ENDOTOXIN-MEDIATED DISTURBANCE OF HEPATIC CYTOCHROME P450 FUNCTION AND DEVELOPMENT OF ENDOTOXIN TOLERANCE IN THE RAT MODEL OF DEXTRAN SULFATE SODIUM-INDUCED EXPERIMENTAL COLITIS

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ABSTRACT:

Hepatobiliary abnormalities have been described in patients with chronic inflammatory bowel diseases. Hepatic cytochrome P450 (P450)-dependent drug-metabolizing enzyme activities and susceptibility to a hepatotoxin, d-galactosamine, were determined in rats with dextran sulfate sodium (DSS)-induced colitis to assess whether liver function is affected in the model of inflammatory bowel disease. Colitis was induced by treatment of rats with 3% DSS in drinking water for 7 days. Liver microsomes for enzyme activities and serum for biological analysis were prepared from the rats with colitis, along with untreated and lipopolysaccharide (LPS)-treated rats. Other rats received intraperitoneal injection of d-galactosamine to assess their susceptibility to the toxic-induced liver injury. Treatment of rats with DSS resulted in not only colitis but also decreases in hepatic P450-dependent drug-metabolizing enzyme activities. Elevated endotoxin was found in portal blood, which was not associated with liver injury. The potency and the isoform selectivity in the suppression of the P450 enzymes by DSS treatment were similar to those of LPS-treated rats. Coadministration of antibiotics, polymyxin B or metronidazole, with DSS protected rats from decreases in some but not all P450 enzyme activities, indicating partial involvement of bacterial endotoxin in the P450 decreases. The rats with colitis were less susceptible than untreated rats to d-galactosamine-induced liver injury and TNF-α production, suggesting development of endotoxin tolerance in DSS-colitis. In conclusion, these results suggest that the DSS-colitis leads to endotoxin-mediated down-regulation of hepatic P450 enzymes and protection against d-galactosamine-induced liver injury, probably due to endotoxin tolerance.

Various hepatobiliary abnormalities have been described in patients with chronic inflammatory bowel diseases. They include increases in serum bilirubin and transaminase activities in patients with ulcerative colitis and Crohn’s disease (Desmet and Geboes, 1987). Liver biopsy specimens from patients with inflammatory bowel diseases showed inflammation of portal areas and primary sclerosing cholangitis (Fausa et al., 1991). The pathogenetic mechanisms responsible for the hepatobiliary alterations are not known, but several hypotheses have been proposed, such as autoimmunity, genetic factors, virus infections, and gut-derived bacterial antigens or toxins. Because gut-derived components are easily accessible to the liver via the portal vein, it is suggested that increases in the permeability of the intestinal epithelium in inflammatory bowel diseases allow bacterial antigens and toxins to enter the lamina propria and cause an inflammatory reaction when the bacterial products such as endotoxin reach the liver. To study the hypothesis that inflammatory mediators and bacterial products are involved in the development of the hepatobiliary abnormalities, experimental models have been applied. For example, Lichtman et al. (1991) developed a rat model of hepatobiliary injury resembling sclerosing cholangitis by surgical creation of jejunal self-filling blind loops that causes overgrowth of small bowel bacteria.

Chemically induced colitis models have been developed and extensively used for elucidation of pathogenetic mechanisms of inflammatory bowel diseases. One of the widely used experimental models resembling human ulcerative colitis has been developed by treatment of animals with dextran sulfate sodium (DSS) in drinking water for 6 to 10 days (Okayasu et al., 1990). Because DSS is a large and negatively charged molecule that cannot easily cross the membranes, it is poorly absorbed after oral administration (Lorentsen et al., 1989; Oshima et al., 1999). Thus, it is generally accepted that DSS exerts its effects on the luminal surface of the colonic mucosa (Okayasu et al., 1990; Ni et al., 1996). In addition to having direct effects on the intestinal epithelial cells in the acute injury (Ni et al., 1996), DSS causes regional inflammation within the colon through the up-regulation of T helper cell 1- and T helper cell 2-type cytokines (Dieleman et al., 1998). These cytokines as well as other inflammatory mediators are involved in the response in the chronic phase of DSS-induced colitis.

