Disturbance of Hepatic and Intestinal UDP-glucuronosyltransferase in Rats with Trinitrobenzene Sulfonic Acid–induced Colitis

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Summary: UDP-glucuronosyltransferase (UGT) is an important class of phase II metabolizing enzymes, playing a pivotal role in detoxifying various substances and in the pathological procedures of some diseases. The present study aims to uncover the potential dysregulation pattern of UGTs in trinitrobenzene sulfonic acid (TNBS)–induced colitis. Colitis was induced by intra-rectally administering a single dose of TNBS (100 mg/kg). The expression and enzyme activity of hepatic UGTs of colitis rats were all down-regulated significantly except UGT1A7, for which the mRNA level was up-regulated. In contrast, UGT isoforms in the small intestine were relatively unaffected. In the colon, where the inflammation occurs, the mRNA level and enzyme activity of UGT1A1 and 1A6 were down-regulated, but those of UGT1A7 and 2B1 up-regulated. The mRNA levels of various transcription factors, including AhR, CAR, PXR, PPARγ, and FXR were all decreased, except for AhR and CAR in the small intestine and colon. Our data suggests that colitis induces an isoform-dependent and tissue-specific dysregulation of UGTs and their related transcription factors.

Keywords: AhR; PPAR; PXR; FXR; nuclear receptors; phase II drug metabolism; UDP-glucuronosyltransferases; cytokines; inflammation; membrane barriers

Introduction

UDP-glucuronosyltransferase (UGT) is a class of phase II enzymes localized on the endoplasmic reticulum (ER) where many compounds are metabolized by conjugation with glucuronic acid.1) The addition of glucuronic acid generally increases the polarity and water solubility of compounds for subsequent excretion into bile or urine2); glucuronidation is thus generally regarded as a “detoxification” reaction. The UGT superfamily is mainly divided into two subfamilies, UGT1 and UGT2, and is expressed in both hepatic and extrahepatic tissues.3) Given that thousands of xenobiotics, including drugs, environmental toxicants, carcinogens and many endobiotics such as steroids, bile acids, bilirubin, and hormones, are substrates of UGTs, UGTs play a pivotal role in regulating various physiological, pathological, and pharmacological processes. Previous studies have shown that genetic lesions of UGT1A1 can result in hyperbilirubinemia, termed Crigler-Najjar syndrome in humans.4) Glucuronidation is also known to be the major detoxification mechanism of many toxic compounds5,6); deficient glucuronidation ability may result in elevated tissue concentrations of toxic substrates and increased risk of tissue damage. Moreover, extensive glucuronidation can be a barrier to oral bioavailability as the first-pass glucuronidation of orally administered agents usually results in poor oral bioavailability and lack of efficacy.7) Thus, the understanding of UGT regulation under both physiological and pathological conditions is of great concern in both experimental and clinical medicine.

However, current information concerning the potential dysregulation of UGTs in various pathological conditions is still very limited. Hepatic mRNA expression of UGT1A1, 1A9, and 2B5 were found down-regulated after LPS exposure; conversely, renal UGT2B5 was induced after LPS treatment.2) Besides, down-regulation of multiple hepatic UGT mRNAs was reported in turpentine-treated rats5) and human liver biopsies with increased inflammation.9) In contrast, we have recently found that the mRNA and protein levels of most hepatic UGT isoforms were significantly up-regulated while the enzyme activities remained unaltered in rats with thioacetamide-induced liver cirrhosis.10) All together, previous...
results imply that the dysregulation patterns of UGT enzymes are probably pathology-dependent and isoform-specific.

Inflammatory bowel diseases (IBDs), consisting of ulcerative colitis (UC) and Crohn’s disease (CD), are characterized by chronic inflammation of the gastrointestinal tract in genetically susceptible individuals exposed to environmental risk factors. The incidence of IBD has been increasing steadily in regions with high prevalence, such as North America and Europe, since initial identification in the 1960s and has now reached the current plateau. Despite the much lower prevalence of IBDs reported in developing regions, a steadily rising incidence of IBDs has been seen in many of those regions like Japan and China. Patients with IBDs are at increased risk of developing colorectal cancer (CRC) whose prevalence is found being intertwined with the course of IBDs.

