Investigation of the role of oligopeptide transporter PEPT1 and sodium/glucose cotransporter SGLT1 in intestinal absorption of their substrates using small GTP-binding protein Rab8 null mice

Yukio Kato, Tomoko Sugiura, Yasuhito Nakadera, Mikihiro Sugiura, Yoshiyuki Kubo, Takashi Sato, Akihiro Harada and Akira Tsuji

Division of Pharmaceutical Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192 (Y.K., T.S., Y.N., M.S., Y.K., A.T.)

and Laboratory of Molecular Traffic, Department of Molecular and Cellular Biology, Institute for Molecular and Cellular Regulation, Gumma University, 3-39-15 Showa, Maebashi, Gunma 371-8512 (T.S., A.H.), Japan
Running title:

PEPT1-mediated cefixime absorption demonstrated in rab8⁻/⁻ mice

Corresponding author:

Prof. Akira Tsuji, Ph.D.

Division of Pharmaceutical Sciences, Graduate School of Natural Science and Technology, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan.

Tel: (81)76-264-5085, Fax: (81)76-234-4010, E-mail: tsuji@kenroku.kanazawa-u.ac.jp

Document statistics:

Number of text pages: 30
Number of tables: 1
Number of figures: 3
Number of references: 33
Number of words:

Abstract: 250
Introduction: 756
Discussion: 1019

Abbreviations:

PEPT1, oligopeptide transporter; Gly-Sar, glycylysarcosine; SGLT1, sodium/glucose cotransporter; α-MDG, α-methyl-d-glycopyranoside;
ABSTRACT

A small GTP-binding protein, Rab8, is essential for apical localization of oligopeptide transporter PEPT1/SLC15A1 and sodium/glucose cotransporter SGLT1/SLC5A1 in small intestine; deficiency of rab8 gene results in mislocalization and reduced expression of these transporters. Here, we examined the role of PEPT1 and SGLT1 in vivo in gastrointestinal absorption of a β-lactam antibiotic, cefixime, and α-methyl-d-glycopyranoside (α-MDG), respectively, using rab8 gene knockout (rab8−/−) mice as experimental animals deficient in those transporters. Plasma concentration of cefixime and α-MDG after oral administration in rab8−/− mice was much lower than that in wild-type mice, whereas such reduction in oral absorption was not observed for antipyrine, membrane permeation of which is not transporter-mediated. Uptake of cefixime from the apical side of isolated small intestine assessed by means of the everted sac method in wild-type mice was decreased in the presence of excess unlabeled glycylsarcosine, a PEPT1 substrate. On the other hand, the uptake in rab8−/− mice was much lower than that in wild-type mice and comparable with that of an extracellular marker, mannitol, suggesting that the apical membrane permeability of cefixime was reduced in rab8−/− mice. Uptake of cefixime in wild-type mice was pH-dependent, being higher at lower pH, whereas that in rab8−/− mice remained at the background level at all pH values examined. These results suggest that PEPT1 and SGLT1 play an important role in gastrointestinal absorption of cefixime and α-MDG, respectively, in vivo in mice. The present findings also illustrate the pharmacokinetic influence of the sorting machinery protein Rab8.
Introduction

Gastrointestinal absorption plays a key role in determining the efficacy of orally administered therapeutic agents. Xenobiotic transporters have recently been identified to be expressed in small intestinal epithelial cells, and are thought to be involved in membrane permeability of at least certain types of therapeutic agents (Sai and Tsuji, 2004; Herrera-Ruiz and Knipp, 2004; Thwaites and Anderson, 2007). Several types of efflux transporters which belong to the ATP binding cassette (ABC) superfamily, including P-glycoprotein, breast cancer resistance protein and multidrug resistance-associated protein, have been confirmed to play a role as a barrier against membrane permeation of therapeutic agents and xenobiotics. On the other hand, the role of influx mechanism(s) in gastrointestinal absorption of drugs has not yet been fully clarified. One reason for this may be the limited number of animal models with knockout or deficiency of genes for influx transporters expressed on apical membranes of small intestine, whereas mice with knockout of various ABC transporter genes are readily available. Most studies on the role of influx transporters have employed in vitro experimental tools, such as gene expression systems, and histochemical/biochemical analyses, such as immunostaining and western blotting.