To characterize liver function in inflammatory bowel disease, we used a rat model of DSS-induced experimental colitis. Cytochrome P450 (P450) enzymes have been shown to be markedly down-regulated during inflammation and thus were recently evaluated as one of...
the negative acute phase proteins (Siewert et al., 2000). Therefore, the present study initially focused on the effects of DSS on P450-dependent monoxygenase activities as possible markers of altered liver function by gut-derived inflammatory mediators. Furthermore, we investigated whether the experimentally induced colitis predisposes rats to liver injury induced by a hepatotoxin, β-galactosamine, which does not require metabolic activation for manifestations of its toxicity.

Materials and Methods

Animals. Male Wistar rats (2 months old) were obtained from Takesugi Experimental Animals (Saitama, Japan). The animals were housed in an air-conditioned room (25°C) under a 12-h light/dark cycle for 1 week prior to use. Food (Oriental Yeast Co., Ltd., Tokyo, Japan) and water were given ad libitum. All animal experiments were performed according to the criteria for humane care as outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health.

Chemicals. DSS (molecular weight, 5000), LPS (Escherichia coli, serotype O111:B4), polyoxin B sulfate, metronidazole, β-galactosamine hydrochloride, 4-nitrophenol, and 4-nitroacetate were purchased from Wako Pure Chemicals (Osaka, Japan); testosterone, phenacetin, 4-acetamidophenol, and propranolol hydrochloride were from Sigma-Aldrich (St. Louis, MO); 6β-, 6β-, and 16α-hydroxytestosterone were from Steraloids (Wilton, NH); 7-hydroxypropranolol was synthesized as hydrochlorides according to the method of Oatis et al. (1981). Glucose 6-phosphate, glucose-6-phosphate dehydrogenase, and NADPH were purchased from Oriental Yeast Co., Ltd. All other chemicals and solvents used were of analytical grade.

Experimental Design. Colitis was induced by feeding rats 3% DSS dissolved in drinking water for 7 days, and the rats were killed by decapitation on the seventh morning. Control rats received water without DSS. Other rats were given LPS intraperitoneally at a dose of 4 mg/kg body weight and the rats were killed 8 or 24 h after the administration. In experiments to examine the effects of antibiotics, polyoxin B or metronidazole at concentrations of 72 mg/L or 400 mg/L, respectively, dissolved in drinking water was coadministered with DSS. A model of acute liver injury was made by the treatment of rats with β-galactosamine intraperitoneally at a dose of 800 mg/kg, and the rats were sacrificed 24 h after the injection. Blood for the assay of the portal endotoxin was sampled from cannulated portal vein under anesthesia with diethyl ether. Blood for the other assays was sampled from carotid artery. The blood was allowed to coagulate, and the samples were then centrifuged to obtain serum. Colonic mucosa was scraped off with a glass slide. Liver microsomal fractions were prepared according to the method of Omura and Sato (1964). Protein concentrations were assayed by the method of Lowry et al. (1951).

Biochemical Analysis. Myeloperoxidase (MPO) activity of colonic mucosa was measured as a marker of tissue granulocyte infiltration according to a modification of the method of Schierwagen et al. (1990). Colonic samples were placed in a 0.5% solution of hexadecyltrimethylammonium bromide in 50 mM potassium phosphate buffer, pH 6.0. After heating for 2 h at 60°C, the samples were homogenized and centrifuged. The supernatant was reacted with 3,3′,5′-tetramethylbenzidine, and the absorbance was determined at 630 nm.

Liver injury was evaluated by serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) leakage as determined spectrophotometrically (glutamic-oxaloacetic and glutamic-pyruvic transaminase; Sigma Diagnostics, St. Louis, MO). Serum tumor necrosis factor α (TNF-α) was determined by enzyme-linked immunosorbent assay (Cytoscreen; BioSource International, Camarillo, CA). Portal blood for assay of endotoxin was collected under pyrogen-free conditions. The endotoxin levels in portal serum were determined using the Endospecy; Sekigaku Co., Tokyo, Japan).