Because patients with IBD may require life-long therapeutic treatments, the understanding of dysregulation patterns of drug-metabolizing enzymes is of great importance for the adjustment of dosage regimens. Current therapeutic drugs for IBD treatment usually consist of steroids and immunomodulators; and most of these drugs are the substrates of UGTs. Moreover, it has been found that the homeostasis of bile acids is disrupted in the case of IBDs, which may lead to consequent damage of intestinal tissue and present as an important cancer promoter. Both intestinal and hepatic UGT isozymes are responsible for maintaining the homeostasis of bile acids. Thus, a good recognition of UGT regulation in IBDs is not only meaningful for better therapeutic regimen design, but may also shed a light on the pathological process of these diseases. Considering its critical role as a metabolism barrier through detoxification of various carcinogenic factors as well as its sensitivity in mRNA expression to infection and inflammation, the expression and function of UGTs need to be characterized under the condition of colitis. However, the potential patterns of both hepatic and intestinal UGTs in the case of IBDs remain completely unknown. From the analysis above, we speculate that the regulation of UGTs is both isoform-specific and pathological condition-dependent; thus it is difficult to predict the possible outcomes of UGT dysregulations in IBDs from the studies of other diseases. Therefore, the present study was designed to uncover the dysregulation pattern of UGTs and their related nuclear receptors in the liver and intestine under colitis by using the trinitrobenzene sulfonic acid (TNBS)-induced colitis rat model.

Materials and Methods

Chemicals: TNBS was purchased from Fluka (Milwaukee, WI); UDP-glucuronic acid (UDPGA), α-saccharic acid 1,4-lactone, alamethicin, estradiol, estradiol 3-O-glucuronide, 4-methylumbelliferone (4-MU), 4-MU O-glucuronide, mycophenolic acid (MPA), and naloxone were all purchased from Sigma-Aldrich (St. Louis, MO). MPA O-glucuronide and naloxone 3-β-d-glucuronide were obtained from Toronto Research Chemicals Inc (Ontario, Canada). All other chemicals and solvents were of analytical grade or the highest grade commercially available.

Animals and treatments: Specific pathogen free (SPF) male Wistar rats (2 months old, 200–220 g) were obtained from Academy of Military Medical Sciences, China. Animals were housed in an air-conditioned room (25°C) under a 12-h light/dark cycle for 1 week before experiments and allowed water and standard chow ad libitum. All animal studies were approved by the Animal Ethics Committee of China Pharmaceutical University and have been carried out in accordance with the Declaration of Helsinki. Colitis was induced using the method described before with a little modification. In brief, rats fasted for 24 h were lightly anesthetized with isoflurane, and then 100 mg/kg TNBS solution in 30% ethanol (0.25 mL/200 g) was administered through a polyethylene catheter (2 mm in outer diameter) inserted rectally 6–8 cm from the anus. Rats of the control group were treated with 30% ethanol alone. All rats were weighed everyday and killed 3 days after the TNBS treatment. Blood for the assay of portal endotoxin, cytokines, and nitric oxide (NO) was sampled from the cannulated portal vein under anesthesia with diethyl ether. Immediately after the excision from the body, the small intestine, and the colon were placed in ice-cold phosphate-buffered saline and cut longitudinally, and then washed with ice-cold buffered saline to remove contents. The entire length of the small intestine and colon were divided equally into three parts longitudinally for PCR, Western blot, and enzyme activity assay. The liver was also harvested for UGT expression and activity detection subsequently.

Assessment of mucosal damage and biochemical analysis: Samples for histology were excited from the distal 6–8 cm of the colon, fixed in 10% buffered formalin, and embedded in paraffin blocks. Slices with 5 µm sections were stained with hematoxylin and eosin. Microscopic damage of the colonic mucosa was scored on a scale ranging from 0 to 5 by two observers blinded to the treatment given and based on the following parameters: percentage of area involved in erosions, mucosal edema, crypt distortion, ulceration and infiltration of mononuclear and polymorphonuclear cells.

