Note

Studies on the Intestinal Absorption Characteristics of Sulfasalazine, a Breast Cancer Resistance Protein (BCRP) Substrate

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Summary: Oral sulfasalazine (SASP) is now used clinically as a probe substrate of a breast cancer resistance protein (BCRP) activity; however the intestinal absorption characteristics of SASP are not well understood. The purpose of this study was to clarify the characteristics of SASP transport in the mouse intestine. The everted ileum was incubated with SASP in the absence or presence of the Bcrp inhibitor Ko134. The steady-state intestinal absorptive clearance was 0.14 µL/min/cm in the absence of Ko134 and increased by 4.8-fold in the presence of Ko134. These results indicate that Bcrp mediates the efflux of SASP in the intestine. The absorptive clearance of SASP did not change in a concentration-dependent manner in the range of 0.1 to 50 µM in wild-type mice. By contrast, the absorptive clearance of SASP decreased significantly in a concentration-dependent manner in the presence of Ko134. Similar results were obtained in Bcrp−/− mice. These results suggest the possible involvement of some influx transporters in the intestinal absorption of SASP. In conclusion, both the influx and efflux transporters are involved in the intestinal absorption of SASP, which would explain why the absorptive clearance did not appear to change at various SASP concentrations in wild-type mice.

Keywords: sulfasalazine; BCRP; intestinal absorption; everted sac; transporter

Introduction

Sulfasalazine (SASP) has long been used in the treatment of inflammatory bowel diseases such as ulcerative colitis and Crohn’s disease, and rheumatoid disease.1,2 After oral administration, SASP is broken down into sulfapyridine and 5-aminosalicylic acid by bacterial azo reductases in the colon and cecum.3,4 5-Aminosalicylic acid is effective for inflammatory bowel diseases, while SASP and sulfapyridine are effective for rheumatoid disease.5-7 The targeting of SASP to the colon is critical for demonstrating its pharmacological action.

In humans, the bioavailability of orally administered SASP is less than 15%.8) The cause of the low bioavailability of SASP has been investigated and was attributed to low solubility and poor permeability.9 A recent study demonstrated that long-term treatment of human T-cells with SASP causes the development of cellular drug resistance that is mediated by the induction of breast cancer resistance protein (BCRP/ABCG2), suggesting that SASP is a substrate of human BCRP.10) BCRP is a member of the ATP-binding cassette family and is expressed widely in tissues such as the liver, kidney, brain, placenta, intestine, and colon.11) Like P-glycoprotein, BCRP is an efflux transporter located at the apical membrane in the small intestine and limits oral absorption. Zaher et al. reported that Bcrp plays an important role in the disposition of SASP in mice.12) In their study, the area under the plasma concentration time-curve (AUC) of SASP after oral administration was 111-fold higher in Bcrp−/− mice compared with wild-type mice. These results suggest that Bcrp limits oral absorption of SASP in the intestine, and mediates the elimination from the systemic circulation.

More recently, it has been suggested that efflux transporters such as Mrp2 and Bcrp limit the oral absorption of SASP, thereby enabling its colonic targeting.13,14) Yamasaki et al. and Urquhart et al. demonstrated a prominent role of BCRP polymorphisms in the intestinal absorption of SASP in humans.15,16) Furthermore, it has been demonstrated that SASP is a substrate of OATP2B1 in vitro using HEK293 cells expressing OATP2B1.17) OATP2B1, a multispecific organic anion influx transporter localized at the brush-border membrane of intestinal epithelial cells, is considered...
to mediate uptake of many endogenous substrates and xenobiotics from the lumen.

In our recent clinical study, we found a non-linearity in AUC of plasma SASP concentration between microdose and therapeutic dose. As one possible mechanism underlying the nonlinear pharmacokinetics of SASP, the saturation of the influx transporter, OATP2B1, was considered.\(^{17}\) However, the contribution of influx transporters to the intestinal SASP absorption has not been elucidated in detail, and only limited information is available on the characterization of SASP intestinal absorption. Thus, this study aimed to examine the intestinal absorption of SASP by using everted sacs from wild-type and Bcrp\(^{-/-}\) mice.