Oligopeptide transporter PEPT1/SLC15A1 is a small-intestinal influx transporter that is thought to be involved in gastrointestinal absorption of various types of drugs, including \( \beta \)-lactam antibiotics. These antibiotics are usually administered orally because of their substantial
gastrointestinal absorption, due to high small-intestinal membrane permeability, and minimal metabolism in the body (Tamai et al., 1995; Saitoh et al., 2002). Involvement of oligopeptide transport systems in the high membrane permeability of cephalexin and amoxicillin, both of which have an amino group in their structure, was first established in our laboratory (Tsuji et al., 1979, 1981; Nakashima et al., 1984a, 1984b; Nakashima and Tsuji, 1985). On the other hand, cefixime has no amino group, but exhibits high membrane permeability despite its hydrophilic character, probably because it shares the same transport systems as dipeptide and α-amino β-lactam antibiotics in small intestine (Tsuji et al., 1987a, 1987b). PEPT1 was cloned as a possible candidate for the oligopeptide transport system (Fei et al., 1994; Miyamoto et al., 1996), and recognizes various β-lactam antibiotics, including cefixime and cephalexin (Sai et al., 1996; Wenzel et al., 1996; Tamai et al., 1997). However, most of the studies that have supported a fundamental role of PEPT1 were in vitro studies, and no direct in vivo evidence has been available up to now.

Glucose is an essential nutrient for all living organisms, and small intestinal transport system(s) for glucose have been researched for a long time. The sodium/glucose cotransporter (SGLT1/SLC5A1) is expressed on apical membranes of small intestine (Takata et al., 1992). Some patients with congenital glucose-galactose malabsorption have mutations in SGLT1 gene, and the transport activity of SGLT1 with those mutations is vanishingly small (Truk et al., 1991; Kasahara et al., 2001; Wright et al., 2003). Therefore, SGLT1 is thought to be essential for the intestinal
absorption of glucose, although only limited information is available on pharmacokinetic role of SGLT1 in the small intestine in vivo.

The small GTP-binding protein Rab8 is a member of the Rab GTPase family. The structure of Rab GTPase is well conserved from yeast to human, and the Rab GTPase family has more than 60 members in humans (Stenmark and Olkkonen, 2001; Grosshands et al., 2006). Many Rab proteins are expressed ubiquitously in tissues and are localized at the cytosolic side of cellular membranes, being involved in intracellular vesicular transport (Stenmark and Olkkonen, 2001; Grosshands et al., 2006). Rab8 was first cloned from a cDNA library of Mardin-Darby canine kidney cells, and has been demonstrated to be involved in protein trafficking from the trans-Golgi network to the basolateral membranes in vitro (Huber et al., 1993; Ang et al., 2003). Sato et al. (2007) recently demonstrated that the deficiency of the rab8 gene in mice leads to reduced expression and missorting of various apical membrane proteins, including PEPT1 and SGLT1 to the intracellular compartment, with minimal effects on basolateral membrane proteins in small intestine. Uptake of substrates of these transporters, glycylsarcosine (Gly-Sar) and α-methyl-d-glycopyranoside (α-MDG), respectively, from the apical membrane was almost entirely abrogated to the background level in small intestine isolated from rab8 gene knockout (rab8−/−) mice (Sato et al., 2007). Therefore, this mouse strain would be a useful tool to analyze the function of PEPT1 and SGLT1 in vivo. The purpose of the present study was to demonstrate an important role
of PEPT1 and SGLT1 in gastrointestinal absorption of their substrates, cefixime and α-MDG, respectively, in vivo by using rab8−/− mice.
MATERIALS AND METHODS

Animals

Wild-type and homozygous mutants (rab8⁻/⁻) were obtained by mating heterozygous male mice with heterozygous female ones. Because the rab8⁻/⁻ mice died within 3-4 weeks after birth, all the animal experiments were performed at 20 days of age. Mice were used without prior deprivation of food, because starvation severely weakened them. All the animal experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of Kanazawa University.

Materials

Cefixime was a gift from Astellas Pharmaceutical Co., Ltd (Osaka, Japan). Cephalexin was purchased from Sigma-Aldrich Japan K.K. (Tokyo, Japan). L-[Methyl-³H]carnitine hydrochloride, antipyrine and [¹⁴C]α-MDG were purchased from GE Healthcare (Buckinghamshire, UK). [¹⁴C]Mannitol was purchased from PerkinElmer Life Sciences (Boston, MA). All other reagents were commercial products of reagent grade.

