0026-895X/05/6703-923–928$20.00
MOLECULAR PHARMACOLOGY
Copyright © 2005 The American Society for Pharmacology and Experimental Therapeutics
Mol Pharmacol 67:923–928, 2005

Vol. 67, No. 3
7393/1196084
Printed in U.S.A.

Role of Breast Cancer Resistance Protein (Bcrp1/Abcg2) in the Extrusion of Glucuronide and Sulfate Conjugates from Enterocytes to Intestinal Lumen

Yasuhisa Adachi, Hiroshi Suzuki, Alfred H. Schinkel, and Yuichi Sugiyama

School of Pharmaceutical Sciences, the University of Tokyo, Tokyo, Japan; and Division of Experimental Therapy, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Received September 16, 2004; accepted December 14, 2004

ABSTRACT

The purpose of this study is to examine the significance of efflux transporters in the small intestine to extrude glucuronide (G) and sulfate (S) conjugates into the intestinal lumen. From this standpoint, we performed in situ intestinal perfusion experiments by using Eisai hyperbilirubinemic rats (EHBRs) in which the multidrug resistance protein 2 (Mrp2/Abcc2) is hereditarily defective and breast cancer resistance protein (Bcrp1/Abcg2) knockout mice. The intestinal lumen of EHBRs and Bcrp1 (−/−) mice was perfused with medium containing 4-methylumbelliferone (4MU) and E3040 [6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridilmethyl) benzothiazole] to determine the efflux of metabolites into the outflow. The efflux of E3040-glucuronide (G) in EHBRs was significantly lower compared with that in normal rats. However, no significant difference was observed for the efflux of 4MU-G, 4MU-sulfate (S), and E3040-S between EHBRs and normal rats. In contrast, the efflux of intracellularly formed 4MU-G, 4MU-S, and E3040-G in Bcrp1 (−/−) mice was significantly lower than that in normal mice. Therefore, Bcrp1 has an important role in extruding glucuronide and sulfate conjugates formed in enterocytes into the intestinal lumen, whereas Mrp2 is responsible for the efflux of some glucuronide conjugates.

It has been recognized that metabolism and active efflux in the small intestine act synergistically to reduce the oral bioavailability of substrate drugs. For example, cytochrome P450 CYP3A4 (Prueksaritanont et al., 1996; Zhang et al., 1999; Doherty and Charman, 2002; Kaminsky and Zhang, 2003) and P-glycoprotein/MDR1/ABCB1 (Wacher et al., 1998; Benet et al., 1999; Suzuki and Sugiyama, 2000; Kusuhara and Sugiyama, 2002) are expressed in the enterocytes and play an important role in reducing the drug absorption because of their similar substrate specificity. In addition, the synergistic action of conjugation and cellular extrusion into the intestinal lumen is also highlighted as a possible mechanism to account for the lower oral bioavailability of substrate drugs; substrate drugs are metabolized to their conjugates in enterocytes and then excreted into the lumen via efflux transporters (Suzuki and Sugiyama, 2000; Kusuhara and Sugiyama, 2002). Among the conjugating enzymes, it is known that UDP-glucuronosyl transferases and sulfotransferases are highly expressed in the gastrointestinal tract (Doherty and Charman, 2002; Kaminsky and Zhang, 2003). For the extrusion of glucuronide and sulfate conjugates formed in enterocytes, possible candidates are multidrug resistance protein 2 (MRP2/ABCC2) and/or breast cancer resistance protein (BCRP/ABCG2) (Suzuki and Sugiyama, 2000; Kusuhara and Sugiyama, 2002). In the human intestine, reverse transcription-polymerase chain reaction analysis revealed that MRP2 is more highly expressed in enterocytes than MDR1, an efflux transporter for neutral and cationic compounds (Taipalensuu et al., 2001).

The purpose of the present study is to examine the role of the efflux transporters Mrp2 and Bcrp1 in the small intestine in the extrusion of glucuronide and sulfate conjugates into the intestinal lumen. From this standpoint, we performed in situ intestinal perfusional experiments in Eisai hyperbilirubinemic rats (EHBRs), Bcrp1 (−/−) mice, and corresponding normal rats and mice, respectively. As test substances, we chose 4-methyl-umbelliferone (4MU) and E3040 which are

This work was supported by Grant-in-Aid 12144201 for Scientific Research on Priority Areas epithelial vectorial transport from the Ministry of Education, Science and Culture of Japan.
Article, publication date, and citation information can be found at http://molpharm.aspetjournals.org.
doi:10.1124/mol.104.007393.

ABBREVIATIONS: MDR (Mdr), multidrug resistance; MRP (Mrp), multidrug resistance associated protein; BCRP (Bcrp), breast cancer resistant protein; EHBR, Eisai hyperbilirubinemic rat; E3040, 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridilmethyl) benzothiazole; 4MU, 4-methylumbelliferone; -G, -glucuronide; -S, -sulfate; IS, internal standard; GF120918, *N*-(4-[2-(1,2,3,4-tetrahydro-6,7-dimethoxy-2-isoquinolinyl)ethyl]-phenyl)-9,10-dihydro-5-methoxy-9-oxo-4-acridine carboxamide.

metabolized in enterocytes by UDP-glucuronosyl transferases and sulfotransferases to yield conjugated metabolites (Mulder et al., 1985; Zimmerman et al., 1991; Takenaka et al., 1995a,b; Chen and Pang, 1997).

Materials and Methods

Chemicals. 4MU, 4MU-G, and 4-MU-S were purchased from Sigma-Aldrich (St. Louis, MO). E3040, E3040-G, and E3040-S were kindly donated by Eisai Co., Ltd. (Tsukuba, Japan). All other chemicals were products of Sigma-Aldrich.