**Materials and Methods**

**Materials:** SASP was purchased from Sigma-Aldrich (St. Louis, MO, USA). Ko134 was kindly provided by SOLVO biotechnology (Budaörs, Hungary). All other chemicals were of analytical grade and are commercially available.

**Animals:** Male ddY mice were purchased from Sankyo Labo Service Corp. (Tokyo, Japan). Female Bcrp\(^{-/-}\) mice were purchased from Tacom Farms (Germantown, NY, USA) and were bred by Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan). Age-matched wild-type mice (FVB strain) were purchased from CLEA Japan (Tokyo, Japan). All mice (10–40 weeks) were housed in rooms maintained at 23°C and 55 ± 5% relative humidity, and were allowed free access to food and water during the acclimatization period. The animal work was performed at Hoshi University and complied with the regulations of the Committee on Ethics in the Care and Use of Laboratory Animals.

**Preparation of everted mouse ileum sacs:** Everted sacs were prepared by a modification of the procedure described previously.\(^{18}\) Mice were anesthetized with ether and sacrificed by exsanguination of the abdominal aorta. The ileum was removed immediately and rinsed in ice-cold Krebs-Ringer-Henseleit bicarbonate buffer (KRB; 118 mM NaCl, 4.75 mM KCl, 2.50 mM CaCl\(_2\), 1.19 mM KH\(_2\)PO\(_4\), 1.19 mM MgSO\(_4\), 25 mM NaHCO\(_3\), 11 mM d-glucose, pH 6.5). Approximately 5-cm segments of the ileum were isolated and everted using a stainless steel rod. Polyethylene tubes were inserted into both ends of the everted segments and ligated.

**In vitro absorptive transport (mucosal-to-serosal) study using everted mouse ileum sacs:** The everted sac was placed in 30 mL of KRB and gassed with O\(_2\)/CO\(_2\) (95:5) at 37°C. The everted ileum was filled initially with 1.5 mL of KRB and perfused with the buffer at 0.1 mL/min using an infusion pump (KD Scientific Inc., Holliston, MA, USA) throughout the transport study. After preincubation in the buffer containing 2 µM Ko134/ethylenediaminetetraacetic acid (Ko134 is 0.07µM).\(^{19}\) Therefore, we used 2 µM Ko134 to inhibit completely the efflux transport by Bcrp. The absorptive clearance was increased by 4.8-fold in the presence of Ko134 (Figs. 1A and 1B). These results indicate that Bcrp limits the intestinal absorption of SASP as reported previously.

The intestinal SASP absorption was also examined at various SASP concentrations. In wild-type mice, the absorptive clearance of 10 µM SASP reached a plateau at 60 min in the absence and presence of 2 µM Ko134, a known inhibitor of Bcrp. The absorptive clearance at 60 min was 0.14 µL/min/cm in the absence of Ko134. The reported 50% inhibitory concentration (IC\(_{50}\)) for the inhibition of BCRP by Ko134 is 0.07µM.\(^{19}\) Therefore, we used 2 µM Ko134 to inhibit completely the efflux transport by Bcrp. The absorptive clearance was increased by 4.8-fold in the presence of Ko134 (Figs. 1A and 1B). These results indicate that Bcrp limits the intestinal absorption of SASP as reported previously.

**Quantification of SASP concentration in samples:** The SASP concentration was analyzed by liquid chromatography/mass spectrometry (LC-MS/MS) in turbo ion spray negative ion mode using with the Prominance LC system (Shimadzu Co., Kyoto, Japan) coupled to an API4000 mass spectrometer (AB SCIEX, San Jose, CA, USA). The mass transition was from m/z 397 to 197. A CAPCELL PAK MGII C\(_{18}\) (2.1 × 50 mm, 5 µm; Shiseido Co., Ltd., Tokyo, Japan) maintained at 40°C was used for the chromatographic separation. The LC separation was achieved using a mobile phase comprising of 10 mM ammonium acetate (pH 8) and acetonitrile (2:8, v/v) at a flow rate of 0.2 mL/min.

