Contribution of Multidrug Resistance-Associated Protein 2 to Secretory Intestinal Transport of Organic Anions

Shirou Itagaki, Makoto Chiba, Masaki Kobayashi, Takeshi Hirano, and Ken Iseki*

Laboratory of Clinical Pharmaceutics & Therapeutics, Division of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University; Kita-12-jo, Nishi-6-chome, Kita-ku, Sapporo 060–0812, Japan.

Various mechanisms can influence the intestinal absorption and oral bioavailability of drugs. The barrier effects of efflux transporters may be one of the critical factors limiting the bioavailability of certain drugs. It has been reported that multidrug resistance-associated protein 2 (Mrp2) is expressed in the mucosal membrane of the epithelium of the small intestine and secretes various drugs into the jejunum lumen. However, it is possible that total intestinal secretion of Mrp2 substrates is accounted for the contribution of Mrp2 and other transporter(s) to the intestinal secretion of Mrp2 substrates. In this study, we found that phenolsulfonphthalein and pravastatin, both Mrp2 substrates, are transported by different transport systems in the intestine. These results suggest that contribution of transporters to the drug transport may be a critical factor affecting drug disposition and drug–drug interaction. In addition to evaluating the substrate specificity of a transporter, it is important to be aware of the contribution of a transporter to drug disposition.

Key words  anion; phenol red; transporter; interaction

Various mechanisms can influence the intestinal absorption and oral bioavailability of drugs. Permeation by diffusion is often predictable from a drug’s physicochemical properties. However, there have been numerous drugs exhibiting lower absorption rates after oral administration than expected from their physicochemical properties. It has been reported that intestinal absorption of some compounds is limited partly because they are preferentially transported in the secretory direction. Studies on the mechanisms of intestinal absorption of various ionic drugs have revealed that drug transporters involved in secretion, P-glycoprotein (P-gp/Abcb1) has been the most extensively investigated. Generally, a substrate of P-gp is thought to be a lipophilic and neutral or cationic drug. In addition to P-gp, several organic anion transport systems are expressed in the apical membrane of the epithelium of the small intestine and secrete various drugs into the lumen. p-Aminohippuric acid (PAH) and phenolsulfonphthalein (PSP) have been widely used as substrates for organic anion transport systems. Naruhashi et al. reported that PAH is preferentially transported in the secretory direction in the intestine. Moreover, it has been reported that PSP is transported from the serosal to mucosal side of the rat intestine and that a P-gp-like transporter is involved in this permeation. It has been reported that multidrug resistance-associated protein 2 (Mrp2/Abcc2) is expressed in the mucosal membrane of the epithelium of the small intestine and secretes various drugs into the jejunum lumen. We have found that PSP is secreted into the intestinal lumen by Mrp2-like transporter and that two Mrp2 substrates, PSP and pravastatin, are likely to be transported by different transport systems at the mucosal membrane. It is possible that the contribution of Mrp2 to the intestinal secretion of PSP is markedly different from that of pravastatin. By comparing the transport properties in normal rats and those in mutants whose Mrp2 function is hereditarily defective (e.g., Eisai hyperbilirubinemic rats (EHBBr)), the substrate specificity of Mrp2 has been clarified. The purpose of this study was to clarify the contribution of Mrp2 to the intestinal secretion of an Mrp2 substrate.

MATERIALS AND METHODS

Chemicals  PSP and pravastatin were purchased from Wako Pure Chemical (Osaka, Japan). Probenecid was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). d-[3H]-Mannitol was purchased from Daiichi Pure Chemicals Co. (Tokyo, Japan). All other reagents were of the highest grade available and used without further purification.

Animals  Male EHBR and male Sprague-Dawley (SD) rats, aged 6 weeks (200—250 g in weight), were obtained from SLC (Hamamatsu, Japan). The housing conditions were described previously. The experimental protocols were reviewed and approved by the Hokkaido University Animal Care Committee in accordance with the “Guide for the Care and Use of Laboratory Animals” as adopted by the National Institutes of Health.

Everted Sac Studies  Transport studies were carried out as described in a previous report. The medium used for all experiments was Tyrode’s buffer (137 mM NaCl, 3 mM KCl, 2 mM CaCl2, 2 mM MgCl2, 12 mM NaHCO3, 0.4 mM NaH2PO4 and 6 mM d-glucose). For everted sac studies, the jejunum was excised from the rat under anesthesia and rinsed in ice-cold saline. The intestinal segments were sliced onto a glass rod and the epithelial surface was exposed. After washing the epithelial surface with ice-cold saline, 5-cm-long everted segments of intestine were isolated. These everted segments were each ligated at one end. Then 100 μl of an experimental solution containing a substrate (100 μM pravastatin, 170 μM PSP or 100 μM d-mannitol) was injected into each segment, and each segment was ligated at the other end. The sac was immersed in 10 ml of the drug-free buffer. The buffer was prewarmed at 37 °C and preoxygenated with O2/CO2 (95:5) mixture gas. Under bubbling with mixture gas, the amount of the substrate transported from the serosal to mucosal surfaces across the intestine was measured by sampling the mucosal buffer periodically for 60 min.