Animals. EHBRs and normal rats were purchased from Clea Japan (Tokyo, Japan). The rats were housed in groups (3–5/cage) under controlled conditions (23°C, 55% air humidity, 12-h light cycle). Bcrp1 (−/−) mice were prepared as described previously (Jonker et al., 2002). FVB mice (control mice) were purchased from Clea Japan. All mice were housed individually in cages with paper bedding (α-dry, Shepherd Specialty Papers, Inc., Kalamazoo, MI) under controlled conditions as described above. The rats and mice were acclimatized for at least 1 week before carrying out experiments and had unrestricted access to water and rodent pellet food (MF; Oriental Yeast Co. Ltd., Tokyo, Japan).

Jejunum Perfusion Experiments. The perfusion experiments were performed according to methods described previously (Loria et al., 1976; Yuasa et al., 1993; Barthe et al., 1999; Adachi et al., 2003). Rats (240–280 g) and mice (23–28 g) were fasted overnight before the perfusion experiment with access to tap water only. Anesthesia was induced by ether for rats and by an i.v. injection of Nembutal (pentobarbital sodium, 50 mg/kg) for mice. The animals were placed on a heating pad to maintain body temperature at 37°C. The abdomen was opened by a midline longitudinal incision and an 8- to 10-cm jejunal segment was isolated and cannulated at both ends with plastic tubing. The segment was rinsed with phosphate-buffered saline, pH 6.4. Saline was dropped onto the surgical area, which was then covered with a paper sheet to avoid loss of fluid. The experiment was initiated by filling the segment with a 1-ml bolus of the perfusion solution followed by perfusion at 0.2 ml/min for rats and 0.1 ml/min for mice using an infusion pump (syringe infusion pump; Harvard Apparatus Inc., Holliston, MA). The perfusion solution consisted of 20.1 mM Na_2HPO_4, 47.0 mM KH_2PO_4, and 101 mM NaCl, pH 6.4, and contained 10 μM 4MU or E3040 with a tracer concentration of ^{14}C-labeled inulin as a nonabsorbable marker (Yuasa et al., 1993; Adachi et al., 2003). The outflow perfusate was collected at 10-min intervals for 30 min. The length of the segment was measured at the end of experiments. Scintillation cocktail (Hionic-fluor; PerkinElmer Life and Analytical Sciences, Boston, MA) was added to the aliquots from the outflow specimens to measure the radioactivities in a liquid scintillation counter (model 2700 TR; PerkinElmer Life and Analytical Sciences).

Data Analysis. The absorbed fraction (F_a) of each ligand was estimated according to the following equation, which corrects for the volume change using [^{14}C]inulin as a nonabsorbable marker (Loria et al., 1976; Yuasa et al., 1993; Barthe et al., 1999; Adachi et al., 2003):

$$F_a = 1 - \frac{C_{in,I}}{C_{out,I}} \times \frac{C_{out}}{C_{in}} \quad (1)$$

where $C_{in,I}$ and $C_{out,I}$ represent the concentration of [^{14}C]inulin in the inflow and outflow solutions, respectively, and C_{in} and C_{out} represent the 4MU and E3040 concentration in the inflow and outflow solutions, respectively.

The apparent membrane permeability clearance for the unit length of intestinal segments was calculated as follows (Yuasa et al., 1993; Adachi et al., 2003):

$$CL_{a,app} = -\frac{Q}{L} \times \ln(1 - F_a) \quad (2)$$

where $CL_{a,app}$ is the apparent membrane permeability clearance, Q is the perfusion rate (0.1 and 0.2 ml/min), and L is the length of perfused segments.

The efflux rate of metabolites was calculated as follows (Chen and Pang, 1997):

$$\text{Efflux rate} = C_{out,M} \times Q \quad (3)$$

where $C_{out,M}$ is the concentration of 4MU and E3040 metabolites, and Q is the perfusion rate (0.1 and 0.2 ml/min).

Analytical Methods. For the determination of 4MU, 4MU-G, and 4MU-S, two methods were used. In the rat experiments, high-performance liquid chromatography equipped with triple stage mass spectrometer analysis was performed on a model API4000 (MDS Sciex, Concord, ON, Canada) equipped with a G1312A pump (Agilent Technologies, Palo Alto, CA). After addition of umbelliferone as internal standard (IS) solution into perfusate, the samples were injected and chromatographic separation was performed on a column (Xterra C18; 4.6 mm i.d. × 50 mm in length) (Waters, Milford, MA). Mobile phase, consisting of 0.1% acetic acid and methanol, was delivered at a flow rate of 0.5 ml/min in a gradient (methanol concentration) of 20% at 0 min, 20% at 2 min, 75% at 2.1 min, 75% at 5 min, 20% at 5.1 min, and 20% at 10 min. 4MU, 4MU-G, 4MU-S, and IS were detected as ion pairs at m/z 175.2/132.9, 351.4/175.3, 254.7/175.1, and 161.3/132.9, respectively. For mouse experiments, high-performance liquid chromatography analysis was carried out using an LC-10Avp pump equipped with an SPD-10Avp detector (Shimadzu, Kyoto, Japan). Perfusate was directly injected onto a column (Inertsil ODS-3; 4.6 mm i.d. × 150 mm long) (GL Science, Tokyo, Japan). Chromatography was achieved using a gradient from 25% (0 min) to 50% (20 min) methanol in 50 mM KH_2PO_4 at a flow rate of 1 ml/min, and UV detection was performed at 313 nm.

