Introduction

Drug absorption, metabolism, and excretion are mainly controlled in the intestinal tract and liver. In these processes, drug-metabolizing enzymes and transporters play a key role in the bioavailability of orally administered drugs. Of these, cytochrome P450s (CYPs) are important drug-metabolizing enzymes in the oxidative metabolism of various drugs. CYP3A4, the predominant human hepatic CYP isoform, is associated with the metabolism of approximately 50% of current drugs on the market (1). Moreover, CYP3A4 is largely expressed in the human small intestine, accounting for an estimated 80% of total intestinal CYP content (2). P-glycoprotein (P-gp), encoded by the multidrug resistance (MDRI) gene, is also widely expressed in human organs such as the liver, small intestine, kidneys, and brain. In these organs, P-gp functions to transport several types of drugs from the intracellular to the extracellular region. For example, the absorption of anticancer agents, anti-arrhythmic agents, and immunosuppressants is restricted by intestinal P-gp in the small intestine (3, 4). In the liver, hepatic P-gp...
regulates the excretion of drugs to the bile duct (5). Interestingly, substrates of both CYP3A4 and P-gp overlap extensively (6). Based on in vivo experiments, researchers have demonstrated that CYP3A and P-gp have possible cooperative functions on drug metabolism and excretion in the liver and small intestine (7, 8).

In previously reported experimental colitis models, the expression levels and activities of CYPs were reduced in the liver (9, 10). Researchers have speculated that an unknown factor, such as a colon-derived bacterial antigen, is transported from the colon to the liver via the portal vein, resulting in liver injury. In addition, dysfunctions of the hepatobiliary system cause down-regulation of hepatic CYPs and P-gp, thereby affecting drug clearance (11 – 13). The hepatobiliary system and small intestine are closely linked to the secretion and absorption of bile acids, referred to as enterohepatic circulation. Through enterohepatic circulation, liver damage is likely to influence the action of the small intestine. However, few reports have investigated whether dysfunctions of the hepatobiliary system affect drug absorption, metabolism, and excretion in the small intestine.

Ulcerative colitis (UC) is chronic inflammatory disease restricted to the colon (14). The main pathogenesis of UC is thought to be an abnormal immune response based on genetic and environmental factors (i.e., dietary antigens, viral and bacterial infections, and intestinal bacterial flora changes). UC patients occasionally exhibit various complications unassociated with the colon, and dysfunction of the hepatobiliary system is a relatively common complication. Approximately 5% of UC patients develop abnormal manifestations in the hepatobiliary system, such as hepatic steatosis, cholangitis primary sclerosing, and cholelithiasis (15 – 17). Thus, in addition to the colonic pathology, UC may have aberrant effects on the hepatobiliary system. However, it remains largely unclear whether this dysfunction of the hepatobiliary system affects drug metabolism and excretion in the liver and small intestine in patients with UC.

In the present study, we examined the mRNA expression levels of inflammatory markers and hepatobiliary transporters and assessed the presence of secondary inflammation in the liver and small intestine in mice with dextran sodium sulfate (DSS)-induced colitis. Furthermore, we investigated changes in the mRNA and protein expression levels of CYP3A and P-gp in these organs and assessed the concentrations of cyclosporine A (CsA), a substrate of both CYP3A and P-gp, in the blood of mice with DSS-induced colitis.

Materials and Methods

Animals

Male C57BL/6 mice (6-week-old) were purchased from CLEA Japan (Shizuoka). The mice were maintained under an artificial 12-h dark–light cycle (lights on at 8:30 AM) at a constant temperature of 24°C ± 1°C and 55% humidity under specific-pathogen–free conditions. They were provided with the laboratory chow CE-2 (CLEA) and water ad libitum. All experimental procedures described were approved by the Animal Care Committee of Kobe Pharmaceutical University.

Induction of DSS-induced experimental colitis in mice

Ulcerative colitis was induced by feeding animals 5% (w/v) DSS (molecular weight, 36,000 – 50,000 Da; MP Biomedicals, Solon, OH, USA) in drinking water. The animals were given free access to water containing DSS for 7 days. Control animals received water without DSS. The animals were weighed and monitored for the appearance of diarrhea and blood in the stool throughout the experimental period.