* To whom correspondence should be addressed. e-mail: ken-i@pharm.hokudai.ac.jp © 2008 Pharmaceutical Society of Japan
Analytical Procedures  Pravastatin and PSP were determined using an HPLC system equipped with a Jasco 880-PU pump and 870-UV UV/VIS detector as described previously.\textsuperscript{10} The column was a Hitachi ODS Gel #3053 (4 mm i.d.×250 mm). Column temperature and flow rate were 55 °C and 0.7 ml/min, respectively. In the assay for pravastatin, a mobile phase containing 30% acetonitrile/30 m\textsuperscript{M} \text{HPO}_4 \text{ was used. The wavelength of the detector for pravastatin was 230 nm. In the assay for PSP, a mobile phase containing 20% acetonitrile/50 m\textsuperscript{M} \text{HPO}_4 \text{ was used. The wavelength of the detector for PSP was 432 nm. [\textsuperscript{3}H]-Man-}

RESULTS AND DISCUSSION

The barrier effects of efflux transporters may be one of the critical factors limiting the bioavailability of certain drugs. Oral drug delivery is generally the most desirable means of administration, mainly because of patient acceptance, convenience in administration, and cost-effective manufacturing. In clinical practice, patients usually take many kinds of drugs at the same time. It is well known that drug–drug interactions involving transporters can often directly affect the therapeutic safety and efficacy of many important drugs. The expression system of transporters is an efficient tool for clarifying the substrate specificity of transporters and for determining the transporter that recognizes the compound. However, various transporters are expressed in the apical membrane of the small intestine and secrete various drugs into the lumen.\textsuperscript{15} It has been reported that Mrp2 is present in the small intestine and that Mrp2 is involved in the secretion of organic anions in the small intestine.\textsuperscript{7,8} However, it is possible that total intestinal secretion of Mrp2 substrates is accounted for the contribution of Mrp2 and other transporter(s) to the intestinal secretion of Mrp2 substrates. Since the contribution of Mrp2 to the intestinal secretion of organic anions. These findings suggest that the contribution of Mrp2 to the intestinal secretion of PSP is markedly different from that of pravastatin. To confirm this hypothesis, we carried out a study using EHBR, whose Mrp2 function is hereditarily defective.\textsuperscript{12}

Firstly, we compared the serosal-to-mucosal permeation of pravastatin across the jejunum from SD rats and that from EHBR. The serosal-to-mucosal permeation of pravastatin across the jejunum from EHBR was not significantly different from that across the jejunum from SD rats (data not shown). Serosal-to-mucosal permeation of pravastatin across the jejunum from EHBR was significantly smaller than that across the jejunum from SD rats (Fig. 1). This finding suggests that Mrp2 plays a role in the jejunal secretion of pravastatin. We then investigated the serosal-to-mucosal permeation of PSP. Different from pravastatin, serosal-to-mucosal permeation of PSP across the jejunum from EHBR was significantly greater than that across the jejunum from SD rats (Fig. 2). In addition to Mrp2, several other transporters are involved in intestinal secretion. Total intestinal secretion of Mrp2 substrates is accounted for the contribution of Mrp2 and other transporter(s) to the intestinal secretion of Mrp2 substrates. Since the contribution of Mrp2 to the serosal-to-mucosal permeation of PSP across the jejunum from EHBR

Fig. 1. Time Courses of Serosal-to-Mucosal Permeation of Pravastatin across the Everted Jejunum from SD Rats and EHBR

The concentration of pravastatin was 100 µM. Each value represents the mean with S.D. of 4 measurements. * p<0.05, significantly different.

Fig. 2. Time Courses of Serosal-to-Mucosal Permeation of PSP across the Everted Jejunum from SD Rats and EHBR

The concentration of PSP was 170 µM. Each value represents the mean with S.D. of 4 measurements. * p<0.05, significantly different.

Fig. 3. Effect of Probenecid on the Serosal-to-Mucosal Permeation of PSP across the Everted Jejunum from EHBR

The concentration of PSP was 170 µM. Results were obtained at the end of a 60-min experiment. Each value represents the mean with S.D. of 4 measurements. * p<0.05, significantly different.
is considered to be minor, the efflux transport of PSP via organic anion transporter(s), which is distinct from Mrp2, in EHBR is likely to be greater than that in SD rats. We then investigated the effect of probenecid, an inhibitor of organic anion transporters, on the serosal-to-mucosal permeation of PSP across the jejunum from EHBR. Secretory transport of PSP was significantly reduced in the presence of probenecid (Fig. 3), suggesting that organic anion transporter(s), which is distinct from Mrp2, plays a major role in the serosal-to-mucosal permeation of PSP across the jejunum from EHBR. The serosal-to-mucosal permeation of PSP across the jejunum from EHBR was decreased to about the same level as that in SD rats in the presence of probenecid (Figs. 2, 3). In EHBR, up-regulation of other intestinal organic anion transport system(s) may compensate for the loss of Mrp2 function. Taking of these findings into consideration, we conclude that contribution of Mrp2 to the jejunal secretion of PSP is minor. An organic anion transporter(s) other than Mrp2 may be responsible for the intestinal secretion of PSP. Further studies are needed to elucidate the mechanism of intestinal secretion of PSP.

Since patients take many kinds of drugs at the same time, it is important to be aware of the potential of drug–drug interactions involving transporters. The results of this study indicated the possibility that contribution of transporters to drug transport is a critical factor affecting drug disposition and drug–drug interaction. Evaluating the substrate specificity of a transporter is not sufficient to study drug–drug interactions involving transporters; it is important to be aware of the contribution of transporters to drug disposition.

In summary, we have demonstrated that PSP and pravastatin, both Mrp2 substrates, are transported by different transport systems in the intestine. Further studies are needed to assess the contribution of other intestinal transporters to intestinal transport of their substrates. Such investigations will provide important information for prescription.

REFERENCES