Pharmacokinetic Studies

Mice were anesthetized with diethylether during drug administration. Cefixime (20 mg/kg
body weight), $[^{14}\text{C}]\alpha$-MDG (250 ng/kg body weight) or antipyrine (20 mg/kg body weight) dissolved in saline was orally administered by gavage. At various intervals up to 240 min after administration, aliquots of about 10 µL of blood were collected through the caudal vein. All blood samples were immediately centrifuged to obtain plasma. For the quantification of cefixime, the plasma samples (5 µL) were mixed with methanol (15 µL). All the mixed solutions were centrifuged at 19,000 g for 15 min at 4 °C. The supernatants were subjected to HPLC analysis. For the quantification of antipyrine, the plasma samples (5 µL) were mixed with acetonitrile (15 µL). After the centrifugation, the supernatants were diluted with mobile phase (at a volume ratio of 1:49) and again centrifuged. The supernatants were then subjected to LC-MS/MS analysis. $[^{14}\text{C}]\alpha$-MDG was determined as radioactivity associated with plasma, which was mixed with Clearsol I (Nacalai Tesque Inc., Kyoto, Japan) as the scintillation fluid. The radioactivity was determined using a liquid scintillation counter, LSC-5100 (Aloka, Tokyo, Japan).

Pharmacokinetic parameters were determined by moment analysis. Since the elimination phase in the plasma concentration profiles of cefixime and $\alpha$-MDG could not be fully chased after oral administration, area under the curve (AUC) was calculated until the end of the in vivo experiment (240 min). Therefore, absolute bioavailability (BA) cannot be calculated, and the apparent BA was estimated as $(\text{AUC}_{\text{po},0-240}/\text{D}_{\text{po}})/(\text{AUC}_{\text{iv},0-240}/\text{D}_{\text{iv}})$, where $\text{D}_{\text{po}}$ and $\text{D}_{\text{iv}}$ are the doses in the cases of oral and intravenous administrations, respectively.
Uptake study in everted intestinal sac

Everted intestinal sacs were prepared from the upper part (within 10 cm below the pylorus) of the small intestine according to the reported method (Nakashima et al., 1984b). After 10 min of preincubation in the transport buffer (125 mM NaCl, 4.8 mM KCl, 1.2 mM KH$_2$PO$_4$, 5.6 mM D-glucose, 1.2 mM CaCl$_2$, 1.2 mM MgSO$_4$ and 25 mM MES (pH 5.0 or 6.0) or 25 mM HEPES (pH 7.4)), the sac was incubated with cefixime, cephalexin or [$^{14}$C]mannitol for designated times under bubbling with O$_2$/CO$_2$ gas at 37 °C. The sac was then washed with ice-cold buffer, weighed, and homogenized (Ultra Turrax, Ika Japan, Nara, Japan) in saline to give a 20% (w/v) homogenate. After deproteinization with methanol (at a volume ratio of 1:2) and centrifugation at 19,000 g for 15 min, the amount of cefixime or cephalexin in the supernatant fraction was determined by HPLC assay. The uptake of [$^{14}$C]mannitol was determined as radioactivity associated with the sac, which was solubilized with Soluene-350 (Packard Co., Canberra, Australia) by incubation at room temperature for 24 h, decolorized with 0.2 mL of H$_2$O$_2$, neutralized with 0.1 mL of 5 N HCl, left at room temperature for 4 h and then mixed with 3 mL of Clearsol I for the radioactivity measurement.

Uptake study in *Xenopus laevis* oocytes injected with cRNA encoding mouse PEPT1
The full-length cDNA of mouse PEPT1 was cloned by PCR amplification of cDNA obtained from mouse kidneys as a template, and subcloned into the EcoRV and Xho I sites of pcDNA3 (Invitrogen, Carlsbad, CA). The full sequence of the insert was verified. The capped cRNA of mouse PEPT1 was synthesized by means of a mCAPTM RNA capping kit (Stratagene, La Jolla, CA). *Xenopus laevis* oocytes were prepared and injected with 50 nL of cRNA (25 ng) or water as described previously (Terasaki et al., 1993). Four days after the cRNA injection, the oocytes were transferred to ND96 solution (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2 and 5 mM HEPES, pH 7.4). After preincubation at 25°C for 15 min, uptake was initiated by replacing the solution with fresh ND96 solution containing 5 mM MES instead of HEPES (pH 5.0 or 6.0) and cefixime at 25°C. At the designated times, the oocytes were rinsed five times with ice-cold ND96 solution. Individual oocytes were disrupted in 35 µL of methanol with a tip sonicator, and the lysate was cleared by centrifugation at 15,000 g for 15 min at 4 °C. The supernatant was then subjected to HPLC analysis.

