Title: Role of breast cancer resistance protein (Bcrp1/Abcg2) in the extrusion of glucuronide and sulfate conjugates from enterocytes to intestinal lumen.

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Running title: intestinal function of Bcrp and Mrp2

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A list of nonstandard abbreviations:

BCRP (Bcrp), breast cancer resistant protein; MRP (Mrp), multidrug resistance associated protein; EHBR, Eisai hyperbilirubinemic rat; 4MU, 4-methylumbelliferone;
PCR, polymerase chain reaction; Fa, absorbed fraction; CLa,app, apparent membrane permeability clearance; HPLC, high performance liquid chromatography; LC-MS/MS, high performance liquid chromatography equipped with triple stage mass spectrometer.
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 (EHBR) in which the multidrug resistance protein 2 (Mrp2/Abcc2) is hereditarily defective and the breast cancer resistance protein (Bcrp1 / Abcg2) knock out mice. The intestinal lumen of EHBR and Bcrp1 (-/-) mice was perfused with medium containing 4-methylumbelliferone (4MU) and E3040 to determine the efflux of metabolites into the outflow. The efflux of E3040-G in EHBR was significantly lower compared with normal rats. However, no significant difference was observed for the efflux of 4MU-G, 4MU-S, and E3040-S between EHBR 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. Consequently, 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.
Introduction

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 (CYP) 3A4 (Preuksaranont 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 due to 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 conjugate 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 (UGT) and sulfotransferases (ST) 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 human intestine, reverse transcription PCR analysis revealed that MRP2 is more highly expressed in enterocytes than MDR1, an efflux transporter for neutral and cationic compounds (Taipalenuu 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 (EHBR), Bcrp1 (-/-) mice and corresponding normal rats and mice, respectively. As test substances, we chose 4-methyl-umbelliferone (4MU) and E3040 which are metabolized in enterocytes by UGT and ST to yield conjugated metabolites (Mulder et al., 1985, Zimmerman et al., 1991, Takenaka et al., 1995a and 1995b, Chen and Pang, 1997).
Material 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 (St. Louis, MO).

Animals.

EHBR and normal rats were purchased from Clea Japan (Tokyo, Japan). The rats were housed in groups (3-5 per 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 (alpha-dry, SHEPHERD SPECIALTY PAPERS, Michigan) 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).
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 8-10 cm jejunal segment was isolated and cannulated at both ends with plastic tubing. The segment was rinsed with phosphate buffered saline (PBS, 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 (Harvard Apparatus Syringe Infusion Pump). The perfusion solution consisted of 20.1 mM Na$_2$HPO$_4$, 47.0 mM KH$_2$PO$_4$, 101 mM NaCl (pH 6.4) and contained 10 µM of 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, Packard, Meriden, CT) was added to the aliquots from the outflow specimens to measure the radioactivities in a liquid scintillation counter (model 2700 TR, Packard, Meriden, CT).

**Data Analysis**

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

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

where $C_{\text{in}, \text{i}}$ and $C_{\text{out}, \text{i}}$ represent the concentration of $[^{14}\text{C}]$inulin in the inflow and outflow solutions, respectively, and $C_{\text{in}}$ and $C_{\text{out}}$ 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):

$$\text{CL}_{a, \text{app}} = -\frac{Q}{L} \times \ln(1 - F_a)$$ (2)

where $\text{CL}_{a, \text{app}}$ 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):

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

where \( C_{\text{out,M}} \) 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 employed. In the rat experiments, LC-MS/MS analysis was performed on a MDS Sciex (Concord, Canada) model API4000 equipped with Agilent G1312A pump (Page Mill, Rd). 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. \( \times \) 50 mm l.) (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, HPLC analysis was carried out using a Shimadzu LC-10Avp pump equipped with a Shimadzu SPD-10Avp detector (Kyoto, Japan). Perfusate was directly injected onto a column (Inertsil ODS-3, 4.6 mm i.d. × 150 mm l.) (GL science, Tokyo, Japan). Chromatography was achieved using a gradient from 25% (0 min) to 50% (20 min) methanol in 50 mM KH\textsubscript{2}PO\textsubscript{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, LC-MS/MS analysis was employed. 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 l.) (Waters, Milford, MA). 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

In order to examine the role of Mrp2 in the efflux of conjugated metabolites, we performed perfusion of the intestinal lumen of EHBR 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. 1A, 1B and 2B), the efflux rate of E3040-G in EHBR 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 EHBR (Figs. 1C and 2C).

Perfusion experiment in mice

In order 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. 4A, 4B and 5A). Particularly, 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 were 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 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, 2003). In addition, the expression level of MRP2 and BCRP in human small intestine was found to be much higher than MDR1 at the mRNA level (Taipalensuu, 2001). Based on these results, we focused on the above 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 EHBR, 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 EHBR, suggesting that Mrp2 is not responsible for the intestinal excretion of these conjugates (Figs. 1A and 1B). It was also found that the intestinal excretion of E3040-G, but not that of E3040-S, was decreased in EHBR. Collectively, 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 (DNP-SG), an Mrp2 substrate, into the intestinal lumen after intravenous administration of its precursor, 1-chloro-2,4-dinitrobenzene (CDNB), was significantly lower in EHBR 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 SD rats compared with EHBR (Gotoh et al., 2000). In addition, the serosal to mucosal transport was greater than that in the opposite direction.
and unidirectional transport disappeared in EHBR (Gotoh et al., 2000). These results indicate the significance of Mrp2 in the intestinal extrusion of DNP-SG.