Determination of the disease activity index (DAI)

Determination of the DAI was performed according to previously described methods (18, 19). Briefly, to evaluate the extent of intestinal inflammation, body weight, stool consistency, and blood in the stool were monitored daily using the criteria described in Supplementary Table 1 (available in the online version only).

Colon length and histological evaluation

After 7 days of treatment with DSS or water, the animals were sacrificed under deep ether anesthesia. The entire colon (from the end of the cecum to the anus) was removed immediately, and the colon length was measured. In addition, colon tissue samples were fixed in 10% formaldehyde and then embedded in paraffin. Seven-micrometer sections were prepared, stained with hematoxylin and eosin, and then microscopically evaluated for infiltration by polymorphonuclear leukocytes in the mucosa and mucosal erosion or ulceration.

Determination of myeloperoxidase (MPO) activity

MPO activity was measured as previously reported, with modifications (20, 21). The animals were sacrificed 7 days after the administration of DSS or water under deep ether anesthesia. The colon was excised quickly and rinsed with cold saline. Colonic samples were then homogenized in a 50 mM phosphate buffer containing 0.5% hexadecyl-trimethyl-ammonium bromide (pH 6.0; Wako, Osaka). Homogenized samples were subjected to freezing and thawing 3 times and then centrifuged at
350 × g for 10 min at 4°C. The protein concentration of the supernatant was measured using a bicinchoninic acid (BCA) assay kit (Thermo Fisher Scientific, Rockford, IL, USA).

MPO activity in the supernatant was determined by adding 5 µl of the supernatant to 95 µl of 10 mM phosphate buffer (pH 6.0) and 50 µl of 1.5 M o-dianisidine hydrochloride (Sigma, St. Louis, MO, USA) containing 0.0005% (w/v) hydrogen peroxide. Changes in the absorbance of each sample at 450 nm were recorded on a spectrophotometer (Multiskan Ascent; Thermo Fisher Scientific). MPO activity was obtained from the slope of the reaction curve, based on the following equation: Specific activity (µmol H₂O₂/min per mg protein) = (OD/min) / (OD/µmol H₂O₂ × mg protein).

Biochemical analysis

After 7 days of treatment with DSS or water, blood samples were collected from the inferior vena cava under deep ether anesthesia. The serum samples were isolated by centrifugation at 3,500 × g for 5 min at room temperature. Serum bile acid, total bilirubin, aspartate transaminase (AST), and alanine aminotransferase (ALT) were analyzed according to the standard biochemical methods at Mitsubishi Chemical Medience, Co. (Tokyo). The small intestinal lumen was washed with 1 ml cold saline, and the washing solution was collected. The concentrations of bile acids and phospholipids in the washing solution were measured by the Bile Acid Test Kit (Diazyme Laboratories, Poway, CA, USA) and Phospholipids C test Wako (Wako, Osaka), respectively.

Preparation of total RNA

Animals were sacrificed at 3, 5, and 7 days after the administration of DSS or water. The liver, small intestine, and colon were excised quickly and washed with cold saline. To examine site-specific differences, the small intestine was divided equally into 3 portions: upper, middle, and lower.

Total RNA was extracted from these tissues using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA), according to the manufacturer’s instructions. The quantity of RNA was measured using an ND-1000 spectrophotometer (NanoDrop Technologies; Wilmington, DE, USA). Two hundred nanograms of total RNA was subjected to reverse transcription (RT) as described below.

Quantitative real-time PCR

Purified total RNA was reverse transcribed with a ReverTra Ace qPCR RT Kit (Toyobo, Osaka) in a total volume of 20 µl. For real-time PCR amplification, 2 µl of cDNA was used as a template. The PCR amplification was performed on a Thermal Cycler Dice Real Time System (TaKaRa Bio, Otsu) with Thunder bird SYBR qPCR Mix (Toyobo). The PCR conditions were as follows: 40 cycles of 15 s at 95°C for annealing and 45 s at 60°C for extension. The primer sequences are shown in Supplementary Table 2 (available in the online version only). Relative expression levels of target genes were calculated by the delta-delta Ct method with β-actin used as a reference gene (22).