**HPLC Analysis**

The HPLC analysis was performed as described previously (Tsuji et al., 1987a, 1987b; Tamai et al., 1988) with some modifications. The system consisted of a constant-flow pump (JASCO PU-2080 *Plus*, JASCO International Co., Ltd., Tokyo, Japan), a UV detector (JASCO
DMD #23689

UV-2075 Plus), an automatic sample injector (JASCO AS-1555-10) and an integrator (Chromatopac CR-7A, Shimadzu Corporation, Kyoto, Japan). The UV detector was set at 260 nm. The reversed-phase column (Cosmosil 5C18-MS-II, 4.6 x 150 mm; Nacalai Tesque Inc.) was maintained at 35°C in a column oven (JASCO CO-2065 Plus). The mobile phase was 10 mM ammonium acetate/methanol (8:92, v/v). The flow rate was 1.0 mL/min.

Measurement of antipyrine by LC-MS/MS analysis

The LC/MS/MS system was equipped with a constant flow pump (Agilent 1200 series G1312A, Agilent Technologies, Tokyo, Japan), an automatic sample injector (G1367B; Agilent Technologies), a column oven (G1316A; Agilent Technologies) and a mass spectrometer (API 3200, Applied Biosystems, Tokyo, Japan). The analytical column was COSMOSIL® 5C18-MS-II (2.0 mm x 50 mm; Nacalai Tesque Inc., Kyoto, Japan). Mobile phase A was 0.01 M ammonium formate and mobile phase B was methanol. The gradient elution time program was as follows: 0-1 min, B 7-70%; 1-9.5 min, B 70%; 9.5-9.6 min, B 70-7%; 9.6-19.6 min, B 7%. The flow rate was 0.2 mL/min. Separation was performed at 40 °C. The multiple reaction monitor was set at 189.2 to 56.0 m/z for antipyrine.
RESULTS

Intestinal absorption of cefixime and α-MDG was reduced in vivo in rab8−/− mice

Since the expression and apical localization of PEPT1 and SGLT1 were reduced in small intestine of rab8−/− mice, compared with wild-type mice (Sato et al., 2007), we hypothesized that oral absorption of cefixime and α-MDG would be decreased in rab8−/− mice, if PEPT1 and SGLT1, respectively, are responsible for gastrointestinal absorption of these compounds. In the present study, we first compared oral absorption of cefixime between wild-type and rab8−/− mice. Plasma concentrations of cefixime after oral administration in rab8−/− mice were lower than those in wild-type mice (Fig. 1A), the AUCpo,0-240 in rab8−/− mice being 40% of that in wild-type mice (Table 1). Further, when cefixime was intravenously injected, both AUC and distribution volume (Vdss) were similar in the two strains, suggesting that distribution and systemic elimination are not very different in these strains (Table 1).

The plasma concentration profile of α-MDG was next examined in wild-type and rab8−/− mice after oral and intravenous administrations. Plasma concentrations of α-MDG after oral administration in rab8−/− mice were higher than those in wild-type mice (Fig. 1B), the AUC calculated from 0 to 240 min in rab8−/− mice being lower than that in wild-type mice (Table 1). The apparent BA in rab8−/− mice was 40% of that in wild-type mice (Table 1). On the other hand, oral absorption of antipyrine, membrane permeation of which is not transporter-mediated, was also
examined as a control study. Plasma concentrations of antipyrine after oral administration in *rab8*^-/-^ mice were slightly, but significantly higher than those in wild-type mice (Fig. 1C).