For the determination of E3040, E3040-G, and E3040-S, high-performance liquid chromatography equipped with triple stage mass spectrometer analysis was used. After addition of phenacetin as IS solution into perfusate the samples were injected onto the column (Xterra C18; 4.6 mm i.d. × 50 mm long) (Waters). Mobile phase, consisting of 10 mM ammonium acetate and acetonitrile, was delivered at a flow rate of 0.5 ml/min in a gradient (acetonitrile concentration) of 10% at 0 min, 10% at 4 min, 60% at 4.5 min, 60% at 6.5 min, 10% at 6.6 min, and 10% at 10 min. E3040, E3040-G, E3040-S, and IS were detected as ion pairs at m/z 299.9/221.1, 475.6/300.2, 380.5/300.1, and 180.1/110.4, respectively.

Results

Perfusion Experiment in Rats. To examine the role of Mrp2 in the efflux of conjugated metabolites, we performed perfusion of the intestinal lumen of EHBRs in which Mrp2 expression is hereditarily defective using medium containing 4MU and E3040. Although no significant difference was observed between the two rat strains for the efflux of 4MU-G, 4MU-S, and E3040-S (Figs. 1, A and B, and 2B), the efflux rate of E3040-G in EHBRs was significantly lower than that in normal rats (Fig. 2A). The concentrations of conjugated metabolites of 4MU and E3040 in intestinal tissue were almost the same at the end of experiments between the two strains (Table 1). The mass balance after perfusion experiments is summarized in Fig. 3. In addition, there was no significant difference in the extent of absorption of 4MU and E3040 between the normal rats and EHBRs (Figs. 1C and 2C).

Perfusion Experiment in Mice. To examine the role of Bcrp1 in the efflux of conjugated metabolites formed in enterocytes, the intestinal lumen of Bcrp1 (−/−) and normal mice was perfused with medium containing 4MU and E3040.

Efflux rate of intracellularly formed 4MU-G, 4MU-S, and E3040-G into the lumen was calculated from the concentration of these conjugates in the outflow perfusate. In Bcrp1 (−/−) mice, the efflux rate of 4MU-G, 4MU-S, and E3040-G was significantly lower than that in normal mice (Figs. 4, A and B, and 5A). In particular, 4MU-S was not detectable in the outflow collected from Bcrp1 (−/−) mice (Fig. 4B). It was also found that the amount of 4MU-G, 4MU-S, and E3040-G associated with the intestinal tissue were almost the same at the end of experiments between two strains (Table 1). In contrast to 4MU-S formation, the sulfate conjugate of E3040 was not detectable in either the outflow or the intestinal tissue in either Bcrp1 (−/−) or normal mice. The mass balance of 4MU and E3040 after perfusion experiments is summarized in Fig. 6.

In addition, the apparent membrane permeability clearance ($CL_{a,app}$) of 4MU and E3040 was also calculated. The time profile for the $CL_{a,app}$ of 4MU and E3040 indicated that there is no significant difference in the extent of absorption of parent compounds between two strains (Figs. 4C and 5B).

Discussion

It has been suggested that conjugating enzymes and efflux transporters may play an important role synergistically in extruding organic anions into the intestinal lumen (Suzuki and Sugiyama, 2000; Kusuhara and Sugiyama, 2002). In the present study, we have focused on the function of Mrp2 and Bcrp1, apically located efflux transporters. It has been reported that both transporters are responsible for the cellular

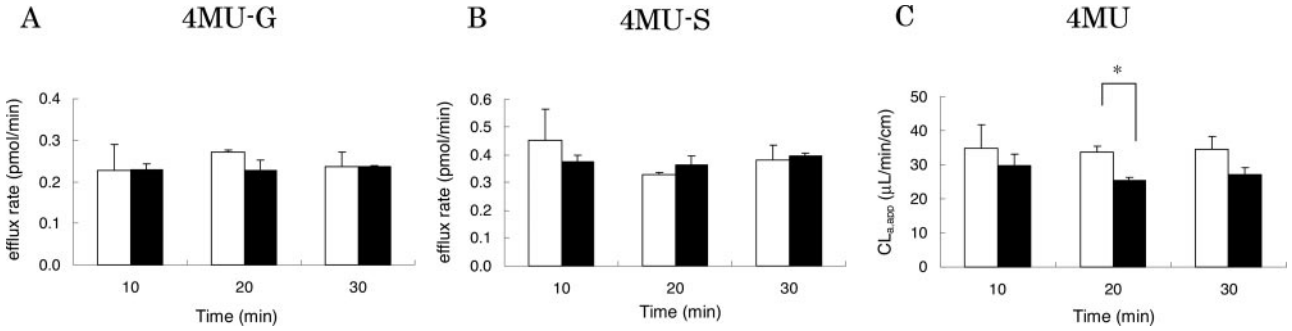

Fig. 1. Time profiles for the efflux rate of 4MU-G and 4MU-S and absorption clearance of 4MU in EHBRs and normal rats. Small intestinal segments were perfused with medium containing 10 μM 4MU to determine the outflow concentrations. A and B represent the efflux rate of 4MU-G and 4MU-S, defined by eq. 3, and C represents the absorption clearance of 4MU, defined by eq. 2, respectively. Each point and vertical bar represents the mean ± S.E. of three independent determinations. Open and closed columns represent the results in normal rats and EHBRs, respectively. Statistical difference between EHBRs and normal rats was compared by two-sided Student's t test with $p < 0.05$ as the limit of significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

