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Effects of dextran sulfate sodium induced experimental colitis on cytochrome P450 activities in rat liver, kidney and intestine

Nan Hu [a, c], Yanjuan Huang [a], Xuejiao Gao [a], Sai Li [a], Zhixiang Yan [a], Bin Wei [a], Ru Yan Associate Professor [a, b, *]

[a] State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Macao, China
[b] UM Zhuhai Research Institute, No.1 Software Road, Zhuhai Hi-tech Zone, Guangdong, China
[c] Department of Pharmacy, The First People's Hospital of Changzhou, Changzhou, Jiang Su, China

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ABSTRACT

Dextran sulfate sodium (DSS) induced experimental colitis presents a histologic resemblance to human ulcerative colitis (UC). Altered cytochrome P450s (CYPs) have been reported in this model and patients with UC. In this study, six CYPs activities were quantitatively determined in microsomes of liver (RLMs), kidney (RRMs) and intestine (RIMs) from rats with colitis at acute (5% DSS for 7 days, UCA) and remission (7-day DSS treatment followed by 7-day cessation, UCR) phases and compared with normal rats. Generally, CYPs activities varied with isoform, organ, and disease status. Hepatic CYP1A2, 2B1, 2C6/11, 2E1 and 3A1/2 activities were reduced by acute colitis and completely or partially restored after DSS was halted. Although DSS treatment decreased the V_{max} of renal CYP2C6/11 and increased that of CYP2D2, their $CL_{int,\ in\ vitro}$ were comparable among normal, acute and remission stages. DSS treatment changed the kinetics of CYP3A1/2-mediated nifedipine metabolism in RRMs from biphasic to classical kinetics. Notably, CYP2D2 activity was elevated in liver and kidney in acute UC, while enhanced in liver and decreased in kidney in remission. In intestine, CYP3A1/2 activity was increased in UCA and further enhanced after DSS withdrawal. These findings highlight the necessity of quantifying enzyme activity for precision drug therapy.

1. Introduction

Cytochrome P450s (CYPs) play an important role in inter-individual differences in drug response, which is one of the key factors to be considered in precision medicine. Many diseases, such as diabetes mellitus, chronic renal failure, hepatitis B virus cirrhosis and inflammatory diseases, have been reported varied CYP expressions and activities [1–3]. A variety of factors, in particular inflammation, participate in the regulation of CYPs under the pathological conditions, and thereby affect the drug disposition, leading to altered pharmacodynamics and toxicity [4].

Ulcerative colitis (UC) belongs to inflammatory bowel disease (IBD) and is characterized by periods of exacerbation (active disease) and remission (inactive disease), which are associated with different inflammatory states. Several CYP isoforms showed altered activities and/or expressions in UC patients, leading to changes in drug metabolism and disposition [5,6]. Both *in vivo* and *in vitro* studies showed that inflammatory cytokines could regulate the CYPs [7,8]. Some cytokines, for example, IL-6, exhibited disease-related alteration and varied in different stages of UC [9]. Correspondingly, Kusunoki and co-workers detected the decreases of mRNA levels of hepatic CYP3A11, 1A2, 2C29, 2D9 and 2E1 in mice during active stage of colitis induced by 10 days of DSS treatment and the subsequent restoration during remission [10]. However, whether the CYPs activities are also altered by UC and vary with disease status remains to be addressed.

The changes of CYPs function and expression have been investigated in experimental colitis animal models [11,12]. One of the mostly used colitis model is dextran sulfate sodium (DSS) induced colitis model. Dextran is a complex polymer of glucose, which is made of straight and branched chains, with highly variable molecular weight (range from 5, 000 to 1.4 million Da). DSS is polyanionic derivative of dextran, produced by esterification with

* Corresponding author. State Key Laboratory of Quality Research in Chinese Medicine, Institute of Chinese Medical Sciences, University of Macau, Taipa, Macao, China.
E-mail address: ruyan@umac.mo (R. Yan).

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chlorosulphonic acid. DSS cannot cross the cellular membranes, thus, is poorly absorbed after oral administration. The exact mechanisms through which DSS induces intestinal inflammation are unclear but may be the result of direct damage of the monolayer of epithelial cells in the colon which leads to the crossing of intestinal contents (e.g., commensal bacteria and their products) into underlying tissue and consequently induce inflammation. DSS induced colitis resembles human IBD in aetiology, pathology, pathogenesis and therapeutic response, thus, it is widely perceived as a good model of experimental colitis [13] (Solomon et al., 2010). It could induce symptoms of colitis, such as watery diarrhea, occult blood in stools, weight loss, etc. in animal models. Depending on the concentration, duration and frequency of DSS administration, animals may develop acute or chronic colitis. The DSS induced colitis model is now commonly used in small animals, such as mouse and rat, and the latter is often used to investigate the pharmacokinetic changes under disease state.

In rats with colitis induced by 3% DSS for 7 days, the activities of hepatic CYP3A2, 2C11, 1A2 and 2E1 were decreased, and CYP2D2 was unchanged [14]. Similarly, the hepatic mRNA and protein expression of CYP1A, 2C, 2D, 2E and 3A, and the CYP3A activity were also significantly decreased in mice treated with 3.5% DSS for 10 days [10,11]. When treated with 3% DSS for 3, 5 and 7 days, the protein and mRNA expression alterations of CYP1A, 2A, 2B, 2C, 2D, 2E, 3A and 4A in mice liver varied with CYP isoform and treatment durations [15]. Hepatic CYP3A was significantly decreased at both mRNA and protein levels in mice receiving 5% DSS at all designated time points [16]. Similar changes were also obtained in rats with trinitrobenzene sulfonic acid (TNBS)-induced colitis which showed the decline of the protein expression and activities of hepatic CYP3A2, 2C11, 1A2 and 2E1 [12]. Taken together, colitis generally diminished hepatic CYPs activity/expression levels. Quantitative information of these changes is critical for dose adjustment, while is lack in previous studies.