**Uptake of cefixime from apical membranes was reduced in small intestine of *rab8*^-/-^ mice**

To confirm the change in the membrane permeability of cefixime in the apical membrane of small intestine in *rab8*^-/-^ mice, everted sacs were prepared from both wild-type and *rab8*^-/-^ mice, and the uptakes of cefixime were compared. Uptake of cefixime in wild-type mice was significantly higher than that in *rab8*^-/-^ mice, and was decreased in the presence of 20 mM unlabeled Gly-Sar (Fig. 2A, 2B). The uptake in the presence of Gly-Sar in wild-type mice and that in *rab8*^-/-^ mice (~0.2 µL/mg protein/15 min) were close to the apparent uptake of an extracellular marker, mannitol (Fig. 2B, 2D). Decreased uptake from apical membrane of *rab8*^-/-^ mice was also observed for another β-lactam antibiotic, cephalexin (Fig. 2C).

Since the driving force of PEPT1-mediated uptake is a proton gradient, the pH dependence of cefixime uptake was next examined in small intestines of both strains. The uptake of cefixime in wild-type mice was higher at lower pH values, whereas that in *rab8*^-/-^ mice did not exhibit pH dependence (Fig. 3) and was close to the apparent uptake of mannitol (Figs. 2D, 3). Such a pH profile is similar to our previous observation in the uptake of cefixime by brush-border membrane vesicles of rat small intestine (Tsuji *et al.*, 1987b).
Cefixime is a substrate of mouse PEPT1

Cefixime is known to be transported by human, rabbit and rat PEPT1 in vitro (Wenzel et al., 1996, Ganapathy et al., 1997; Tamai et al., 1997), but there has been no direct demonstration that cefixime is transported by mouse PEPT1. In the present study, mouse PEPT1 was cloned, and cRNA of the mouse PEPT1 was injected into Xenopus laevis oocytes. The cefixime uptake by oocytes injected with cRNA for mouse PEPT1 was 54.2 ± 10.4 nL/oocytes/90 min (mean ± S.E.M., n = 10-11 oocytes), whereas that by water-injected oocytes was under the determination limit (< 0.5 nL/oocytes/90 min). These cefixime uptakes were decreased in the presence of 20 mM unlabeled Gly-Sar to the level under the determination limit.
DISCUSSION

Oligopeptide transporter PEPT1 is believed to play a predominant role in gastrointestinal absorption of β-lactam antibiotics, but direct evidence in vivo has not yet been obtained. The rab8<sup>−/−</sup> mice exhibit greatly reduced expression and intracellular mislocalization of PEPT1 in small intestine (Sato et al., 2007), and therefore, should be suitable to demonstrate the role of the transporter in drug absorption. In the present study, the uptake of cefixime from the apical side of small intestine isolated from rab8<sup>−/−</sup> mice was found to be quite small (Fig. 2A). Indeed, it could mainly represent nonspecific adsorption of cefixime, since the absolute value of the uptake was similar to that of mannitol (Fig. 2A, 2D). The uptake of cefixime in wild-type mice was inhibited by the dipeptide Gly-Sar and depended on the extracellular pH value, but that in rab8<sup>−/−</sup> mice was not inhibited by Gly-Sar and was pH-independent (Figs. 2D, 3). These results are consistent with the observation that oral absorption of cefixime was much lower in rab8<sup>−/−</sup> mice than in wild-type mice (Fig. 1A, Table 1). Because cefixime is not metabolized in the body and is mainly excreted in urine in unchanged form (Sakamoto et al., 1985), the decrease in AUC<sub>po,0-240</sub> and apparent BA values (Table 1) can be explained by the decrease in gastrointestinal absorption. Thus, the present findings suggest that PEPT1 plays an important role in gastrointestinal absorption of cefixime in mice, if we consider the mislocalization and reduced expression of PEPT1 in rab8<sup>−/−</sup> mice (Sato et al., 2007). This conclusion was further supported by the in vitro finding that Gly-Sar inhibited the uptake of
DMD #23689

cefixime in oocytes injected with cRNA for mouse PEPT1. The decreased uptake of another
β-lactam antibiotic, cephalexin, in small intestine of rab8+/− mice (Fig. 2C) also supports a role of
PEPT1 in gastrointestinal absorption of β-lactam antibiotics.

