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Alterations of testosterone metabolism in microsomes from rats with experimental colitis induced by dextran sulfate sodium

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ABSTRACT

Down-regulation of some hepatic cytochrome P450s (CYP450s) was observed in patients and animals with ulcerative colitis (UC). This study examined changes of CYP450s activities in microsomes of liver (RLMs), intestine (RIMs) and kidney (RRMs) from rats with experimental acute colitis induced by 5% dextran sulfate sodium (DSS) for 7 days and those receiving DSS treatment followed by 7-d cessation through measuring 6α-(CYP1A1), 7α-(CYP2A1), 16α-(CYP2C11) and 2β-/6β-(CYP3A2) hydroxytestosterone (OHT) formed from testosterone. Both pro-(IL-1β, IL-6, TNF-α) and anti-(IL-4, IL-10) inflammatory cytokines were elevated in acute colitis, while the production of the former was enhanced and that of the latter declined by DSS withdrawal. In RLMs, the CYP2A1 activity was significantly increased at DSS stimulation and partially returned to normal level when DSS treatment was terminated. Activity of other CYP450s were decreased by acute colitis and remained after DSS withdrawal. In RRMs, formations of 6α-, 16α- and 2β-OHT significantly declined in acute colitis and DSS termination further potentiated the down-regulation, while 7α-OHT formation was suppressed at DSS stimulation and remained after DSS withdrawal. The formation of 6β-OHT only showed significant decrease after DSS withdrawal. Two metabolites (6α- and 6β-OHT) formed in RIMs and 6β-OHT formation was significantly decreased by DSS stimulation and continued after DSS treatment halted. These findings indicate that the alterations of CYP450s activities vary with organ, CYP isoforms and colitis status, which arouse cautions on efficacy and toxicity of drug therapy during disease progression.

1. Introduction

Ulcerative colitis (UC), one of the major forms of chronic inflammatory bowel disease (IBD), is an intractable gastrointestinal disorder with the symptoms of erosion and ulcers in the colonic mucosa [1]. Patients with UC have an increased risk of developing colon cancer. A recent report has indicated the increasing number of UC patients in Japan [2]. Although many factors, such as environmental factors, infectious microbes, genetic susceptibility and dysregulated immune system, have been reported to be associated with UC [3], the exact cause of UC is still unclear. Rodent colitis induced by dextran sulfate sodium (DSS) is a well-established experimental model, which is highly stable and reproducible, and has been extensively used to study inflammatory bowel disease. Receiving continuous DSS treatment, rodents produce significant signs and symptoms resembling human UC, including weight loss, diarrhea, bloody feces and mucosal ulceration, as well as impaired cytokine profile [4].

CYP450s play crucial roles in biotransforming xenobiotics (drugs and environmental chemicals) and endobiotics (bile acids, fatty acids, prostaglandins, etc.) [5]. CYP450s are predominantly expressed in the liver. In addition, CYP450s are expressed appreciably in the small intestinal mucosa, kidney, lung and brain. Thus, CYP450s activities in these organs determine the local and systemic exposures of many drugs. Down-regulation in the activity and expression of some CYP450s has been reported in inflammation, which is associated with varied drug exposure and toxicity. It was reported that activity of liver CYP450s were markedly decreased in isoform-specific manner during inflammation in rodents [6]. Several hepatic CYP450s, such as CYP3A2, 2C11, 1A2, and 2E1 activities were decreased, while CYP2D2 activity was unaffected in rats with DSS-induced experimental colitis [7]. The down-regulation in activities of these hepatic CYP450s were also observed in rats with colitis induced by trinitrobenzene sulfonic acid (TNBS) [8]. In DSS-treated mice, hepatic mRNA and protein levels of CYP1A, 2C, 2D, 2E and 3A, as well as the CYP3A activity,

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were significantly decreased [9]. UC is characterized by periods of exacerbation (active disease) and remission (inactive disease) and progresses with dynamic immune status. Many disease-related factors, for example, IL-6 expression and eosinophil granulocyte activity, varied in different stages of UC [10,11]. The regulatory effects of many inflammatory mediators on activity and expression of hepatic CYP450s have been well characterized [12]. However, to our best knowledge, there is no report addressing whether the hepatic CYP450s activity alters with the dynamic immune status during UC progression.

Intestine and kidney are important extra-hepatic organs involved in drug absorption and elimination. The alterations in drug eliminating function in extra-hepatic organs were regarded as a compensatory mechanism in diseases. However, activities of intestinal CYP450s in inflammatory disease were relatively less addressed. Xu and co-workers reported a down-regulation of the mRNA and activity of intestinal cyp3a11 in lipopolysaccharide (LPS)-treated mice [13]. Alterations of renal CYP450s activities have been reported in diabetes, pregnancy and hypertension [14–16]. So far, the impact of colitis on intestinal and renal CYP450s activity has not been established.