Samples for myeloperoxidase (MPO) assay were harvested from the distal colon. MPO activity, a widely used marker to quantify neutrophil accumulation in tissue, was determined according to the O-dianisidine method described before.

Portal blood for assay of endotoxin was collected under pyrogen-free conditions. The endotoxin levels in portal serum were determined by the limulus amebocyte lysate test kit (Chinese Horseshoe Crab Reagent Manufactory, Co., Ltd., Xiamen). Portal blood for assay of NO formation was determined by the Griess reaction with little modification.

Enzyme-linked immunosorbent assay for cytokines: For enzyme-linked immunosorbent assay (ELISA) of IL-6, tissues (0.1 g) from the liver, small intestine and colon were homogenized in 1 mL Tris-EDTA (10 mM Tris-HCl, and 1 mM EDTA, pH 7.4) containing 0.05% sodium azide, 1% Tween-80, 2 mM PMSF, and 1 µg/mL of each of the following protease inhibitors: aprotinin, leupeptin, and pepstatin A. The lysate was centrifuged (15,000 × g, 4°C) for 15 min, and the supernatant was transferred to the ELISA plates. IL-6 concentrations were determined using commercial ELISA kits (R&D Systems, Abingdon, UK) and were expressed in unit/mg protein. The protein content was assayed by the BCA protein assay kit (Beyotime Institute of Biotechnology, Haimen, China) as described by the manufacturer. Serum interleukin-6 (IL-6), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) were determined using commercial ELISA kits (R&D Systems, Abingdon, UK) following the instructions of the manufacturer.

Preparation of total RNA and quantitative reverse transcriptase PCR (real-time PCR): Total tissue RNA extraction was performed using the RNAiso Plus reagent (TaKaRa Biotechnology Co., Ltd, Dalian, China) according to the manufacturer’s protocol. Purified total RNA was reverse-transcribed using the PrimeScript RT Reagent Kit (TaKaRa Biotechnology Co., Ltd.)
All the subcellular fractions were stored at −80°C before use.19) Each primer’s specificity was monitored using product melting curves in each reaction well.

**Western blot analysis:** For Western blot analysis of UGT1A1 and UGT1A6, 100 mg tissue was homogenized in 1 mL of ice-cold RIPA lysis buffer containing 1% protease inhibitor cocktail (Beyotime Institute of Biotechnology) and 1% phosphatase inhibitor cocktail (Beyotime Institute of Biotechnology). The tissue homogenate was centrifuged at 4°C and the supernatant was centrifuged at 9,000 g for 20 min to isolate pooled postmitochondrial fractions (S9). The enzymatic activity was determined by HPLC analysis according to the manufacturer’s protocol. Primer sequences are as follows (forward 5'-3', reverse 5'-3'): UGT1A1, ACCTGTCTGCTGCTTGTT; UGT1A6, AACTTCACTGACTACCTTG; GTAGGGACACACTCTCT; UGT1A7, GTGAGGATTTGTATTAAGG; TCTTTGGAGAACTTTAG; UGT2B1, AAAAAAGCATGTGGTGCC- CAC, GCCGAAGATACAAACGGTGTA; PXR: GATGATCA- TTGGTGATCAGGTGCTGCTG; GAGTGTGATGTTCCAGATTGTG; AhR, GGGTCTCCTATGCGTCCCT; TGGCTCTCTGATGCTATGATGAT; PPARγ, GACATTGCCATCTCTACT; CTCATCATGCGCGCTTCTC; FXR, CGTCTTTATATACCAACTC, ATTCGGCAGTGTTCATAG; CAR, CAGATCTCATCAACTCAGC; GACCGATCTCCTACTTT; IL-6, CACAAAGTCCGAGAGGAGAGAC, CAGAATTGCCATGGACACAC; IL-1β, CCTCTGGCAAGTCTCAGTT- CAGTCTC, GAAGTGCACGCTGTTTCTT; TNF-α, GAGAGA- TTGGCTGCTGCTGAAAC, TGGAGAACCATGATCAGGCTA; actin, TGGCTCTGCTGCTGATTCT; GGCACATACGCACTCCT- CTC. Real-time PCR was performed using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Bedford, MA) and SyBr Green reagent kit (TaKaRa Biotechnology Co., Ltd.) to determine UGT mRNA expression by the method described previously.13 Each primer’s specificity was monitored using product melting curves in each reaction well.

