Effect of Rabeprazole on MDR1-Mediated Transport of Rhodamine 123 in Caco-2 and Hvr100-6 Cells

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The aim of our study was to investigate the effects of rabeprazole, a proton pump inhibitor, on MDR1 expressed on human colon carcinoma cell line, Caco-2, and MDR1-overexpressing human cervical carcinoma cell line, HeLa cells selected by exposure to 100 nM vinblastine (Hvr100-6 cells). Inhibitory effects of rabeprazole on MDR1-mediated transport of Rhodamine 123 were examined in these cells. A thousand micro molar rabeprazole increased Rhodamine 123 uptakes in Caco-2 and Hvr100-6 cells by 68% and 185%, respectively. No significant effects of rabeprazole were observed at the concentration of 1–100 μ M. Since rabeprazole did not show any effects on Rhodamine 123 transport *via* MDR1 at the plasma levels (approximately 1 μ M), it was considered that the drug interaction with MDR1 substrates would be minimal even though the interaction occurred in the patients with rabeprazole treatment.

Key words MDR1; inhibitor; Rhodamine 123; Caco-2 cell; Hvr100-6 cell

Rabeprazole, a proton pump inhibitor (PPI) for acid related diseases, possesses suppressive activity on gastric acid secretion by inhibiting H^+, K^+ -ATPase.^{1,2)} It has been reported that metabolism of rabeprazole is less dependent on cytochrome P-450 (CYP) systems, CYP2C19, compared with other PPIs, omeprazole and lansoprazole.^{2,3)}

Omeprazole, lansoprazole and pantoprazole, were currently found to inhibit the ATP-dependent efflux transporter, MDR1, which is located mainly in intestine, where it functions as drug efflux pump.⁴⁾ These PPIs might enhance absorption of the MDR1 substrates, such as digoxin, tacrolimus, and clarithromycin, by inhibiting MDR1-mediated transport. It has been speculated that coadministration of omeprazole elevated digoxin plasma levels *via* inhibiting MDR1-mediated intestinal efflux-transport.^{4,5)} It is unknown so far whether rabeprazole is an inhibitor of MDR1 as well as omeprazole and lansoprazole.

The aim of our study, therefore, was to characterize rabeprazole as a MDR1 inhibitor by using human colon carcinoma cell line, Caco-2, and MDR1-overexpressing human cervical carcinoma cell line, HeLa-Ohio (HeLa) cells selected by exposure to 100 nM vinblastine (Hvr100-6 cells). Effect of rabeprazole on MDR1-mediated transport was assessed by using Rhodamine 123, a typical MDR1 substrate.

MATERIALS AND METHODS

Chemicals Rabeprazole was kindly given from Eisai (Tokyo, Japan). Rhodamine 123 was purchased from Molecular Probes, Inc. (Eugene, OR, U.S.A.). All other reagents were obtained commercially and were of analytical grade requiring no further purification.

Cells and Cell Culture The human colon carcinoma cell line Caco-2 was obtained from American Type Culture

Collection (Rockville, MD, U.S.A.). Caco-2 cells (47–52 passage) were maintained in culture medium consisting of Dulbecco's modified Eagle's medium (D-MEM; Cat. No. 12800-017, Invitrogen Corp., Carlsbad, CA, U.S.A.) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Lot. No. 41K2300, Sigma-Aldrich Corp., St. Louis, MO, U.S.A.), 100 U/ml penicillin G, 100 μ g/l streptomycin sulfate, and 0.1 mM non-essential amino acids (Invitrogen). Cells (2×10^4 cells/cm²) were seeded on plastic culture dishes (100 mm in diameter), grown in humidified atmosphere of 5% CO₂–95% air at 37 °C, and subcultured with 0.05% trypsin–0.02% EDTA (Invitrogen). The human cervical carcinoma cell line HeLa was obtained from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). HeLa cells (396–401 passage) were maintained in a culture medium consisting of D-MEM supplemented with 10% heat-inactivated FBS (Lot. No. 41K2300, Sigma-Aldrich) and 100 mg/l of kanamycin sulfate (Invitrogen). A vinblastine-resistant subline of HeLa, Hvr100-6, was established by stepwise increases of the concentration of vinblastine sulfate in the culture medium. Hvr100-6 cells (82–87 passage) were derived and maintained with 100 nM vinblastine sulfate. These cell lines were seeded into culture flasks (Corning-Costar Corp., Cambridge, MA, U.S.A.) at a density of 4 and 12×10^4 cells/cm², respectively, grown in a humidified atmosphere of 5% CO₂–95% air at 37 °C, and subcultured every 3 or 4 d with 0.05% trypsin–0.02% EDTA.