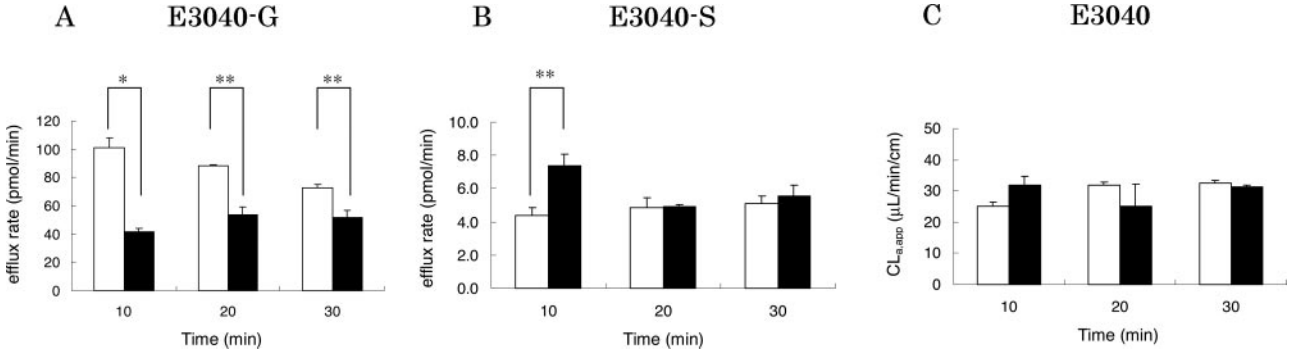

Fig. 2. Time profiles for the efflux of E3040-G and E3040-S and absorption clearance of E3040 in EHBRs and normal rats. Small intestinal segments were perfused with medium containing 10 μM E3040 to determine the outflow concentrations. A and B represent the efflux rate of E3040-G and E3040-S, defined by eq. 3, and C represents the absorption clearance of E3040, defined by eq. 2, respectively. Each point and vertical column represent the mean ± S.E. of three independent determinations. Open and closed columns represent the results in normal rats and EHBRs, respectively. Statistical difference between EHBRs and normal rats was compared by two-sided Student's t test with $p < 0.05$ as the limit of significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

TABLE 1

Mucosal concentration of glucuronide and sulfate conjugates remaining in the intestine

Amount of glucuronide and sulfate conjugates of 4MU and E3040 remaining in intestine was determined at the end of experiments. Results are given as the mean ± S.E. of three independent experiments. Statistical difference between Bcrp1 (−/−) mice and normal mice or EHBR and normal rats were compared by two-sided Student's t test with $p < 0.05$ as the limit of significance.

	4MU-G	4MU-S	E3040-G	E3040-S
		pmol/g tissue		
EHBR	917 ± 263	1.25 ± 0.38	861 ± 25	2.26 ± 0.44
Normal rats	989 ± 363	0.697 ± 0.092	778 ± 47	1.79 ± 0.32
Bcrp1 (−/−) mice	768 ± 149	73.4 ± 16.1	114 ± 9	N.D.
Normal mice	751 ± 158	56.5 ± 34.0	90.0 ± 17.0	N.D.

N.D., not detected.

extrusion of glucuronide and sulfate conjugates (Suzuki and Sugiyama, 2000; Nakatomi et al., 2001, Suzuki, 2003) and that sulfate conjugates are much preferred substrates compared with glucuronides for Bcrp (Suzuki et al., 2003). In addition, the expression level of MRP2 and BCRP in human small intestine was found to be much higher than MDR1 at