Similar down-regulation was also reported for another small intestinal nutrient transporter,
SGLT1, in rab8−/− mice (Sato et al., 2007). Therefore, the oral absorption of SGLT1 substrate
α-MDG was also examined in the present study, and was found to be decreased in rab8−/− mice (Fig.
1B, Table 1). However, gastrointestinal absorption of both cefixime and α-MDG was not
completely blocked in rab8−/− mice (Fig. 1A, 1B); the apparent BA values for both compounds in
rab8−/− mice remained as high as 40% of those in wild-type mice (Table 1). Thus, rab8 gene
deficiency alone does not result in complete loss of absorption of these compounds. One possible
explanation may be that small numbers of PEPT1 and SGLT1 molecules, which could not be
detected in the previous immunohistochemical analysis (Sato et al., 2007), are still present in apical
plasma membranes and are involved in membrane permeation of these compounds in rab8−/− mice.

Even a small amount of PEPT1 or SGLT1 present in the small intestine could be enough to mediate
significant absorption of these compounds during their movement inside the gastrointestinal tract.

Another possible explanation may be increased passive diffusion through the intestinal membranes
of rab8−/− mice, since oral absorption of antipyrine, the membrane permeation of which is thought to
represent passive diffusion, was slightly higher in rab8−/− mice than in wild-type mice (Fig. 1C).
The \( \text{rab8}^- \) mutation is lethal, and therefore, \( \text{rab8}^- \) mice at 20 days after birth were used in the present study. At this time, small intestines of \( \text{rab8}^- \) mice were swollen and longer than those in wild-type mice. Such morphological changes in the small intestine of \( \text{rab8}^- \) mice may be relevant to the change in antipyrine absorption. An increase in passive diffusion would lead to underestimation of the contribution of PEPT1 and SGLT1 to the overall absorption of cefixime and \( \alpha \)-MDG, respectively, in the present study. The developmental changes in expression of PEPT1 after birth in rodents may also be relevant to the estimation of the contribution of PEPT1. Limited information is available on developmental changes in PEPT1 expression in mouse small intestine, whereas the expression level of PEPT1 in rat small intestine is known to increase biphasically: it increases within 1 week after birth and, then after a transient decrease, it gradually increases again from two to 4 weeks after birth (Miyamoto et al., 1996; Shen et al., 2001). Therefore, the expression level of PEPT1 at 20 days could be lower than the adult level, and this may lead to underestimation of the contribution of PEPT1.

We recently proposed that both PEPT1 and carnitine/organic cation transporter (OCTN2/Slc22a5) are regulated by a PDZ (PSD95/Dlg/ZO-1) domain-containing scaffold protein, PDZK1, since the expression and apical localization of both transporters are down-regulated in small intestine of \( pdzk1 \) knockout mice, leading to delayed oral absorption of substrate drugs of PEPT1 and OCTN2 (Sugiura et al., 2008). PDZK1 physically interacts with PEPT1 and OCTN2
DMD #23689

(Sugiura et al., 2008; Kato et al., 2006), whereas it is not known whether a direct interaction of PEPT1 and SGLT1 with Rab8 occurs. Thus, the molecular mechanisms of apical localization of small intestinal transporters have recently been at least partly revealed, and not only transporter, but also the intracellular sorting machinery proteins could have a significant influence on drug absorption. In the cases of both Rab8 and PDZK1, at least two transporters are regulated by one intracellular protein. This implies that multiple transporters may be orchestrated by a single adaptor protein. Although the physiological meaning of such a transporter network is unknown, this concept may be relevant to the recent observation that PEPT1 substrate tripeptides (Gln-Cys-Pro and Gln-Ser-Pro) can exert post-transcriptional regulation of SGLT1 at the trans-Golgi network (Vernaleken et al., 2007). The association between two different transporters may thus have pharmacological significance.

In the present study, gastrointestinal absorption in vivo and uptake from the apical membrane of isolated small intestine in vitro of cefixime were examined and found to be much smaller in rab8−/− mice, compared with wild-type mice. Gastrointestinal absorption of α-MDG in rab8−/− was also lower than that in wild-type mice. It is concluded that PEPT1 and SGLT1 are involved in the gastrointestinal absorption of these substrates in vivo, if we consider the mislocalization and reduced expression of PEPT1 and SGLT1 in rab8−/− mice.
ACKNOWLEDGEMENT

We thank Ms Lica Ishida for technical assistance.
REFERENCES


*Biochim Biophys Acta* **1324**: 296-308.


Nakashima E and Tsuji A (1985) Mutual effects of amino-beta-lactam antibiotics and glycylglycine on the transmural potential difference in the small intestinal epithelium of rats. *J*


intestinal peptide transporter PepT1 and in human intestinal Caco-2 cells. *J Pharmacol Exp Ther* 277: 831-839.

