, imipenem and panipenem inhibited valproic acid transport across the Caco-2 cell monolayers, probably, at the basolateral membrane. The results support the hypothesis that the carbapenems intravenously administered inhibit the intestinal absorption of valproic acid, which contributes to the decrease in plasma concentration of valproic acid after oral administration.

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