The extrahepatic CYPs are also important for overall drug disposition and exposure. They may serve as compensation mechanisms when the hepatic CYPs are altered by diseases. However, compared with the extensive study on hepatic CYPs, there is rather less information available for extrahepatic CYPs in diseases, including IBD. Immunohistochemical studies of colon biopsy showed that the expression of CYP2B6, 2E1 and 3A4 were higher and CYP2C9 and 1A1 were lower in UC patients, while all these CYP isoforms were expressed higher in colon of patients with Crohn's disease (CD) than their healthy counterparts [17]. Higher CYP3A4 and 3A5 mRNA expressions were also detected in CD non-inflamed duodenal biopsies compared with normal controls [18]. In contrast, decreased mRNA, protein, or activity of intestinal CYP3A was detected in mice with DSS-induced colitis [11] or LPS stimulation [19]. In addition, inflammation could also affect the CYPs in kidney. It has been reported that renal CYP2E1 and CYP4A activities were significantly increased in mice by LPS administration [20]. Thorough investigation of extrahepatic CYPs in experimental colitis models is lack.

CYPs catalyzing drug metabolism are classified into three families (CYP1, 2, and 3). They participate in metabolism of more than 85% of drugs in the market. The CYPs are dominantly expressed in the liver. The most abundant isoforms expressed in human liver are CYP3A, 2C, 1A2, 2E1, 2A6, 2D6 and 2B6, accounting for 40%, 25%, 18%, 9%, 6%, 2%, and less than 1% of total hepatic CYPs, respectively. Among them, CYP1A2 is liver specific isoform. Small intestine and kidney are two main extrahepatic organs expressing CYPs. In human intestine, CYP3A4 is the most abundant (80%), followed by CYP2C9 (15%), 2C19 (2.9%), 2J2 (1.4%) and 2D6 (1%) of total immunoquantified CYPs [21]. The information about CYPs in human kidney was relatively few. It was reported that the CYP2B6 and 3A5 were expressed in kidney of human, however, data regarding other CYPs were equivocal [22]. Probe substrates phenacetin, bupropion, tolbutamide, dextromethorphan, chlorzoxazone and nifedipine were commonly used for investigating human CYP1A2, 2B6, 2C9, 2D6, 2E1 and 3A4 activities, respectively. Because of the small differences in the primary amino acid sequences of the CYPs across species, some CYPs involved in drug metabolism differ between rats and humans [23]. In rats, it was CYP2B1, not CYP2B6, that catalyzed bupropion hydroxylation [24]. Tolbutamide, dextromethorphan and nifedipine were found to be metabolized by rat CYP2C6/11, 2D2 and 3A1/2, respectively [25,26].

Therefore, in this study, the changes of main CYP activities in liver, kidney and intestine of DSS-induced colitis rat model were investigated at different statuses of colitis by measuring the metabolism of specific probe substrates of CYP1A2, CYP2B1, CYP2C6/11, CYP2D2, CYP2E1 and CYP3A1/2 in microsomal proteins using an HPLC-MS/MS method.

2. Materials and methods

2.1. Materials

DSS (M.W. 36, 000-50, 000 Da) was purchased from MP Biomedicals, LLC (Aurora, Ohio, USA). Nifedipine, phenacetin, dextrorphan, bupropion, chlorzoxazone, tolbutamide, (±)-hydroxybupropion, dextrorphan tartrate, glucose 6-phosphate, glucose-6-phosphate dehydrogenase and nicotinamide adenine dinucleotide phosphate ($NADP^+$) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Paracetamol were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Dehydronifedipine, 4-hydroxytolbutamide and 6-hydroxychlorzoxazone were provided by Toronto Research Chemicals Inc. (Toronto, Canada). Rat Cytokine/Chemokine Immunoassay Kit was supplied by Millipore (Bedford, MA, USA). Acetonitrile and formic acid of high-performance liquid chromatography grade were purchased from Merck (Darmstadt, Germany). Purified water was prepared using a Milli-Q water purification system from Millipore Corporation (Millipore, Bedford, MA). All other chemicals and reagents were of analytical grade and commercially available.

2.2. Animals

Male Sprague-Dawley rats (180–200 g) were purchased from Guangzhou University of Chinese Medicine Laboratory Animal Center (Guangzhou, China). The animals were kept in specific pathogen free (SPF) grade room at 22–24 °C with a 12/12h light/dark cycle and 55–60% relative humidity. The animal experiment protocol was reviewed and approved (file no.: ICMS-AEC-2013-05) by the Animal Ethics Committee of the Institute of Chinese Medical Sciences, University of Macau (Macao, China). The rats were acclimatized to the animal facility for ten days before the beginning of the experiment.

2.3. Induction and assessment of colitis in rats

Colitis was induced by *ad libitum* oral administration of 5% DSS (w/v) in drinking water for indicated time periods (Fig. 1) as previously reported [27]. Animals were randomly assigned to three experimental groups (n = 6) to receive drinking water for 7 days before being given 7 days of 5% DSS in drinking water to induce acute colitis (UCA group), 5% DSS for 7 days followed by drinking water for another 7 days to allow recovery (UCR group), and drinking water alone for consecutive 14 days (Normal group). DSS solution was freshly prepared daily. The body weight, stool feature,