**Preparation of subcellular fractions (S9) and UGT activity detection:** Tissues from 8 rats were combined and homogenized in 1 mL of ice-cold RIPA lysis buffer containing 1% protease inhibitor cocktail (Beyotime Institute of Biotechnology) and 1% phosphatase inhibitor cocktail (Beyotime Institute of Biotechnology). The tissue homogenate was centrifuged at 4°C and the supernatant was centrifuged at 12,000 × g and were kept as tissue extracts. Protein concentration was measured by BCA assay. Protein lysates (80 μg/lane) were loaded onto 10% SDS-polyacrylamide gels (Beyotime Institute of Biotechnology), subjected to electrophoresis, and transferred to Immoblot polyvinylidene difluoride (PVDF) membranes (0.2 μm; Bio-Rad Laboratories). PVDF membranes were incubated in primary antibody overnight at 4°C, after which they were washed three times for 5 min in Tris-buffered saline containing 0.5% Tween 20. Membranes were then incubated with the appropriate secondary antibody for 1 h. Subsequently, membranes were washed three times for 5 min, and immunostaining was detected by chemiluminescence using the ECL Plus Western Blotting Detection System (GE Healthcare BioSciences KK, Piscataway, NJ) for UGT. Image capture was performed with an LAS-3000 system (FUJIFILM Corporation, Tokyo, Japan), and image analysis was carried out with MultiGauge software (FUJIFILM Corporation). The antibodies used were as follows: anti-UGT1A1 (sc-27419, Santa Cruz Biotechnology, Santa Cruz, CA) and anti-UGT1A6 (sc-27434, Santa Cruz Biotechnology).

**Results**

**Histopathological and inflammatory changes of TNBS-induced colitis:** Male Wistar rats treated intracolonically with TNBS (100 mg/kg) in 30% ethanol developed ulcerative colitis-like symptoms. TNBS-treated animals exhibited severe inflammation damage, as assessed from the colonic histopathological scores (range 0–5) (Fig. 1C). The histological features of the colons of the control group rats were typical of a normal structure, whereas the inflamed colon of rats with TNBS-induced colitis showed evidence of mucosal edema, crypt distortion, thickening of the colon wall, and a high level of leukocyte and polymorphonuclear infiltration (Figs. 1A and 1B). MPO activity of colonic mucosa, an additional marker of colitis severity and mucosal injury, was also increased in TNBS-treated rats (Fig. 1D). Endotoxin in the portal blood of the rats treated with TNBS was higher than that in control rats, indicating that TNBS-induced colitis resulted in portal endotoxemia (Table 1). Similar increases in portal concentration were observed for NO and proinflammatory cytokines, including IL-6, TNF-α, and IL-1β (Table 1). The expression of IL-6 was dramatically up-regulated in the liver, small intestine and colon; MPB and naloxyone. The concentration of UDPGA was confirmed to reach a plateau level for each UGT activity. S9 fractions were preincubated with alamethicin for 30 min at 4°C. The concentrations of estradiol, 4-MU, MPA, and naloxyone were 20, 100, 200, and 20 μM, respectively, all of which were concentrations below apparent Km values. Other experimental conditions were the same as the incubation conditions described by Shiratani et al. before. The enzymatic activity was determined by HPLC analysis as described previously.20,21}

**Fig. 1.** (Color online) Colon histopathological examinations and MPO activity
(A) Normal control and (B) TNBS model, hematoxylin and eosin staining of colon tissues, original magnification: 200×; (C) Histopathological scores; (D) MPO activity, results are means ± S.E.M. of 6 rats, **p < 0.01.
while the expression of IL-1β and TNF-α exhibited a different expression pattern in specific tissues (Fig. 2).