Uptake of Rhodamine 123 in Caco-2, HeLa and Hvr100-6 Cells Uptake of Rhodamine 123 was determined as described previously.^{6–8)} In the uptake experiments, cells (2×10^5 cells) were seeded on 24-well plates (Cat. No., 3526, Corning Costar) in 1 ml/well of culture medium, and incubated for 48 h in a humidified atmosphere of 5% CO₂–95% air at 37 °C. After incubation, cells were washed three times

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with warmed Hanks' balanced salt solution (HBSS; 137 mM NaCl, 5.4 mM KCl, 1.3 mM CaCl_2 , 0.4 mM MgSO_4 , 0.5 mM MgCl_2 , 0.3 mM Na_2HPO_4 , 0.4 mM KH_2PO_4 , 4.2 mM NaHCO_3 , 5.6 mM Glucose, 0.06 mM phenol red and 25 mM HEPES), and the uptake experiments were started by addition of fresh HBSS containing 10 μM Rhodamine 123, without or with 1, 10, 100 and 1000 μM rabeprazole and further incubated for 60 min at 37 °C. Uptake experiments were stopped by aspiration of HBSS from the well, followed by washing three times with ice-cold phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4 , 8.1 mM NaHPO_4). After stopping the uptake experiments, cells were solubilized with 1 ml of 0.3 M NaOH, and the aliquots (500 μl) were neutralized with 500 μl of 0.3 M HCl. Aliquots (200 μl) of the neutralized cell lysate solution were transferred to 96-well black plates (Cat. No. 3915, Corning Costar) and the fluorescence intensity of Rhodamine 123 was measured with an excitation wavelength of 485 nm and emission wavelength of 535 nm using the Spectra Fluor (Tecan Switzerland, Switzerland). Protein content was determined by the Lowry method,⁹⁾ and bovine serum albumin was used as the standard.

RESULTS

The uptake of Rhodamine 123 in Caco-2 cells was increased by co-existence with 1000 μM rabeprazole (68%), although no remarkable effects were observed in the presence of 1–100 μM rabeprazole (Fig. 1). Similar results were observed in Hvr100-6 cells (Fig. 2). A thousand micro molar rabeprazole increased the uptake of Rhodamine 123 by 185%, though 1–100 μM rabeprazole showed no remarkable effects. Co-existence with 10 μM cyclosporine increased Rhodamine 123 uptakes in Caco-2 and Hvr100-6 cells by 20% and 420%, respectively.

DISCUSSION

Both cells, Caco-2 and Hvr100-6, in the presence of 1000 μM rabeprazole increased the uptake of Rhodamine 123, suggested that rabeprazole has inhibitory effects on MDR1-mediated Rhodamine 123 transport. The uptakes of Rhodamine 123, however, were not affected by co-existence with 1–100 μM rabeprazole. This means that rabeprazole does not show inhibitory effects on MDR1-mediated Rhodamine 123 transport in the range of plasma concentration of rabeprazole (1.0–1.3 μM) after 20 mg oral administration.³⁾ Since the value of 1000 μM for rabeprazole is extremely higher than their plasma concentration, it is speculated that circulating rabeprazole does not affect MDR1 in patients receiving rabeprazole. It also must be noted that the inhibitory effect of 1000 μM rabeprazole may be derived from decreased MDR1 activity via the direct or indirect effects on the cells, such as depletion of intracellular ATP.

Pauli-Magnus *et al.* reported that MDR1-mediated digoxin transport in Caco-2 cells was inhibited by omeprazole, pantoprazole and lansoprazole with IC_{50} values of 17.7–62.8 μM , respectively, which were close to plasma levels (0.7–4.2 μM) in patients receiving these PPIs.^{3,4)} IC_{50} /plasma concentration ratio calculated from previous study were 5.7–24.2 and 15.0–27.3 for omeprazole and lansoprazole, respectively, which were extremely low compared with that of present

Fig. 1. Effects of Rabeprazole on Rhodamine 123 Uptake in Caco-2 Cells

Cells were incubated with HBSS containing 10 μM Rhodamine 123 without or with the indicated concentrations of rabeprazole for 60 min at 37 °C. Each bar represents the mean $\pm$ S.E. ($n=3$ or 4). CsA: cyclosporine.

Fig. 2. Effects of Rabeprazole on Rhodamine 123 Uptake in HeLa and Hvr100-6 Cells

Cells were incubated with HBSS containing 10 μM Rhodamine 123 without or with the indicated concentrations of rabeprazole for 60 min at 37 °C. Each bar represents the mean $\pm$ S.E. ($n=4$). CsA: cyclosporine.

rabeprazole study (IC_{50} /plasma concentration ratio for rabeprazole is >100), though the substrate of MDR1 was different between previous digoxin and present Rhodamine 123. These observations supported drug interaction between omeprazole and digoxin in the previous clinical case report.⁵⁾ Coadministration of omeprazole increased digoxin AUC by 30% in two of the ten tested subjects, whereas coadministration of rabeprazole required no dose adjustment for digoxin.¹⁰⁾ Similar inhibitory effects of PPIs on MDR1-mediated transport may also explain the drug-interaction between tacrolimus and PPIs in our previous case report, where the blood concentration of tacrolimus was dramatically increased by co-administration of lansoprazole but not rabeprazole.¹¹⁾

The metabolic profile of rabeprazole also differs somewhat from other PPIs, because rabeprazole has shown a lesser contribution of CYP2C19 to the overall metabolism than omeprazole or lansoprazole. Thus, pharmacokinetic characterization of rabeprazole would be less affected by CYP2C19 genotype status as well as MDR1-mediated drug transport system. Taken together, the risk for drug–drug interaction of rabeprazole via MDR1 and CYP systems is lower than those of other PPIs.

In conclusion, rabeprazole has an inhibitory effect on MDR1-mediated Rhodamine 123 uptake at 1000 μM , which is extremely higher than plasma concentration under clinical

application. It, therefore, was considered that the drug interaction with MDR1 substrates would be minimal even though the interaction occurred in the patients with rabeprazole treatment.

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