FOOTNOTES

This study was supported in part by a Grant-in-Aid for Scientific Research provided by the Ministry of Education, Science and Culture of Japan and in part by a Grant from Japan Research Foundation for Clinical Pharmacology.
Fig. 1  Plasma concentration-time profiles of cefixime (A), α-MDG (B) and antipyrine (C) after single oral administration in wild-type (○) and rab8⁻/⁻ (●) mice.

Cefixime (20 mg/kg), [¹⁴C]α-MDG (25 µg/kg) and antipyrine (20 mg/kg) were orally administered in both strains of mice. Plasma concentrations of cefixime and antipyrine were measured by HPLC and LC-MS/MS, respectively, and that of α-MDG was measured as total radioactivity. Values represent the mean ± S.E.M. (n = 4-9).

*, Significantly different from wild-type mice by Student’s t-test (p < 0.05).

Fig. 2  Uptake of cefixime (A, B) and cephalexin (C) from apical side of small intestine

Small intestines were isolated from wild-type (open column) and rab8⁻/⁻ (closed column) mice, and uptake of each compound (1 mM) was measured using the everted sac method in the absence (A, C) or presence (B) of 20 mM Gly-Sar at pH 6.0. Panel D shows the apparent uptake of an extracellular marker, [¹⁴C]mannitol (5 µM). Values represent the mean ± S.E.M. (n = 3-7).

*, Significantly different from wild-type mice by Student’s t-test (p < 0.05).

#, Significantly different from the uptake in the absence of Gly-Sar by Student’s t-test (p < 0.05).

Fig. 3  Effect of pH of the incubation medium on uptake of cefixime from apical side of small

- 28 -
Small intestines were isolated from wild-type (○) and \textit{rab8}^{−/−}(●) mice, and uptake of cefixime (1 mM) was measured at various pH conditions. Values represent the mean ± S.E.M. (n = 3-6).

*, Significantly different from wild-type mice by Student’s t-test (p < 0.05).
### Table 1. Pharmacokinetic parameters of cefixime and α-MDG

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cefixime a)</th>
<th></th>
<th>α-MDG b)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wild-type</td>
<td>rab8⁻/⁻</td>
<td>Wild-type</td>
<td>rab8⁻/⁻</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>20 (po) / 10 (iv)</td>
<td></td>
<td>0.025 (po) / 0.025 (iv)</td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;po,0-240&lt;/sub&gt; c)</td>
<td>1.94 ± 0.20</td>
<td>0.800 ± 0.005*</td>
<td>9.28 ± 0.61</td>
<td>5.18 ± 0.88*</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;iv,0-240&lt;/sub&gt; c)</td>
<td>2.93 ± 0.15</td>
<td>3.15 ± 0.19</td>
<td>11.0 ± 1.9</td>
<td>15.0 ± 2.0</td>
</tr>
<tr>
<td>V&lt;sub&gt;des&lt;/sub&gt; (mL/kg)</td>
<td>225 ± 11</td>
<td>263 ± 47</td>
<td>ND d)</td>
<td>ND d)</td>
</tr>
<tr>
<td>Apparent BA e) (%)</td>
<td>33.1 ± 3.8</td>
<td>12.7 ± 1.7*</td>
<td>84.3 ± 15.3</td>
<td>34.6 ± 7.4*</td>
</tr>
</tbody>
</table>

a) Mean ± S.E.M (n = 3).
b) Mean ± S.E.M (n = 4-6).
c) Units of these parameters were mg · min/mL and µg · min/mL for cefixime and α-MDG, respectively.
d) Not determined because the elimination phase could not be fully observed.
e) Calculated as (AUC<sub>po,0-240</sub>/D<sub>po</sub>)/(AUC<sub>iv,0-240</sub>/D<sub>iv</sub>)

* Significantly different from wild-type mice by Student’s t-test (p < 0.05).
Fig. 1
Fig. 2

(A) Uptake (μL/mg tissue/15 min)

(B) Uptake (μL/mg tissue/15 min)

(C) Uptake (μL/mg tissue/10 min)

(D) Uptake (μL/mg tissue/15 min)

This article has not been copyedited and formatted. The final version may differ from this version.
Fig. 3

![Graph showing the relationship between pH of medium and uptake (μL/mg tissue/15 min).](image-url)