In contrast, it was shown that efflux rates of glucuronide and sulfate conjugates were significantly decreased in Bcrp1 (-/-) mice (Fig. 4A, 4B 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 1/5 and 1/2 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. demonstrated that the oral absorption of topotecan, a Bcrp1 substrate, was enhanced by the simultaneous administration of GF120918, a potent inhibitor of Bcrp1, even in mdr1a/1b knockout
mice (Jonker et al., 2000). In addition, the oral bioavailability of PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine), a food carcinogen, was higher in Bcrp1 (-/-) mice compared to normal mice (van Herwaarden, 2003). Jonker et al. 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 (Jonker et al., 2002). Bcrp1 transports pheophorbide a and is highly efficient in limiting its uptake from ingested food (Jonker et al., 2002). These observations strongly suggest that Bcrp 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 can’t compare the results between kidney and intestine, since E-3040-S was not significantly formed in enterocytes (Fig. 5).

Recently, the function of SNPs type BCRP proteins was extensively studied to explain the interindividual difference of pharmacokinetic profiles of substrates (Honjo et al., 2001; Iida et al., 2002; Zamber et al., 2003). Further investigations are now underway to determine whether pharmacokinetics are 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.
Reference


Footnotes

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Figure legend

Figure 1

Time profiles for the efflux rate of 4MU-G and 4MU-S and absorption clearance of 4MU in EHBR and normal rats.

Small intestinal segments were perfused with medium containing 10 µM 4MU to determine the outflow concentrations. Panels A and B represent the efflux rate of 4MU-G and 4MU-S, defined by Eq. (3), and panel 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 bars represent the results in normal rats and EHBR, respectively. Statistical difference between EHBR 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).

Figure 2

Time profiles for the efflux of E3040-G and E-3040-S and absorption clearance of E3040 in EHBR and normal rats.

Small intestinal segments were perfused with medium containing 10 µM E3040 to determine the outflow concentrations. Panels A and B represent the efflux rate of
E3040-G and E3040-S, defined by Eq. (3), and panel C represents the absorption clearance of E3040, defined by Eq. (2) respectively. Each point and vertical bar represents the mean ± S.E. of three independent determinations. Open and closed bar represent the results in normal rats and EHBR respectively. Statistical difference between EHBR 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).

Figure 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. Each data was taken from Figs. 1 and 2. Panel A, 4MU in normal rats; panel B, 4MU in EHBR; panel C, E3040 in normal rats; panel D, E3040 in EHBR. 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).
Figure 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. Panels A and B represent the efflux rate of 4MU-G and 4MU-S, defined by Eq. (3), and panel 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 bars 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)

Figure 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. Panel A represents the efflux rate of
E3040-G, defined by Eq. (3), and panel B represents the absorption clearance of E3040, defined by Eq. (2) respectively. Each point and vertical bar represents the mean ± S.E. of three independent determinations. Open and closed bars 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 (*\( p<0.05 \); **\( p<0.01 \); ***\( p<0.001 \)).

Figure 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. Each data was taken from Figs. 4 and 5. Panel A, 4MU in normal mice; panel B, 4MU in Bcrp1 (-/-) mice; panel C, E3040 in normal mice; panel 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 (*\( p<0.05 \); **\( p<0.01 \); ***\( p<0.001 \)).
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.

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

N.D.: Not detected
Fig. 1
Fig. 2
**Fig. 3**

A. SDR

- **4MU** (82% of dose)
- **4MU-G** (12% of dose)
- **4MU-S** (0.02% of dose) absorption (5% of dose)

B. EHBR

- **4MU** (76% of dose)
- **4MU-G** (12% of dose)
- **4MU-S** (0.02% of dose) absorption (11% of dose)

C. SDR

- **E3040** (78% of dose)
- **E3040-G** (4% of dose)
- **E3040-S** (0.2% of dose) absorption (17% of dose)

D. EHBR

- **E3040** (77% of dose)
- **E3040-G** (2% of dose)
- **E3040-S** (0.3% of dose) absorption (19% of dose)
Fig. 4

A 4MU-G

B 4MU-S

C 4MU

MOL 7393
**Fig. 5**

Panel A: E3040-G

- **efflux rate (pmol/min)**
  - Bars represent time points (10, 20, 30 minutes).
  - **E3040-G**

Panel B: E3040

- **CL_{app} (µL/min/cm)**
  - Bars represent time points (10, 20, 30 minutes).
  - **E3040**

*Significance levels: *p < 0.05, **p < 0.01*
**Fig. 6**

**A** Normal mice

- 4MU (49% of dose)
- 4MU-G (13% of dose)
- 4MU-S (2% of dose)

**B** Bcrp1 (-/-) mice

- 4MU (45% of dose)
- 4MU-G (3% of dose)
- 4MU-S (Not Detected)

**C** Normal mice

- E3040 (49% of dose)
- E3040-G (13% of dose)
- E3040-S (Not Detected)

**D** Bcrp1 (-/-) mice

- E3040 (60% of dose)
- E3040-G (6% of dose)
- E3040-S (Not Detected)

Absorption:
- 4MU: (35% of dose)
- 4MU-G: (38% of dose)
- 4MU-S: (34% of dose)

Mucosa to lumen:
- 4MU: 30 nmol
- E3040: 30 nmol

Lumen to mucosa:
- 4MU: (35% of dose)
- E3040: (38% of dose)

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