Preparation of microsomes and the plasma membrane fraction

The liver and small intestine were excised at the same time points as preparation of total RNA. For preparation of microsomes, the liver and the upper section of the small intestine were homogenized using a glass potter (30 strokes) in a buffer containing 10 mM potassium phosphate buffer (pH 7.4), 0.15 M potassium chloride, and protease inhibitor cocktail (Nacalai Tesque, Kyoto). The homogenate was centrifuged at 10,000 × g for 20 min at 4°C. The microsomal fraction was obtained by centrifuging the supernatant at 100,000 × g for 60 min at 4°C, and the pellet was suspended in potassium phosphate buffer (pH 7.4). Preparation of the small intestinal plasma membrane fraction was performed as previously reported (23), with modifications. Briefly, the upper section of the small intestine was homogenized using a glass potter (30 strokes) in a buffer containing 50 mM Tris-HCl (pH 7.4), 250 mM sucrose, and protease inhibitor cocktail. The homogenate was centrifuged at 3,000 × g for 10 min at 4°C. The plasma membrane fraction was obtained by centrifuging the supernatant at 15,000 × g for 30 min at 4°C, and the pellet was suspended in potassium phosphate buffer (pH 7.4).

Preparation of the small intestinal plasma membrane fraction was performed as previously reported (23), with modifications. Briefly, the upper section of the small intestine was homogenized using a glass potter (30 strokes) in a buffer containing 50 mM Tris-HCl (pH 7.4), 250 mM sucrose, and protease inhibitor cocktail. The homogenate was centrifuged at 3,000 × g for 10 min at 4°C. The plasma membrane fraction was obtained by centrifuging the supernatant at 15,000 × g for 30 min at 4°C, and the pellet was suspended in potassium phosphate buffer (pH 7.4).

Western blotting

Microsomal proteins (30 µg) and plasma membrane proteins (70 µg) were boiled in a quarter-volume of sample buffer [1 M Tris-HCl (pH 7.4), 640 mM 2-mercaptoethanol, 0.2% bromophenol blue, 4% sodium dodecyl sulfate (SDS), and 20% glycerol] and separated on 6% – 10% SDS polyacrylamide gels. The proteins on the gels were transferred to polyvinylidene difluoride membranes. The membranes were then blocked with 1% ECL Prime Blocking Reagent (GE Healthcare UK Ltd., Buckinghamshire, England) in Tris buffered saline (TBS) containing 0.5% Tween 20 for 1 h at room temperature. Anti-CYP3A goat polyclonal antibody L-14 (1:350; Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-P-gp mouse monoclonal antibody C219
Measurement of interleukin (IL)-1β levels

After 7 days of treatment with DSS or water, the liver was excised quickly and washed with cold saline. The liver was homogenized using a glass potter (30 strokes) in phosphate-buffered saline containing 0.05% Tween 20 (Nacalai Tesque) and protease inhibitor cocktail. The homogenate was centrifuged at 10,000 × g for 10 min at 4°C. The protein concentration of the supernatant was measured using a BCA assay kit.

The protein level of IL-1β was measured using a Ready-Set-Go mouse IL-1β enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA, USA), according to the manufacturer’s protocol. The concentration of IL-1β was calculated using standard curves.

Measurement of whole-blood CsA concentrations

After 7 days of treatment with DSS or water, CsA (Neoral; Novartis, Tokyo) was orally administered at a dose of 1 mg/kg. Blood samples were collected from the inferior vena cava under deep ether anesthesia at 30 and 60 min after the administration of CsA. The concentration of CsA in whole-blood was measured by the enzyme multiplied immunoassay technique (EMIT) using a Viva-E analyzer (Siemens Healthcare Diagnostics, Newark, DE, USA) and a CsA-specific assay kit (Siemens Healthcare Diagnostics) according to the manufacturer’s instructions (24).

Statistical analyses

Data are presented as the mean values ± standard error (S.E.M.) of at least 3 independent determinations for each experiment. Statistical significance was analyzed by Student’s t-test or Mann-Whitney U-test with SPSS version 20.0 (IBM, Tokyo). A probability level of 0.05 was used as the criterion for significance.

Results

Induction of DSS-induced experimental colitis

Animals treated with 5% DSS for 7 days displayed symptoms of colitis. In mice treated with DSS, weight loss and bloody stools were observed from day 3, and diarrhea was observed from day 4. DSS-treated mice exhibited significant increases in DAI scores compared with control mice on days 3 – 7 after administration of DSS (Fig. 1A). The colon length of DSS-treated animals was 4.3 ± 0.2 cm, significantly shorter than that of control mice (6.8 ± 0.3 cm, Fig. 1B).