Assay of Enzymatic Activities. Testosterone 2α-, 6β-, and 16α-hydroxylation, phenacetin O-deethylation, 4-nitrophenol hydroxylation, and propranolol 7-hydroxylation activities were determined according to the reported high-performance liquid chromatography (HPLC) methods (Tassaneeyakul et al., 1993; Masubuchi et al., 1994, 1995) with minor modifications. The standard incubation mixture (1 ml) contained 0.5 mg/ml liver microsomes, 10 mM glucose 6-phosphate, 2 units/ml glucose-6-phosphate dehydrogenase, 5 to 10 mM MgCl2, and each substrate in 0.15 M potassium phosphate buffer (pH 7.4), except for phenacetin O-deethylation (assayed in a 1-ml mixture with 0.15 M Tris-HCl buffer, pH 7.4) and p-nitrophenol 4-hydroxylation (assayed in a 0.5-ml mixture with 0.15 M potassium phosphate buffer, pH 6.8). Substrate concentrations used were 50 μM testosterone, 10 μM phenacetin, 50 μM 4-nitrophenol, 20 μM propranolol. After equilibration at 37°C for 5 min, the reaction was started by adding NADPH (final concentration of 0.5 mM), and the incubation was performed for the following periods: testosterone, 2.5 min; phenacetin, 10 min; 4-nitrophenol, 10 min; propranolol, 2 min. All of the assays were performed under conditions where metabolite formation was linear with much of incubation time. The metabolites were extracted into ethyl acetate, the organic layer was evaporated to dryness, and the residue was dissolved in 0.1 ml of a mobile phase for the HPLC. The HPLC conditions were the same as the reported methods (Tassaneeyakul et al., 1993; Masubuchi et al., 1994, 1995), except that the reversed-phase column (Inertsil ODS 3; GL Sciences Ltd., Tokyo, Japan) was used for the assays in this study.

Statistical Analysis. The experimental groups were compared by analysis of variance, followed by Newman-Keuls multiple comparisons test to determine significant differences between the group means.

Results

Induction of Colitis by DSS. Treatment of rats with 3% DSS by drinking water for 1 week resulted in ulcerative colitis-like symptoms. Severe bloody diarrhea was macroscopically observed and mucosal injury was demonstrated by a marked increase in MPO activities (Table 1). These results indicated that induction of colitis by giving DSS was also observed in the present study. Endotoxin levels detected in the portal blood of the rats treated with DSS were higher than those in control rats (Table 1). Thus, DSS-induced colitis resulted in portal endotoxemia. Despite the elevated portal endotoxin, there was no sign of liver injury in the DSS-colitis rats as judged by liver weight and serum ALT leakage. In contrast, the LPS-treated rats (4 mg/kg, intraperitoneally) caused liver toxicity as evidenced by ALT leakage (Table 1). The body weight in the treated rats was not different from control rats (Table 1).

Decreases in P450 Activities in DSS-Colitis Rats. The following liver microsomal oxidation activities were measured as indices of specific P450 enzymes expressed in normal liver (Reinke and Moyer, 1985; Imaoka et al., 1988; Sesardic et al., 1990; Masubuchi et al., 1993): testosterone 6β-hydroxylation, CYP3A2 (Fig. 1A); testosterone 16α- and 2α-hydroxylation, CYP2C11 (Fig. 1B and C); phenacetin O-deethylation, CYP1A2 (Fig. 1D); 4-nitrophenol hydroxylation, CYP2E1 (Fig. 1E); propranolol 7-hydroxylation, CYP2D2 (Fig. 1F). All of the enzyme activities, except CYP2D2, in DSS-colitis rats were lower than those in control rats. LPS administration also decreased the enzyme activities. CYP2C11 and CYP1A2 were decreased by 8 h after injection, whereas CYP3A2 and CYP2E1 were decreased by 24 h. The treatment with DSS, like LPS, was ineffective in decreasing CYP2D2. The levels of decreased enzyme activities in the DSS-colitis rats were comparable with those found 24 h after treatment with LPS.