Isomform-differentiated dysregulation of UGTs in rats treated with TNBS: RT-PCR analyses were performed to determine the mRNA levels of UGT isoforms in tissues (including liver, small intestine and colon) of TNBS-treated rats and the control rats (Figs. 3A, 3B and 3C). The UGT isoforms we chose here have been previously reported with high expression levels in rats.22) As shown in Figure 3A, TNBS significantly decreased the mRNA levels of hepatic UGT1A1 (29% of control), 1A6 (17% of control), and 2B1 (62% of control) when compared to control group, whereas the mRNA level of UGT1A7 was up-regulated (145% of control). No significant changes were found for the UGT mRNA levels in the small intestine (Fig. 3B). In the colon, mRNA levels of UGT1A1 (14% of control) and 1A6 (26% of control) were found dramatically decreased; in contrast, UGT1A7 (172% of control) and UGT2B1 (388% of control) were significantly up-regulated.

Enzymatic activities of UGTs were determined using selective substrates, β-estradiol, 4-MU, MPA, and naloxone for UGT1A1,23) UGT1A6,23) UGT1A7,24) and UGT2B1,25) respectively (Supplementary Table S1). As shown in Figure 3D, the hepatic enzymatic activities of all UGT isoforms were down-regulated in rats treated with TNBS. Isoform-differentiated regulation was found for the enzyme activities of UGTs in colon tissue; TNBS treatment led to the decreased activities of UGT1A1 and 1A6 but enhanced activities of UGT1A7 and 2B1. The small intestine was less influenced by TNBS treatment. Results of enzyme activity assay were generally consistent with that of the mRNA determinations except for UGT1A7 in the liver.

To further validate these findings, Western blot analysis was performed to measure the protein levels of UGT1A1 and 1A6, whose antibodies were commercially available (Fig. 4). We observed an evident decrease of UGT1A1 (50% of control) and UGT1A6 (83% of control) in the colon tissues of rats treated with TNBS. Protein levels of all the UGTs detected in the liver were down-regulated, in accordance with the decreased mRNA expression and enzyme activity. Consistently, protein levels of UGTs in the small intestine were found with little change.

Dysregulations of ligand-activated transcription factors: Emerging evidence has demonstrated that ligand-activated transcription factors, such as pregnane X receptor (PXR), peroxisome proliferator-activated receptor γ (PPARγ), constitutive androstane receptor (CAR), farnesoid X receptor (FXR), and aryl hydrocarbon receptor (AhR), can regulate UGT transcription. Therefore, we further extended our research to assessing the expression levels of such transcription factors under the condition of colitis. As shown in Figure 5A, mRNA levels of all the five transcription factors in the liver were significantly decreased in rats treated with TNBS. In accordance with that observed for UGTs, mRNA levels of such transcription factors in the small intestine were less influenced by TNBS treatment, although the decrease of expression of PXR, PPARγ and FXR reached a significant level (Fig. 5B). Of interest, it was found that these transcription factors in the inflamed colons were differently regulated; PXR, PPARγ and FXR were down-regulated, whereas AhR was found to have been up-regulated (Fig. 5C).

Discussion

UGTs are major phase II drug metabolism enzymes that catalyze the glucuronidation of numerous endogenous and exogenous com-
pounds. This classic detoxification process is now known to play an important role in metabolism and different disease states in humans. But until recently, little was known about the potential dysregulation of UGTs in various pathological conditions.

Inflammation can alter drug metabolism in humans and rodents, resulting in significantly increased or decreased drug efficacy and potential toxicity. Significant decreases in hepatic UGT activities were reported after LPS administration in rats and isolated perfused mouse liver, but no effect on UGTs was found in mouse hepatocytes. LPS exposure and Citrobacter rodentium infection produce similar down-regulation of hepatic UGT isoforms. But renal UGT mRNAs were not significantly decreased during these exposures. As previously reported, different UGT isoforms may be differentially modulated, with differences in magnitude, time course, and effect, depending on the type and position of inflammation. Many UGT isoforms were abundantly expressed in the gastrointestinal tract, but even less information exists regarding glucuronidation in the gastrointestinal tract regulation during inflammation, especially local inflammation in the distal colon.