MPO activity in the DSS-treated group, an index of neutrophil infiltration, increased about 4-fold over that of the control (Fig. 1B). Histological evaluation revealed the absence of pathological damage in the colonic mucosa of control animals. In contrast, the mucosa of DSS-treated mice showed edema, erosions, ulcerations, and infiltrates of polymorphonuclear leucocytes, with loss of the mucosal surface (Fig. 1C).

Changes in the expression of tumor necrosis factor (TNF)-α, IL-1β, IL-6, cyclooxygenase (COX)-2, and inducible nitric oxide synthase (iNOS) mRNAs in the liver and small intestine of mice with DSS-induced colitis

To examine the effects of DSS-induced colitis on the liver and small intestine, we analyzed the expression patterns of TNF-α, IL-1β, IL-6, COX-2, and iNOS mRNAs. As shown in Fig. 2A, the expression levels of TNF-α, IL-1β, IL-6, COX-2, and iNOS mRNAs in the colon were significantly increased at 3 days after administration of DSS. The expression of these inflammatory mediators reached a peak on day 5 after DSS treatment and gradually decreased at later time points (Fig. 2A).

In the liver, the levels of TNF-α and IL-6 mRNA were significantly increased at 3 days after administration of DSS (Fig. 2B). The expression levels of TNF-α and IL-6 were also elevated on days 5 and 7, respectively. Increased IL-1β mRNA was observed from day 3 and was maintained until day 7 (Fig. 2B). After 7 days of treatment with DSS, the mRNA levels of IL-1β, IL-6, COX-2, and iNOS were significantly elevated by 2.1 – 3.5-fold that of the control (Fig. 2B). In a preliminary experiment, the protein level of IL-1β was significantly increased in DSS-treated mice on day 7 (Supplementary Fig. 1: available in the online version only). In addition, DSS-treated mice exhibited significant increased level of serum AST and ALT compared with that of control mice (Supplementary Table 3: available in the online version only).

In the upper or middle sections of the small intestine, the mRNA levels of inflammatory mediators were not
iNOS mRNA expression in the upper section was significantly increased by 12.2-fold that of the control on day 7, whereas the change in iNOS expression in the middle section did not reach statistical significance (Fig. 2: C and D). DSS treatment did not significantly alter the expression levels of inflammatory markers in the lower section of the small intestine throughout the study period (Fig. 2E).

Changes in the mRNA expression of the hepatobiliary transporter in mice with DSS-induced colitis

DSS-induced colitis resulted in hepatic inflammation, which may affect the mRNA expression levels of hepatobiliary transporters. Thus, we examined the mRNA expression of 5 representative hepatobiliary transporters: bile salt export pump (Bsep), multidrug resistance 2 (Mdr2), multidrug resistance-associated protein 2 (Mrp2), sodium taurocholate cotransporting polypeptide (Ntcp), and organic solute transporter (Ost) α/β. As shown in Fig. 3, the expression level of Mrp2 mRNA was significantly decreased as compared to that of the control after 3 and 7 days of DSS administration. Additionally, Bsep and Mdr2 mRNA levels were decreased from day 5. After 7 days of treatment with DSS, Bsep, Mdr2, Mrp2, and Ntcp mRNA levels were significantly decreased by 54.0% – 71.0% that of the control. In contrast, no significant alterations in Ostα/β expression were observed between DSS-treated mice and control mice.

The concentrations of bile acid, total bilirubin, and phospholipids in serum and small intestinal lumen

Decreased expression of hepatobiliary transporter was observed in the liver of mice with DSS-induced colitis. Therefore, we measured the concentrations of bile acid, total bilirubin, and phospholipids in serum and small intestinal lumen to assess functional change in the hepatobiliary system. As shown in Table 1, there were no significant differences in the concentrations of bile acid and total bilirubin in serum between control and DSS-treated mice. In contrast, in the small intestinal lumen, the bile acid and phospholipids concentrations

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**Fig. 1.** Changes in DAI score (A), colon length and MPO activity (B), and histological evaluation (C) during DSS-induced colitis. Experimental colitis was induced by feeding animals 5% DSS in drinking water for 7 days. DAI score (A) and colon length and MPO activity (B) were determined as described in the Materials and Methods. Histological evaluation was performed using hematoxylin and eosin staining (magnification, 100 ×), and representative histological images are shown (C). Data are presented as the mean values ± S.E.M. for 5 – 6 mice per group. The significance of differences was evaluated by Student’s t-test or Mann-Whitney U-test (*P < 0.05 vs. control).
Decrease of CYP3A and P-gp by Colitis

with DSS-treated mice were significantly decreased as compared to those in the control.