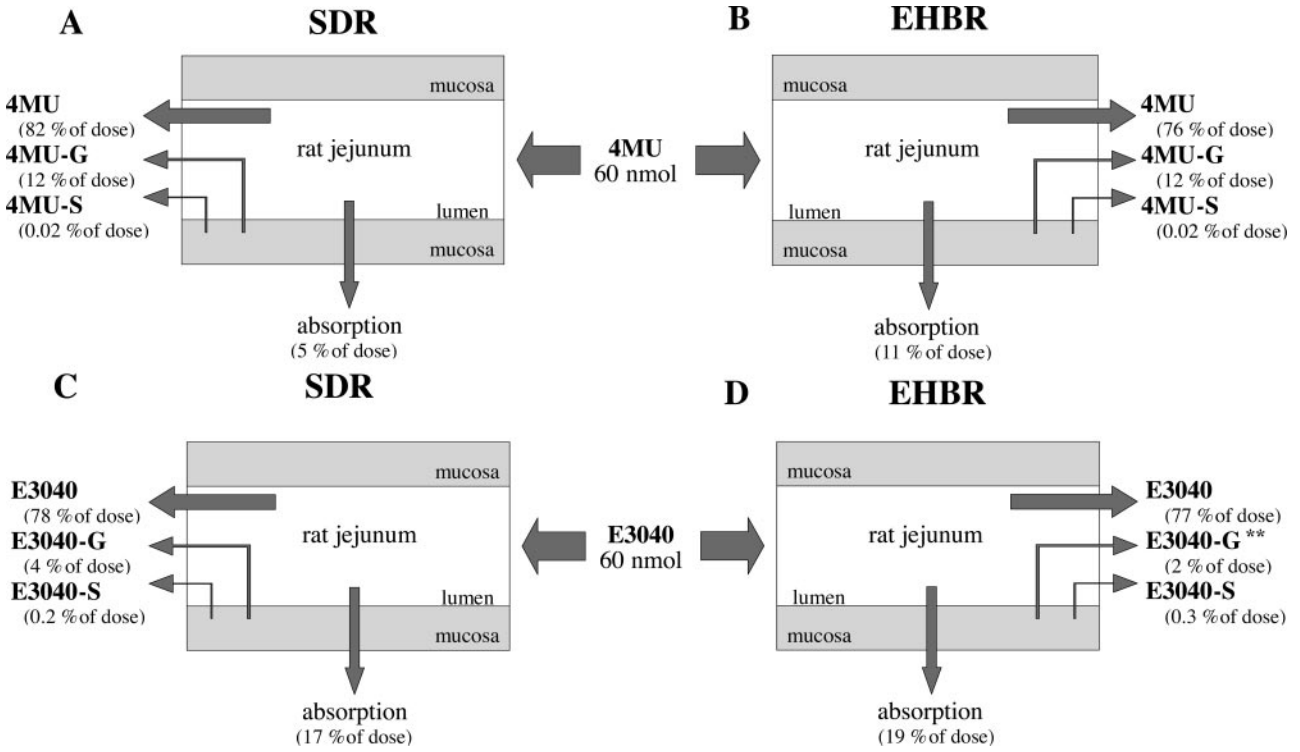

Fig. 3. Mass balance of 4MU and E3040 absorption in rat intestinal perfusion experiments. The intestinal disposition of 4MU and E3040 and their metabolites determined at the end of experiments is summarized. Absorption was defined by subtracting the recovered amount from the input amount. Data were taken from Figs. 1 and 2. A, 4MU in normal rats. B, 4MU in EHBRs. C, E3040 in normal rats. D, E3040 in EHBRs. Statistical difference between Bcrp1 (−/−) mice and normal mice was compared by two-sided Student's t test with $p < 0.05$ as the limit of significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

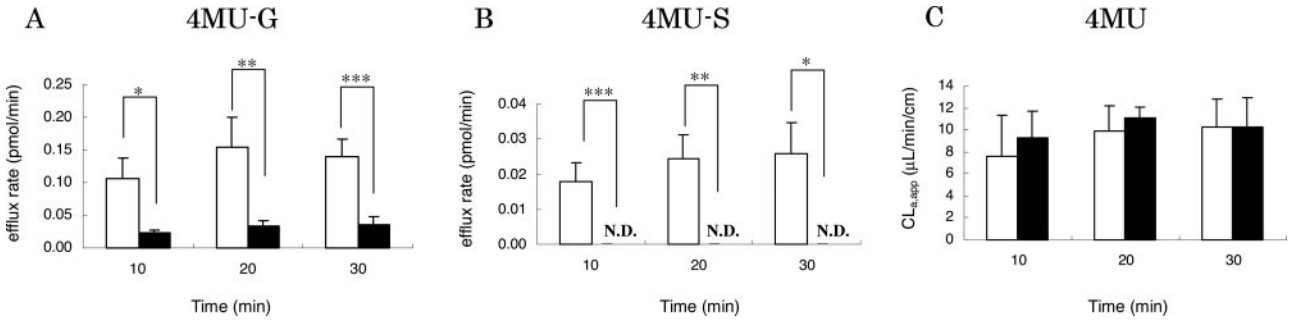

Fig. 4. Time profiles for the efflux rate of 4MU-G and 4MU-S and absorption clearance of 4MU in Bcrp1 (−/−) mice and normal mice. Small intestinal segments were perfused with medium containing 10 μM 4MU to determine the outflow concentrations. A and B represent the efflux rate of 4MU-G and 4MU-S, defined by eq. 3, and C represents the absorption clearance of 4MU, defined by eq. 2, respectively. Each point and vertical column represent the mean ± S.E. of three independent determinations. Open and closed columns represent the results in normal mice and Bcrp1 (−/−), respectively. Statistical difference between Bcrp1 (−/−) and normal mice was compared by two-sided Student's t test with $p < 0.05$ as the limit of significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). N.D., not detected (<20 nM).

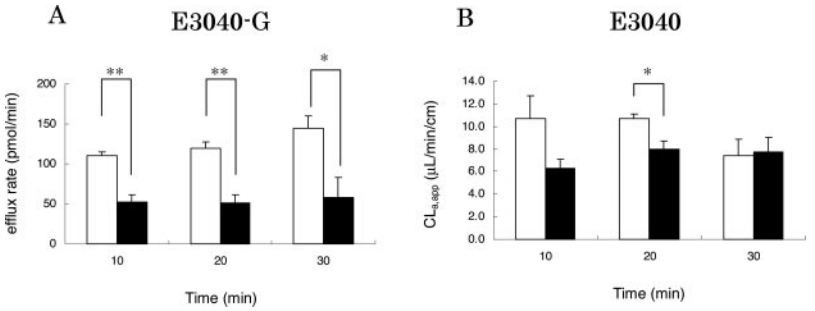

Fig. 5. Time profiles for the efflux rate of E3040-G and absorption clearance of E3040 in Bcrp1 (−/−) and normal mice. Small intestinal segments were perfused with medium containing 10 μM E3040 to determine the outflow concentrations. A, efflux rate of E3040-G, defined by eq. 3. B, absorption clearance of E3040, defined by eq. 2, respectively. Each point and vertical column represent the mean ± S.E. of three independent determinations. Open and closed columns represent the results in normal mice and Bcrp1 (−/−) mice, respectively. Statistical difference between Bcrp1 (−/−) mice and normal mice was compared by two-sided Student's t test with $p < 0.05$ as the limit of significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

the mRNA level (Taipalensuu et al., 2001). Based on these results, we focused on the above-mentioned two transporters as candidates involved in intestinal efflux of glucuronide and sulfate conjugates. In the present study, we have quantitatively determined the contribution of Mrp2 and Bcrp1 to the secretion of glucuronide and/or sulfate conjugates into the intestinal lumen by using an in situ intestinal perfusion technique in EHBRs, Bcrp1 (−/−) mice, and corresponding control animals. To clarify which efflux transporters are responsible for the efflux of conjugates, we also performed kinetic analyses. We previously demonstrated that the function of the Mdr1 product can be quantitatively estimated by this perfusion method (Adachi et al., 2003).