It has been reported that endogenous substances leaked from damaged colons in the rats with TNBS colitis led to down-regulation of hepatic P450s with differential susceptibility to the inflammatory stimuli. Because P450 metabolism is significantly suppressed during inflammation, glucuronidation may be similarly affected, for the regulation of P450s and UGTs have much in common and both of them are regulated by a cluster of largely overlapping transcription factors. To uncover the dysregulation pattern of UGTs and their relative nuclear receptors in the liver and intestine in the conditions of IBDs, a TNBS-induced colitis rat model was used.

Our study found that the local inflammation in the distal colon could not only disturb UGTs in the colon but also in the liver, and the disturbance was pathology-dependent and isoform-specific. Increased concentration of portal endotoxin after TNBS treatment, which had been reported in previous studies, suggests the development of mild endotoxemia in this colitis model. In addition, MPO activity in colonic mucosa and portal NO metabolites probably released from the injured colon were increased. NF-κB is a critical molecule regulating inflammatory and angiogenic processes. Sustained activation of NF-κB is detected in the intestinal lamina propria of patients with Crohn’s disease. The functional importance of NF-κB in colitis is based on its ability to regulate the promoters of a variety of genes whose products, such as pro-inflammatory factors, are critical for inflammatory processes. In the present study, we demonstrated the local inflammatory response by assessing the mRNA levels of TNF-α, IL-1β and IL-6 in specific tissues. In the liver, the significantly up-regulated expression levels of IL-6 and IL-1β, with minor changes in TNF-α expression were observed. In the colon, the expression levels of IL-6, IL-1β and TNF-α were all up-regulated significantly in TNBS rats. In the small intestine of TNBS rats, only the increased expression of IL-6 was observed. Higher levels of portal

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**Fig. 3. mRNA level (upper panel) and enzyme activity (lower panel) assay of UGTs**

Rats were intracolonically treated with 100 mg/kg of TNBS dissolved in 30% ethanol or 30% ethanol alone. The mRNA expression (A, B, C) and enzyme activities (D, E, F) of UGT1A1, 1A6, 1A7 and 2B1 in the liver, small intestine and colon were determined. For the intestine, the whole organ was cut longitudinally into three equal parts for PCR, Western blot and activity assay. Results of mRNA expression are means ± S.E.M. of 5 to 8 rats. *p < 0.05, **p < 0.01. Results of UGT activity were determined toward various substrates in pooled rat S9 (n = 8).
proinflammatory cytokines were also observed, which indicated a close relationship between the exposure of gut-derived mediators and the impaired UGT enzymes in the liver. The effects on UGTs during colitis may be inflammation-dependent, similar to cytokine-mediated P450 suppression after LPS treatment. Therefore, the relatively unaffected UGT mRNAs and protein contents in the small intestine are probably due to the indistinctive pathological damage. It has been reported that UGT1A1, 1A6, and 1A7 have mRNA expression in rat peritoneal macrophages. Macrophage populations include alveolar macrophages, Kupffer cells, peritoneal macrophages and bone marrow stroma, which also have UGT expression. Of these UGT isoforms, mRNA of UGT1A7 was specifically increased after LPS treatment. In our research, we found that UGT1A7 mRNA expression was also up-regulated in both the liver and intestine in colitis rats. Obviously, our study has provided a supplement to the specific regulation of UGT1A7 under inflammation by providing in vivo data. In this work, we have also noticed that MPA metabolism in the liver showed a down-regulated trend (Fig. 3D). To explain this contradiction, it is worth mentioning that MPA is a selective but not specific substrate of UGT1A7. Although UGT1A7 was the most efficient UGT isoform for MPAG synthesis, it can also be glucuronidated by UGT1A1, presumably due to a higher expression of UGT1A1 in the liver of rats, the MPAG production illustrated the same tendency with the changes of the UGT1A1 mRNA expression. Compared with UGT1A7, UGT2B1 was also out of tune in colitis rats, as it was down-regulated in the liver but up-regulated in the colon. To find reasons for the differential regulation in the liver and intestine, more studies will be needed.