Down-regulation of CYP3A and P-gp in the liver and small intestine of mice with DSS-induced colitis

Increased expression of various inflammatory markers was observed in the liver and upper small intestine of mice with DSS-induced colitis. Therefore, we examined mRNA and protein expression levels of CYP3A and P-gp (mdr1a), which are responsible for metabolism and excretion of clinically important drugs, in these organs. The expression of CYP3A11 mRNA in the liver was significantly decreased by 65.0% that of the control on day 3. Thereafter, the level of CYP3A11 mRNA was gradually decreased, reaching 96.0% inhibition on day 7 (Fig. 4A). The level of mdr1a mRNA tended to decrease in DSS-treated mice; however, this change did not reach statistical significance (Fig. 4A). Likewise, down-regulation of CYP3A protein was detected in DSS-treated mice. CYP3A protein expression was significantly decreased by 60.0% and 62.7% that of the control on days 5 and 7, respectively (Fig. 5A). In contrast, in the upper section of the small intestine, the expression levels of CYP3A11 and mdr1a mRNA were not significantly changed as compared to those of the control after 3 and 5 days of DSS administration (Fig. 4B). However,
on day 7, CYP3A11 and mdr1a mRNA levels were significantly decreased by 94.0% and 53.0% that of the control, respectively (Fig. 4B). In addition, down-regulation of CYP3A and P-gp protein levels was observed after 7 days of treatment with DSS, with the inhibition ratio of these targets being about 50.0% (Fig. 5B).

Nuclear receptor mRNA expression in the liver and small intestine of mice with DSS-induced colitis

It is well known that the transcriptional activation of CYP3A and P-gp is mediated by nuclear receptors such as the pregnane X receptor (PXR), constitutive androstane receptor (CAR), and retinoid X receptor α (RXRα). Therefore, we next examined the mRNA expression of these nuclear receptors in the liver and small intestine on day 7, when down-regulation of CYP3A11 and mdr1a mRNA expression was observed. In the liver, the expression of PXR mRNA was significantly decreased by 49.0% that of the control (Fig. 6A). In contrast, the expression of CAR and RXRα transcripts was not different from that of the control (Fig. 6A). The expression levels of these mRNAs were not significantly changed as compared to the control in the upper section of the small intestine (Fig. 6B).

Changes in whole-blood CsA concentrations in mice with DSS-induced colitis

In a preliminary experiment, blood samples were collected from the inferior vena cava in control mice at 30, 60, 120, and 240 min after administration of CsA, and it was found that whole-blood CsA concentrations peaked at 60 min (data not shown). To evaluate the absorption of CsA, Fig. 7 shows the changes of CsA up to 60 min in control and DSS-treated mice. At 30 and 60 min, whole-blood CsA concentrations were higher in DSS-treated mice than in the control mice. In addition, the concentration of CsA in DSS-treated mice

Table 1. The concentrations of bile acid, total bilirubin, and phospholipids in serum and small intestinal lumen

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control</th>
<th>DSS</th>
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<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
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<tr>
<td>Bile acid (uM)</td>
<td>2.27 ± 0.09</td>
<td>1.93 ± 0.17</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>&lt; 0.1</td>
<td>&lt; 0.1</td>
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<tr>
<td>Small intestinal lumen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile acid (uM)</td>
<td>124.5 ± 5.5</td>
<td>52.3 ± 15.1*</td>
</tr>
<tr>
<td>Phospholipids (mg/dL)</td>
<td>280.9 ± 37.0</td>
<td>136.3 ± 31.8*</td>
</tr>
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The concentrations of bile acid, total bilirubin, and phospholipids were determined as described in the Materials and Methods. Data are presented as the mean values ± S.E.M. for 3 – 4 mice per group. *Significantly different from the control at P < 0.05.
Fig. 4. Decreased expression of CYP3A11 and mdr1a mRNAs in the liver and upper small intestine of mice with DSS-induced colitis. Animals were sacrificed on days 3, 5, and 7 after the administration of DSS or water. Total RNA was extracted from the liver (A) and upper small intestine (B). Expression levels of CYP3A11 and mdr1a mRNAs were determined by real-time PCR as described in the Materials and Methods. The reported mRNA levels represent the ratio of DSS-treated mice vs. control mice (control = 1). Data are presented as the mean values ± S.E.M. for 4 – 5 mice per group. White columns represent expression in control mice, and black columns represent expression in DSS-treated mice. *Significantly different from the control at $P < 0.05$.