Protection against Loss of Enzyme Activities in DSS-Colitis Rats by the Coadministration of Antibiotics. To examine the role of enteric bacteria and their cell wall components in the loss of the liver monoxygenase activities, an antibiotic, polyoxin B or metronidazole, was coadministered with DSS. The former binds to endotoxin and has been used to neutralize it, and the latter has been used as a treatment against anaerobic bacteria. Neither of the antibiotics affected basal P450 enzyme activity (shown in the legend of Fig. 2). Coadministration of polyoxin B with DSS abrogated the loss of testosterone 6β-hydroxylation (Fig. 2A) and 4-nitrophenol hydroxylation (Fig. 2D) activities in DSS-colitis rats almost completely. On the other hand, testosterone 16α- (Fig. 2B) and phenacetin O-deethylation (Fig. 2C) activities were still depressed in the rats given...
Liver microsomes were obtained from rats given 3% DSS in drinking water for 7 days (DSS) or with polymyxin B (+Pol) or metronidazole (+Met) for 7 days. The microsomes were subjected to the assay of testosterone 6β-hydroxylation (A), 16α-hydroxylation (B), phenacetin O-deethylation (C), and 4-nitrophenol hydroxylation (D) activities. Results represent the percentage of the control activity without DSS. Control activities are: 2.32 ± 0.13 nmol/min/mg protein (A, DSS); 2.58 ± 0.36 nmol/min/mg protein (A, +Pol); 1.96 ± 0.23 nmol/min/mg protein (A, +Met); 1.08 ± 0.09 nmol/min/mg protein (B, DSS); 1.18 ± 0.24 nmol/min/mg protein (B, +Pol); 1.04 ± 0.25 nmol/min/mg protein (B, +Met); 0.176 ± 0.008 nmol/min/mg protein (C, DSS); 0.176 ± 0.008 nmol/min/mg protein (C, +Pol); 0.180 ± 0.018 nmol/min/mg protein (C, +Met); 1.42 ± 0.12 nmol/min/mg protein (D, DSS); 1.31 ± 0.18 nmol/min/mg protein (D, +Pol); 1.70 ± 0.17 nmol/min/mg protein (D, +Met). Results are means ± S.E. Numbers of rats used are 4 (control, control + Pol), 5 (control + Met); 6 (DSS + Met); and 10 (DSS). *p < 0.05, **p < 0.01, significantly different from control.
colitis, suggesting that tolerance to endotoxin occurs in the colitis. In the present study we observed that a panel of diverse P450 enzymes underwent disturbance as assessed by microsomal drug-metabolizing enzyme activities in livers of DSS-colitis rats. The decrease in P450 enzyme activities was not accompanied by liver injury, even though DSS treatment had increased endotoxin levels in portal vein, confirming development of mild endotoxia in this colitis model. The disturbance of P450 enzymes without liver injury in the DSS-treated rats can be accounted for by high susceptibility of the P450 enzymes to inflammatory responses.

P450 enzyme activities in the DSS-colitis rats were comparable with those in LPS-treated rats, indicating that exposure of gut-derived mediators to the liver impairs P450 enzymes to the same extent as those by the bolus intraperitoneal dose of LPS. Except for CYP2D2, which appears less susceptible than others, apparent isozyme selectivity was not found. CYP3A2 and CYP2E1, slow responders to exogenous LPS, were found to be protected by polymyxin B against the impairment of P450 enzymes in the DSS-colitis rats, suggesting the involvement of endotoxin, as well as by metronidazole. It is conceivable that a decrease in luminal bacteria results in a decrease in portal entry of peptidoglycan-polysaccharide polymers, common bacterial cell wall components, which have been suggested to elicit inflammatory stimuli as well as endotoxin (Lichtman et al., 1992). Inflammatory mediators other than bacterial cell wall components could include proinflammatory cytokines, leukotrienes, and eicosanoids, which were shown to be highly expressed in injured colon (Rachmilewitz et al., 1989; Kabashima et al., 2002) and may be released into the portal vein. Levels of TNF-α, one of these possible mediators, were close to the detection limit and were still low in the DSS-colitis rats. However, the involvement of TNF-α cannot be excluded because it is possible that exposure of low but continuous TNF-α as well as endotoxin to liver results in down-regulation of P450 enzymes.