The appearance of 4MU-S and 4MU-G in the outflow was almost the same between normal rats and EHBRs, suggesting that Mrp2 is not responsible for the intestinal excretion of these conjugates (Fig. 1, A and B). It was also found that the intestinal excretion of E3040-G, but not that of E3040-S, was decreased in EHBRs. On the whole, although glucuronide conjugates are substrates for Mrp2, the functional significance of Mrp2 in intestinal glucuronide excretion depends on the substrates. From this standpoint, the functional significance of Mrp2 in intestinal excretion of conjugated metabolites should also be discussed. In our previous investigation, it has been demonstrated that the efflux of intracellularly formed 2,4-dinitrophenyl-*S*-glutathione, an Mrp2 substrate, into the intestinal lumen after intravenous administration of its precursor, 1-chloro-2,4-dinitrobenzene, was significantly lower in EHBRs than that in normal rats (Gotoh et al., 2000). This in vivo observation was further confirmed using Ussing chamber and everted intestinal sack studies. We have demonstrated that transport in the serosal-to-mucosal direction was 2-fold higher in Sprague-Dawley rats compared with EHBRs (Gotoh et al., 2000). In addition, the serosal-to-mucosal transport was greater than that in the opposite direction, and unidirectional transport disappeared in EHBRs (Gotoh et al., 2000). These results indicate the significance of Mrp2 in the intestinal extrusion of 2,4-dinitrophenyl-*S*-glutathione.

In contrast, it was shown that efflux rates of glucuronide and sulfate conjugates were significantly decreased in Bcrp1 (−/−) mice (Figs. 4, A and B, and 5A). In particular, the efflux of 4MU-S, a Bcrp substrate (Suzuki et al., 2003), decreased to an undetectable level in Bcrp1 (−/−) mice. This observation indicates that Bcrp1 has an important role in extruding sulfate conjugates. In addition, extrusion of 4MU-G and E3040-G was reduced to one fifth and one half that of normal mice, respectively. There is no significant difference in each conjugate concentration in intestinal mucosa between Bcrp1 (−/−) and normal mice. It was thus demonstrated that the efflux activity for these conjugates across the apical membrane of enterocytes was reduced in Bcrp1 (−/−) mice. Although the extent of oral absorption of 4MU and E3040 was almost the same between normal and Bcrp1 (−/−) mice (Figs. 4C and 5B), the results of the present study indicate the synergistic role of conjugating enzymes and Bcrp.

The significance of Bcrp1 in reducing the oral absorption and renal secretion of substrate has also been demonstrated. Jonker et al. (2000) demonstrated that the oral absorption of topotecan, a Bcrp1 substrate, was enhanced by the simulta-