Fig. 5. Down-regulation of CYP3A and P-gp in the liver and upper small intestine of mice with DSS-induced colitis. Hepatic CYP3A protein levels were examined on days 3, 5, and 7 after the administration of DSS or water (A). In the upper small intestine, CYP3A and P-gp protein levels were analyzed on day 7 after DSS treatment (B). The protein levels of CYP3A and P-gp were determined by western blotting as described in the Materials and Methods. Densitometric quantification of CYP3A and P-gp was performed, and expression values were normalized to those of β-actin as a loading control. Representative blots of 4 independent experiments are shown. Data are presented as the mean values ± S.E.M. for 4 mice per group. White columns represent expression in control mice, and black columns represent expression in DSS-treated mice. *Significantly different from the control at $P < 0.05$. 
was significantly increased as compared to that in control mice at 60 min after administration. The whole-blood CsA concentrations at 60 min after CsA administration in the control and DSS-treated mice were 57.6 ± 6.0 and 102.5 ± 7.4 ng/ml, respectively.

Discussion

In this study, we used a mouse model of DSS-induced colitis to investigate the following 3 points: 1) the expression levels of inflammatory markers in the liver and small intestine; 2) changes in mRNA and protein expression levels of CYP3A and P-gp in the liver and the small intestine and expression levels of nuclear receptors associated with CYP3A and P-gp; and 3) whole-blood concentrations of CsA, a substrate of both CYP3A and P-gp.

DSS-induced experimental colitis, a model of human UC, is accompanied by erosion, ulceration, infiltration of inflammatory cells, and up-regulation of inflammatory marker genes (25–28). In this study, the expression levels of TNF-α, IL-1β, IL-6, COX-2, and iNOS mRNA in the mouse colon were significantly increased after the administration of DSS, and experimental colitis was clearly observed. However, the expression of these inflammatory markers reached a peak on day 5 after DSS treatment and decreased on day 7. One possible explanation for this discrepancy is that colonic mucosal tissue could be insufficient to produce cytokines due to the loss of the mucosal surface by severe injury (29). In this study, DAI scores were increased in DSS-treated mice gradually and the most severe symptoms of colitis were induced on day 7. In the livers of DSS-treated mice, inflammatory markers were increased, and their expression profiles were similar to those observed in the colon. In previous studies of patients with UC, bacterial products have been shown to translocate from the intestinal tract into the portal blood (30, 31). In addition, endotoxin and IL-6 are increased in the portal blood of animals with experimentally induced colitis (9, 32). These findings indicated the existence of the second inflammatory response in the livers of mice with DSS-induced colitis through the delivery of colon-derived bacterial factors and/or inflammatory cytokines.

After the administration of DSS, significantly increased expression of iNOS was found in the upper small intestine on day 7, but not before. DSS is a high molecular weight compound with a negative electric charge, and little DSS is absorbed from the small intestinal tract (33). In addition, it is generally accepted that DSS has an effect on the colonic mucous membrane (25). In our study, DSS treatment resulted in insignificant changes in inflammatory markers in the small intestine, except for the upper section on day 7. Thus, the increased expression of iNOS that was only observed in the upper small intestine on day 7, while those of the other inflammatory markers were not, suggests that up-regulation of iNOS was not associated with DSS treatment.