Testosterone (TS) is a major androgen primarily secreted by the testes of males and the ovaries of females and plays a key role in human health and well-being. TS undergoes monohydroxylation which is catalyzed by different CYP450 isoforms at different sites [24,25]. In rat liver and intestinal microsomes, TS could be metabolized to 6α-, 7α-, 6β-/2β- and 16α-/2α-hydroxytestosterones (OHTs) by CYP1A1 [17], CYP2A1 [18,19], CYP3A2 [20,21], and CYP2C11 [8,22,23], respectively. Although the competition for the same substrate among different CYP450 isoforms may lead to overestimate activities of some CYPs while underestimate those of others, and minor contribution of other CYPs in formations of some monohydroxylated metabolites could not be ruled out, TS metabolism still serves as a convenient and useful tool for simultaneous determination of multiple CYP450s activities by measuring the metabolites formed by respective CYP450 isoform [24,25]. Exogenous TS has been successfully used to investigate some CYP450s activities in diseases, such as cardiac hypertrophy [22], diabetic mellitus [26], and heart failure [27].

Thus, in the present study, the activities of several CYP450 isoforms in microsomes of liver, intestine and kidney from rats treated with DSS for consecutive 7 days and those allowed to recover for additional 7-day after DSS cessation to achieve altered immune status were examined and compared with normal rats by measuring TS metabolism using an LC-MS/MS method. In addition, the levels of some inflammatory mediators under each condition were determined, attempting to assess the role of inflammatory mediators in the observed alterations in CYP450s activity.

2. Materials and methods

2.1. Materials

DSS (MW 36,000–40,000 Da) was purchased from MP Biomedicals (Santa Ana, CA, USA). Testosterone, 6α-OHT, 7α-OHT, 16α-OHT, 2β-OHT, 6β-OHT were purchased from Steraloid (Wilton, NH, USA). Glucose 6-phosphate (G-6-P), glucose-6-phosphate dehydrogenase (G-6-PD) and nicotinamide adenine dinucleotide phosphate (reduced form, $NADP^+$) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Hexadecyl-trimethylammonium bromide (HTAB), o-dianisidine dihydrochloride, sodium acetate and human leukocyte myeloperoxidase were supplied by Sigma–Aldrich (St. Louis, MO, USA). Dithiothreitol (DTT), phenylmethanesulfonyl fluoride (PMSF) and protease inhibitor (PI) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Milliplex Rat Cytokine/Chemokine Immunoassay Kit was supplied by Millipore (Bedford, MA, USA). Formic acid and methanol (MeOH) were HPLC-grade and purchased from Merck (Darmstadt, Germany). Ultra-pure water was obtained from a Milli-Q Plus water purification system (Millipore, Bedford, MA, USA). All other chemicals were analytical grade and obtained commercially.

2.2. Animals

Male Sprague-Dawley rats (six-week old, 200–220 g) were supplied by Animal Experimental Center, Guangzhou Academy of Medical Science (Guangzhou, China). The rats were housed in an air-conditioned room (temperature 22 ± 2 °C, relative humidity 50 ± 10%) under a 12/12 h light/dark cycle. Animals were acclimatized to the environment for 1 week with free access to standard chew and water before initiating DSS stimulation. The experimental protocol was approved (reference No.: ICMS-AEC-2013-05) by the Animal Ethics Committee, Institute of Chinese Medical Sciences at the University of Macau.

2.3. Induction of colitis in rats by DSS

Experimental colitis was induced by administration of 5% DSS (w/v) in drinking water *ad libitum* for indicated time periods. Animals were randomly divided into three groups (six animals per group) to receive drinking water (Normal group), 5% DSS in drinking water for 7 days to induce acute colitis (UC-A group), or 7-day DSS stimulation followed by 7-day drinking water to allow recovery (UC-R group), respectively. DSS solution was freshly prepared daily. The body weight was measured and behavior of each animal was recorded daily to obtain disease activity index (DAI). Rats were fasted overnight prior to sacrifice at day 7 (UC-A group) or day 14 (Normal group, UC-R group). On the last day of experiment, blood samples were collected and colon, liver, kidney and small intestine were removed and prepared as described below. One rat from the UC-A group died during the experiment due to severe reaction to DSS insult thus microsome preparation and data calculation of this group were based on other five animals survived.

2.4. Disease activity index (DAI)

Stool consistency, rectal bleeding and rectocele were evaluated daily throughout the experimental period according to the DAI scoring system described previously [28,29]. The score for each rat was determined as follows: stool consistency (0: normal, well formed pellets, 1: pasty stools that do not stick to the anus, 2: loose stools that stick to the anus, and 3: bloody loose stools), rectal bleeding (0: normal,1: +, slight bleeding, 2: ++, moderate bleeding, 3: +++, severe bleeding, 4: gross), rectocele (0: normal, 1: +, mild rectocele, 2: ++, severe rectocele). DAI values for rats from the same group were added together and then divided by the number of rats to give an average score for the group. The higher the score is, the more severely colitis happens. The data collected from the animals receiving the same and complete DSS treatment were used for comparison. Animals that died before the end of the experiment due to DSS insult were excluded from the study and data were not included for calculation.

2.5. Determination of cytokines in serum

On the last day, 200 μL of blood sample was withdrawn from each rat via orbital sinus. After centrifugation at 1000*g* for 10 min, serum was decanted for cytokine determination immediately. The levels of interleukin 1β (IL-1β), interleukin 4 (IL-4),