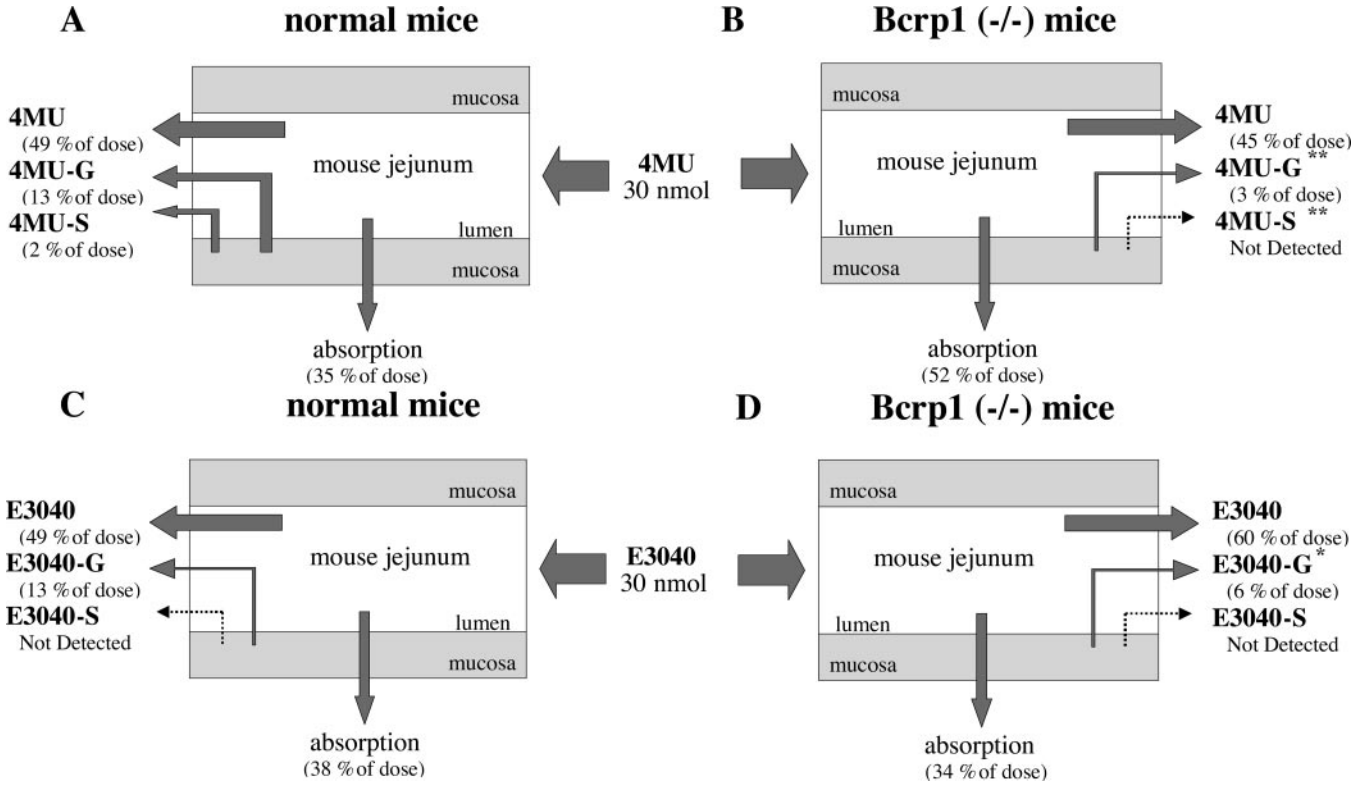

Fig. 6. Mass balance of 4MU and E3040 absorption in mouse intestinal perfusion experiments. The intestinal disposition of 4MU and E3040 and their metabolites determined at the end of experiments was summarized. Absorption was defined by subtracting recovered amount from input amount. Data were taken from Figs. 4 and 5. A, 4MU in normal mice. B, 4MU in Bcrp1 (−/−) mice. C, E3040 in normal mice. D, E3040 in Bcrp1 (−/−) mice. Statistical difference between Bcrp1 (−/−) mice and normal mice was compared by two-sided Student's *t* test with $p < 0.05$ as the limit of significance (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

neous administration of GF120918, a potent inhibitor of Bcrp1, even in mdr1a/1b knockout mice (Jonker et al., 2000). In addition, the oral bioavailability of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, a food carcinogen, was higher in Bcrp1 (−/−) mice compared with normal mice (van Herwaarden et al., 2003). Jonker et al. (2002) also demonstrated that mice lacking Bcrp1 become extremely sensitive to the dietary chlorophyll-breakdown product pheophorbide a, resulting in severe lethal phototoxic lesion on light-exposed skin. Bcrp1 transports pheophorbide a and is highly efficient in limiting its uptake from ingested food (Jonker et al., 2002). These observations strongly suggest that Bcrp1 restricts the absorption of toxic substrates in intestine. Furthermore, the fact that the renal clearance of E3040-S administrated by i.v. infusion is 2.4-fold lower in Bcrp1 (−/−) mice compared with normal mice suggests that Bcrp1 also has a significant role in renal secretion (Mizuno et al., 2004). It is unfortunate that we cannot compare the results between kidney and intestine, because E3040-S was not significantly formed in enterocytes (Fig. 5). The function of single-nucleotide polymorphism-type BCRP proteins has been extensively studied to explain the interindividual difference of pharmacokinetic profiles of substrates (Honjo et al., 2001, Iida et al., 2002, Zamber et al., 2003). Investigations are now underway to determine whether pharmacokinetics is influenced by BCRP genotypes in humans.

In conclusion, we have quantified the contribution of Bcrp1 and Mrp2 to restricting the intestinal absorption of its substrate drugs. It was clearly demonstrated by in situ intestinal perfusion experiments that Bcrp1 has an important role in extruding glucuronide and sulfate conjugates produced in enterocytes to intestinal lumen, although Mrp2 may be responsible for the intestinal excretion of some glucuronide conjugates. These results suggest the synergistic role of conjugating enzymes and efflux transporters in extruding xenobiotics.

References

Adachi Y, Suzuki H, and Sugiyama Y (2003) Quantitative evaluation of the function of small intestinal P-glycoprotein: comparative studies between in situ and in vitro. *Pharm Res (NY)* **20:**1163–1169.

Barthe L, Woodley J, and Houin G (1999) Gastrointestinal absorption of drugs: methods and studies. *Fundam Clin Pharmacol* **13:**154–168.

Benet LZ, Izumi T, Zhang Y, Silverman JA, and Wacher VJ (1999) Intestinal MDR transport proteins and P450 enzymes as barriers to oral drug delivery. *J Control Release* **62:**25–31.

Chen J and Pang KS (1997) Effect of flow on first pass metabolism of drugs: single pass studies on 4-methylumbelliferone conjugation in the serially perfused rat intestine and liver preparations. *J Pharmacol Exp Ther* **280:**24–31.

Doherty MM and Charman WN (2002) The mucosa of the small intestine. How clinically relevant as an organ of drug metabolism? *Clin Pharmacokinet* **41:**235–253.

Gotoh Y, Suzuki H, Kinoshita S, Hirohashi T, Kato Y, and Sugiyama Y (2000) Involvement of an organic anion transporter (canalicular multispecific organic anion transporter/multidrug resistance-associated protein 2) in gastrointestinal secretion of glutathione conjugates in rats. *J Pharmacol Exp Ther* **292:**433–439.

Honjo Y, Hrycyna CA, Yan QW, Medina-Perez WY, Robey RW, van der Laar A, Litman T, Dean M, and Bates SE (2001) Acquired mutations in the MXR/BCRP/ABCP gene alter substrate specificity in MXR/BCRP/ABCP-overexpressing cells. *Cancer Res* **61:**6635–6639.

Iida A, Saito S, Sekine A, Mishima C, Kitamura Y, Kondo K, Harigae S, Osawa S, and Nakamura Y (2002) Catalog of 605 single-nucleotide polymorphisms (SNPs) among 13 genes encoding human ATP-binding cassette transporters: ABCA4, ABCA7, ABCA8, ABCD1, ABCD3, ABCD4, ABCE1, ABCF1, ABCG1, ABCG2, ABCG4, ABCG5 and ABCG8. *J Hum Genet* **47:**285–310.