In most cases, down regulation of CYP activities and protein levels are accompanied or preceded by decreases in the respective CYP mRNAs. The transcription of CYP2C11, 3A2, and 2E1 is suppressed to 20%, 30%, and 10% of control, respectively, within 1–2 h of LPS treatment in rats. The swiftness and magnitude of these effects suggest that transcriptional suppression is the primary mechanism for the decline of CYP mRNAs. There is relatively little information concerning regulation of Phase II enzymes during inflammation; however, emerging evidence has demonstrated that the regulation of P450s and UGTs has much in common and both of them are transcriptionally regulated by overlapping transcription factors. Negative crosstalk between NF-κB and the AhR that prevented AhR from activating the CYP1A1 genes was previously demonstrated. One study showed that NF-κB activation inhibits SXR and its target gene CYP3A4; however, down-regulation of

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CYP3A4 mRNA expression was not associated with changes in SXR mRNA expression but resulted from its transactivation.\(^46\) This is consistent with a recent report that shows that SXR gene expression is unaffected by TNF-\(\alpha\) in intestinal cells.\(^47\) Another report showed that TNF-\(\alpha\) was able to significantly reduce mRNA for nuclear receptor coactivators SRC-1 and SRC-2, thus limiting mRNA levels.\(^49\) NRs such as PXR are down-regulated in hepatoocytes by LPS or inflammatory cytokines,\(^50,51\) which is likely to be responsible for the inflammatory down-regulation of PXR agonist-inducible UGT expression.\(^52\) These results show that there is not likely to be a single common mechanism for the down-regulation of CYP and UGT genes by inflammatory mediators. In this study, we found a dramatic down-regulation of these transcription factors except for AhR in the colon. The decrease of the mRNA expression of the transcription factors could represent one possible molecular mechanism underlying colitis-induced repression of UGT enzymes. To assess the transcription factors functionally regulated, reporter assays will be needed in our future studies. A growing number of studies has recently detailed that AhR might participate in the innate immune system, which is capable of recognizing a wide variety of pathogens and rapidly inducing various antimicrobial and inflammatory responses.\(^53–55\) It has been demonstrated that AhR can be induced in macrophages stimulated by LPS, and also in naïve T cells by transforming growth factor (TGF) plus IL-6.\(^56–58\)

UGTs are involved in the conjugation of important endobiotic signaling molecules including bilirubin, steroid hormones, bile acids and eicosanoids, and of a plethora of xenobiotic biochemicals and marketed drugs.\(^59\) Immunofluorescence analysis has confirmed that UGTs are expressed selectively in the epithelial cell layer of the human colon, where the resorbed compounds would be available as substrates for the large pool of UGTs. In addition, UGT1A protein detected by Western blot has been found to be expressed at levels comparable to the human liver. The colon therefore exerts a “scavenger function” at the distal end of the digestive system.\(^59\) These considerations would assign a significance of UGTs to the human colon. In this paper, we also measured the \(\beta\)-glucuronidase activity of intestinal flora (Supplementary Fig. S1) which produce cytotoxic or carcinogenic agents by releasing noxious metabolites from nontoxic glucuronides and prolong the lifetime of toxicants in the body, and found elevated enzyme activities in the TNBS-treated group of rats, which is consistent with the previous findings.\(^61\) We believe that UGTs may serve to protect against de-conjugation of compounds designated to exit the body because enterohepatic circulation requires gastrointestinal means of glucuronidation to counteract the activities of bacterial \(\beta\)-glucuronidases. Deficient glucuronidation ability and increased bacterial \(\beta\)-glucuronidase activity in colitis may result in elevated tissue concentrations of those toxic substrates. In addition, the imbalance between UGTs and \(\beta\)-glucuronidase may be a direct risk factor for toxin accumulation and the pathological process in the development of colorectal cancer (CRC) for patients with ulcerative colitis and Crohn’s disease.\(^52\)

In conclusion, TNBS-induced experimental colitis leads to the disturbance of UGTs in the liver, small intestine and distal colon in an isoform-dependent and tissue-specific manner, and the regulation may be transcription factor-mediated. UGTs are part of the metabolism barrier in gastrointestinal system because of their detoxification function for a broad range of endogenous and exogenous compounds. Therefore, our data have provided insight into the pharmacokinetics and the target organ toxicity of the UGT substrates in colitis, and may have significant influences on the adjustment of dosage regimen design for IBD treatment.

References


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