The small intestinal tract and hepatobiliary system forms an enterohepatic circulation based on the secretion
and re-absorption of bile acids. We hypothesized that liver damage caused by colonic DSS treatment could alter the excretion of bile acids, thereby affecting the further function of the small intestine. Two major types of hepatobiliary transporters are expressed in the hepatocyte. Bsep, Mdr2, and Mrp2 are localized to the canalicular membrane and export the components of bile, such as bile acids, bilirubin, and phospholipids, to the lumen (34). Conversely, Ntcp and Ostαβ are localized to the basolateral membrane as bile acid uptake transporters into the hepatocytes (34 – 36). In this study, significant down-regulation of Bsep, Mdr2, and Mrp2 mRNAs was observed on days 3 or 5 after treatment with DSS. The expression of Ntcp, but not that of Ostαβ, was significantly decreased on day 7. In addition, the concentrations of bile acid and phospholipids in the small intestinal lumen were significantly decreased in DSS-treated mice as compared to that in the control mice. These data suggested that the local dysfunction of bile acid and phospholipids excretion into the bile canaliculus occurred by downregulation of export transporters, but not systemic dysfunction such as cholestasis in DSS-treated mice. A reason for the latter may be due to a normal hepatic uptake of bile acid by uptake transporters. With our experimental settings, the period of downregulation of these transporters in the liver was consistent with that of the increased expression of inflammatory markers. Furthermore, TNF-α, IL-1β, and IL-6 have been shown to affect decreased mRNA levels of Bsep, Mdr2, Mrp2, and Ntcp in the mouse liver (37 – 39). Taken together, these data indicated the possible dysfunction of bile acid excretion through the disruption of hepatic transporters in DSS-induced experimental colitis.

In the livers of DSS-treated mice, significant downregulation of CYP3A was detected on days 5 and 7. In the upper small intestine, both of CYP3A and P-gp were decreased on day 7 after treatment with DSS. Importantly, the timing of changes in CYP3A and P-gp expression was consistent with that of up-regulation of inflammatory markers in the liver and upper small intestine. The transcriptional activation of CYP3A and P-gp is mediated by nuclear receptors, and PXR, CAR, and RXRα have a key role in this mechanism (40, 41). In the livers of DSS-treated mice on day 7, significant suppression of PXR was observed, but there was no suppression of CAR or RXRα. Several studies have reported that up-regulation of TNF-α, IL-1β, and IL-6 leads to decreased expression of PXR, which in turn results in the down-regulation of CYP3A and P-gp (39, 42). In addition, nucleo-cytoplasmic translocation is the other important factor in the gene regulation of PXR (43). In the livers of mice treated with DSS, our results support that the downregulation of CYP3A is, at least in part, dependent on expression of PXR.

Despite this, the expression levels of PXR, CAR, and RXRα were not decreased in the upper small intestine, although significantly reduced function of both CYP3A and P-gp was observed on day 7. This implied the presence of other mechanisms that affect the regulation of CYP3A and P-gp in the upper small intestine, but not the liver. Notably, the expression of iNOS was changed in parallel, as described above. NO is produced by iNOS, reacts with oxygen radicals, and forms cytotoxic nitrogen species. In previous studies, NO-mediated degradation of CYPs and P-gp was shown to be promoted by reactive nitrogen species (44, 45). However, future studies are needed to determine the exact mechanisms of the dysfunction of CYP3A and P-gp in the upper small intestine of DSS-treated mice.

CsA, an immunosuppressive drug used in patients with UC, is a substrate of both CYP3A and P-gp (6). In this study, we evaluated the concentration of CsA in the blood in the context of CYP3A and P-gp dysfunction in the liver and small intestine of DSS-treated mice. In DSS-treated mice, the concentration of CsA at 60 min after administration was significantly increased compared to that in the control mice. This finding indicated that CsA absorption is likely to be increased in DSS-treated mice. It is well known that the oral bioavailability of CsA is primarily controlled by CYP3A and P-gp in the liver and small intestine (7, 46). After 7 days of treatment with DSS, significant downregulation of CYP3A and P-gp was detected in the liver and upper small intestine. These data suggested that the decreased activity of CYP3A and P-gp in the liver and upper small intestine may affect CsA metabolism and excretion, causing the increased drug level in blood.

In conclusion, we found a novel inflammatory response in the upper small intestine of mice with DSS-induced colitis, in addition to the well-known second response in the liver. These secondary inflammations were accompanied by the down-regulation of CYP3A and P-gp in the liver and upper small intestine, supported by the increased expression of inflammatory markers in these organs. Furthermore, we showed that the down-regulation of CYP3A and P-gp in the liver and upper small intestine of mice with DSS-induced colitis may influence the bioavailability of CsA. Thus, the present findings provide useful information for the clinical use of CsA.

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References


Decrease of CYP3A and P-gp by Colitis