Jonker JW, Buitelaar M, Wagenaar E, van der Valk MA, Scheffer GL, Scheper RJ, Plosch T, Kuipers F, Oude Elferink RPJ, Rosing H, et al. (2002) The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. *Proc Natl Acad Sci USA* **99:**15649–15654.

Jonker JW, Smit JW, Brinkhuis RF, Maliepaard M, Beijnen JH, Schellens JHM, and Schinkel AH (2000) Role of breast cancer resistance protein in the bioavailability and fetal penetration of topotecan. *J Natl Cancer Inst* **92:**1651–1656.

Kaminsky LS and Zhang Q (2003) The small intestine as a xenobiotic-metabolizing organ. *Drug Metab Dispos* **31:**1520–1525.

Kusuhara H and Sugiyama Y (2002) Role of transporters in the tissue-selective distribution and elimination of drugs: transporters in the liver, small intestine, brain and kidney. *J Control Release* **78:**43–54.

Loria RM, Kayne HL, Kibrick S, and Broitman SA (1976) Measurement of intestinal absorption in mice by a double-label radioisotope perfusion technique. *Lab Anim Sci* **26:**603–606.

Mizuno N, Suzuki M, Kusuhara H, Suzuki H, Takeuchi K, T Niwa, Jonker JW, and Sugiyama Y (2004) Impaired renal secretion of 6-hydroxy-5,7-dimethyl-2-methylamino-4-(3-pyridilmethyl) benzothiazole (E3040) sulfate in breast cancer resistance protein (Bcrp1/Abcg2) knockout mice. *Drug Metab Dispos* **32:**898–901.

Mulder GJ, Brouwer S, Weitering JG, Scholtens E, and Pang KS (1985) Glucuronidation and sulfation in the rat *in vivo*. The role of the liver and the intestine in the *in vivo* clearance of 4-methylumbelliferone. *Biochem Pharmacol* **34:**1325–1329.

Nakatomi K, Yoshikawa M, Oka M, Ikegami Y, Hayasaka S, Sano K, Shiozawa K, Kawabata S, Soda H, Ishikawa T, et al. (2001) Transport of 7-ethyl-10-hydroxycamptothecin (SN-38) by breast cancer resistance protein ABCG2 in human lung cancer cells. *Biochem Biophys Res Commun* **280:**827–832.

Prueksaritanont T, Gorham LM, Hochman JH, Tran LO, and Vyas KP (1996) Comparative studies of drug metabolizing enzymes in dog, monkey, and human small intestine and in Caco-2 cells. *Drug Metab Dispos* **24:**634–642.

Suzuki H and Sugiyama Y (2000) Role of metabolic enzymes and efflux transporters in the absorption of drugs from the small intestine. *Eur J Pharm Sci* **12:**3–12.

Suzuki M, Suzuki H, Sugimoto Y, and Y Sugiyama (2003) ABCG2 transports sulfated conjugates of steroids and xenobiotics. *J Biol Chem* **278:**22644–22649.

Taipalensuu J, Tornblom H, Lindberg G, Einarsson C, Sjoqvist F, Melhus H, Garberg P, Sjostrom B, Lundgren B, and Artursson P (2001) Correlation of gene expression of ten drug efflux transporter family in normal human jejunum and in human intestinal epithelial caco-2 cell monolayers. *J Pharmacol Exp Ther* **299:**164–170.

Takenaka O, Horie T, Kobayashi K, Suzuki H, and Sugiyama Y (1995a) Kinetic analysis of hepatobiliary transport for conjugated metabolites in the rat perfused liver of mutant rats (EHBR) with hereditary conjugated hyperbilirubinemia. *Pharm Res (NY)* **12:**1746–1755.

Takenaka O, Horie T, Suzuki H, and Sugiyama Y (1995b) Different biliary excretion systems for glucuronide and sulfate of a model compound; study using Eisai hyperbilirubinemic rats. *J Pharmacol Exp Ther* **274:**1962–1969.

van Herwaarden AE, Jonker JW, Wagenaar E, Brinkhuis RF, Schellens JHM, Beijnen JH, and Schinkel AH (2003) The breast cancer resistance protein (Bcrp1/ABCG2) restricts exposure to the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. *Cancer Res* **63:**6447–6452.

Wacher VJ, Silverman JA, Zhang Y, and Benet LZ (1998) Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. *J Pharm Sci* **87:**1322–1330.

Yuasa H, Matsuda K, and Watanabe J (1993) Influence of anesthetic regimens on intestinal absorption in rats. *Pharm Res (NY)* **10:**884–888.

Zamber CP, Lamba JK, Yasuda K, Farnum J, Thummel K, Schuetz JD, and Schuetz EG (2003) Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. *Pharmacogenetics* **13:**19–28.

Zhang Q, Dunbar D, Ostrowska A, Zeislosft S, Yang J, and Kaminsky LS (1999) Characterization of human small intestinal cytochromes P-450. *Drug Metab Dispos* **27:**804–809.

Zimmerman CL, Ranta S, Leboeuf E, and Pang KS (1991) High-performance liquid chromatographic method for the direct determination of 4-methylumbelliferone and its glucuronide and sulfate conjugation. *J Chromatogr B* **563:**83–94.

Address correspondence to: Prof. Yuichi Sugiyama, Department of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. E-mail: sugiyama@mol.f.u-tokyo.ac.jp