Although liver injury was not observed in DSS-colitis rats, we considered that the portal endotoxia facilitated the liver injury induced by other compounds, because small amounts of LPS are known to enhance susceptibility to various hepatotoxic agents (Roth et al., 1997). D-Galactosamine has been known to inhibit both protein and RNA synthesis in hepatocytes, leading to damage of the plasma membrane (Decker and Kepler, 1974). In addition, endogenous (e.g., gut-derived) circulating endotoxin, as well as exogenously administrated LPS, is known to aggravate D-galactosamine hepatic injury and results in increased mortality of D-galactosamine-treated animals (Galanos et al., 1979; Freudenberg and Galanos, 1988; Stachlewitz et al., 1999). In the present study, contrary to expectation, hepatotoxicity of D-galactosamine was not enhanced but was highly attenuated in the DSS-colitis rats. The increase in TNF-α by D-galactosamine, which was shown to be involved in D-galactosamine-induced liver injury in rats (Stachlewitz et al., 1999), was also suppressed in the rats with DSS-colitis.

Liver injury after D-galactosamine in mice was observed only when LPS was injected simultaneously with, or after D-galactosamine (Galanos et al., 1979). It was also observed that pretreatment with LPS protected the mice from the lethal toxicity of a second LPS administration together with D-galactosamine, demonstrating the development of tolerance to LPS (Freudenberg and Galanos, 1988). On the other hand, gut-derived endotoxin is involved in pathogenesis of D-galactosamine-induced liver injury in rats (Stachlewitz et al., 1999). We thus suggested that continuous low exposure to endotoxin in DSS-colitis established tolerance, which protected the rats against endotoxin-mediated D-galactosamine toxicity. Because bacterial endotoxin activates Kupffer cells (hepatic macrophages) that mediate D-galactosamine hepatotoxicity (Stachlewitz et al., 1999), the macrophages may become unresponsive to endotoxin, resulting in suppression of TNF-α generation, which is probably responsible for suppressing the toxicity. Recent studies proposed that endotoxin tolerance was mediated by down-regulation of LPS signaling via toll-like receptor 4 (Nomura et al., 2000). The present results suggested possible involvement of the suppression of TNF-α in the endotoxin tolerance. Further studies are required to determine the mechanism for the endotoxin tolerance in the colitis model.

In summary, DSS-induced experimental colitis leads to down-regulation of hepatic P450 enzymes in rats, which is mediated by endotoxin associated with colitis. D-Galactosamine-induced liver injury is attenuated in rats with colitis, suggesting that the colitis induces endotoxin tolerance. Our studies do show that DSS-induced colitis is a useful model not only for studying pathogenesis of ulcerative colitis, but also as a tool to alter the liver function by endogenous inflammatory mediators without injection of LPS.

References


"Fig. 3. D-Galactosamine-induced liver injury and serum TNF-α concentration in DSS-colitis rats."

Rats were given 3% DSS in drinking water for 7 days. D-Galactosamine (800 mg/kg i.p.) was injected into the DSS-treated (DSS/GAL) or untreated (GAL) rats on the 7th day, and the blood samples were collected 24 h after the injection. The samples were subjected to serum leakage of ALT (A) and AST (B) as the indices of liver injury and serum TNF-α concentrations (C). Results are means ± S.E. of five (DSS) or six (Control, GAL, DSS/GAL) rats. **, p < 0.01, significantly different from the rats without D-galactosamine; ##, p < 0.01, significantly different from the rats without DSS."
Disturbed Hepatic P450s in Rats with Colitis