The data acquisition was performed using Analyst ver. 1.4.2 (AB SCIEX, San Jose, CA, USA).

**Statistical analysis:** Each value is expressed as the mean ± standard error (S.E.) of 4–7 determinations. For group comparisons, analysis of variance (ANOVA) with a one-way layout was applied. Significant differences in the mean values were evaluated by Student’s unpaired t-test or Dunnett’s test for multiple comparisons. A p value of less than 0.05 was considered significant.

**Results and Discussion**

In wild-type mice, the intestinal absorption rate of 10 µM SASP reached a plateau at 60 min in the absence and presence of 2 µM Ko134, a known inhibitor of Bcrp. The absorptive clearance at 60 min was 0.14 µL/min/cm in the absence of Ko134. The reported 50% inhibitory concentration (IC\(_{50}\)) for the inhibition of BCRP by Ko134 is 0.07µM.\(^{19}\) Therefore, we used 2 µM Ko134 to inhibit completely the efflux transport by Bcrp. The absorptive clearance was increased by 4.8-fold in the presence of Ko134 (Figs. 1A and 1B). These results indicate that Bcrp limits the intestinal absorption of SASP as reported previously.

The intestinal SASP absorption was also examined at various SASP concentrations. In wild-type mice, the absorptive clearance of SASP was similar in the range of 0.1 to 50 µM (Fig. 2). By contrast, the absorptive clearance of SASP decreased significantly along with the SASP concentration in the mucosal side in the presence of Ko134 (Fig. 3). Such concentration dependence in the absorptive clearance of SASP was also observed in Bcrp\(^{-/-}\) mice (Fig. 4). Thus, the saturation of the absorptive rate at a high concentration of SASP in the presence of the specific Bcrp inhibitor and in Bcrp\(^{-/-}\) mice suggests the involvement of some influx transporters in the absorption of SASP. Taken together, our data suggest that both influx and efflux transporters are involved in the absorption of SASP. As a result, clearly neither process of intestinal absorption of SASP mediated by influx or efflux transporters was observed in wild-type mice (Fig. 2).

Recently, involvement of MRP2 in the intestinal absorption of SASP has been reported.\(^{13,14}\) However, considering the affinity and the expression of these efflux transporters,\(^{20}\) the contribution of MRP2 to SASP intestinal absorption is considered to be low in our study because we used the ileum for the experiments.

Kusuhara et al., reported that SASP was a substrate of OATP2B1 and its K\(_{m}\) was 1.7 µM.\(^{17}\) In our study, the K\(_{m}\) of influx transporter-mediated SASP absorption was estimated to be approximately 1 µM (Figs. 3 and 4) and this value was almost the same as the value calculated in their study. Jani et al. investigated the detailed kinetic characterization of SASP using membrane vesicles prepared from mammalian cells selectively overexpressing ABCG2, and calculated K\(_{m}\) of SASP to ABCG2 was about 0.7 µM.\(^{21}\) Therefore, the
Influx and efflux transporters involved in SASP absorption may have similar affinity for SASP.

In clinical use, the intestinal concentration of SASP was calculated to be 2.5 mM when the intestinal volume was assumed to be 1.92 L,22) which was far greater than its $K_m$ to OATP2B1 and saturated. Under this estimation, SASP may inhibit the absorption of OATP2B1 substrate drugs. In a clinical study of talinolol, which is substrate of OATP1A2 and OATP2B1 in humans and Oatp1a5 in rats,23,24) concomitant use of SASP decreased the AUC for talinolol.25) Although this mechanism is unclear, the drug-drug interaction must be noted when SASP is coadministered with a substrate of OATP2B1. Furthermore, in the clinical study of Kusuhara et al.,17) the dose-normalized AUC of SASP was much smaller in therapeutic dose than that in microdose. As they described in their reports, BCRP is considered not to be saturated even in the therapeutic dose and this nonlinearity is caused by the saturation of the influx transporter OATP2B1 at a therapeutic dose. Therefore, the absorption of SASP is mainly regulated by BCRP in clinical use.

In conclusion, this study suggests that both influx and efflux transporters are involved in the intestinal absorption of SASP.

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**References**

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