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Ulcerative colitis-induced hepatic damage in mice: Studies on inflammation, fibrosis, oxidative DNA damage and GST-P expression

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ABSTRACT

There exists a close relationship between ulcerative colitis and various hepatic disorders. The present study was aimed to evaluate the hepatocellular damage in experimental colitis model. Ulcerative colitis was induced in Swiss mice by cyclic treatment with 3% w/v dextran sulfate sodium in drinking water. The severity of colitis was assessed on the basis of disease activity index and colon histology. The effect of ulcerative colitis on the liver was assessed using various biochemical parameters, histological evaluation, sirius red staining, immunohistochemical staining with peroxisome proliferator-activated receptor γ, 8-oxo-7,8-dihydro-2′-deoxyguanosine and placental glutathione S-transferase, comet assay (alkaline and modified), Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick End Labeling assay and western blot analysis to detect the protein expression of nuclear factor kappa B, cyclooxygenase-2, nuclear erythroid 2-related factor 2 and NADPH: quinone oxidoreductase-1. Dextran sulfate sodium induced severe colitis in mice as evident from an elevated disease activity index and histological abnormalities. Ulcerative colitis increased the permeability of colon as apparent from a significant reduction in the expression of tight junction protein, occludin. Further, the bacterial translocation assay as well as the analysis of lipopolysaccharide level revealed the existence of various bacterial species in the liver of ulcerative colitis-induced mice. There was a significant increase in the plasma alanine and aspartate transaminases and liver triglyceride levels, expression of peroxisome proliferator-activated receptor γ, inflammatory markers, oxidative stress, fibrosis, oxidative DNA damage and apoptosis in the liver of mice. Moreover, there was an increase in the expression of nuclear factor kappa B and cyclooxygenase-2 and a reduction in the expression of nuclear erythroid 2-related factor 2 and NADPH: quinone oxidoreductase-1 in the liver of severe ulcerative colitis-induced mice. The results of the present study provide evidence that ulcerative colitis is accompanied with hepatic damage in mice.

1. Introduction

Ulcerative colitis (UC), an inflammatory bowel disease, is a gastrointestinal disorder affecting a part of the colon or the entire colon in an uninterrupted manner [1]. Chronic intestinal inflammation may pose an elevated risk of developing colorectal cancer, which is the third most common malignancy in humans [2]. Besides colorectal cancer, long-term intestinal inflammation is associated with the development of several extra-intestinal manifestations [3,4]. Among the most frequent and serious extra-intestinal manifestations of inflammatory bowel disease are the hepatobiliary abnormalities [5–7]. It is well reported that immunological, genetic and environmental factors influence the development of extra-intestinal manifestations in the patients with inflammatory bowel diseases [8–10]. Approximately 5–10% of patients suffering from inflammatory bowel diseases develop hepatobiliary disorders [11]. The gastrointestinal tract and the

Abbreviations: UC, ulcerative colitis; LPS, lipopolysaccharide; DSS, dextran sulfate sodium; PPAR γ, peroxisome proliferator-activated receptor γ; NF-κB, nuclear factor kappa B; COX-2, cyclooxygenase-2; Nrf2, nuclear erythroid 2-related factor 2; NQO-1, NADPH: quinone oxidoreductase-1; GST-P, placental glutathione S-transferase; End-III, Endonuclease-III; FPG, formamidopyrimidine DNA glycosylase; H&E, hematoxylin and eosin; DMSO, dimethylsulphoxide; NMPA, normal melting point agarose; LMPA, low melting point agarose; EDTA, ethylenediamine tetraacetic acid; HBSS, Hank's balanced salt solution; DAI, disease activity index; VRBG, violet red bile glucose; BHI, brain heart infusion; MRSA, De Man-Rogosa-Sharpe agar; TCBS, Thiosulfate–citrate–bile salts–sucrose; cfu, colony forming unit; ALT, alanine transaminase; AST, aspartate transaminase; MPO, myeloperoxidase; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; TL, tail length; TM, tail moment; OTM, olive tail moment; % DNA, % DNA in comet tail; 8-oxo-dG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; DAB, 3,3′-diaminobenzidine tetrahydrochloride; TUNEL, Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick End Labeling.

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hepatobiliary system are closely linked anatomically and the mesenteric venous drainage ascends via the portal vein into the liver. This makes the liver and the biliary system the direct targets for damage during an exaggerated colonic inflammatory response seen in inflammatory bowel disease. Further, UC is associated with an increased intestinal permeability [12], which may provide a favorable environment for the translocation of gut flora to the liver. It has been reported that exposure to cyclic dextran sulfate sodium (DSS) treatment leads to an increased bacterial translocation to the liver, which is associated with liver injuries in rat [13]. Increased bacterial translocation is associated with an elevated lipopolysaccharide (LPS) level, which in turn is reported to cause inflammation and tissue injury in both experimental and disease conditions [14,15].

It has been reported that infection, inflammation and tissue injury can lead to changes in the lipid metabolism profiles, such as hypertriglycemia, accelerated lipolysis and decreased fatty acid oxidation in the liver [16–18]. However, few reports indicate that UC exerts inflammatory responses, fibrosis, genotoxic assault and hepatocellular damage in the liver [13,19,20]. Hence, the present study was aimed to investigate the possible consequence of DSS-induced UC on the liver of mice. The presence of bacteria in liver due to increased permeability of the colon, as evident from a reduced expression of occludin, in UC-induced mice was assessed through the bacterial translocation assay as well as by measuring the LPS level in the liver homogenate. Effect of UC on the liver of mice was evaluated by the assessment of various biochemical parameters, inflammatory markers, oxidative stress, fibrosis, DNA damage, cellular damage and apoptosis. Further, oxidative DNA damage as well as various molecular markers, such as peroxisome proliferator-activated receptor γ (PPAR γ), nuclear factor kappa B (NF-κB), cyclooxygenase-2 (COX-2), nuclear erythroid 2-related factor 2 (Nrf2), NADPH: quinone oxidoreductase-1 (NQO-1) and placental glutathione S-transferase (GST-P) in the liver of UC-induced mice were also investigated. The present study clearly depicts that UC leads to hepatocellular damage in mice.

2. Materials and methods

2.1. Animals

All the animal experiment protocols were approved by the Institutional Animal Ethics Committee (IAEC) and the experiments on animals were performed in accordance with the Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA) guidelines. Experiments were performed on male Swiss mice (25–28 g) procured from the Central Animal Facility (CAF) of the institute. All the animals were kept under controlled environmental conditions at room temperature (22 ± 2 °C) with 50 ± 10% humidity and controlled cycle of 12 h light and 12 h dark. Standard laboratory animal feed (purchased from commercial supplier) and water were provided *ad libitum*. Animals were acclimatized to the experimental conditions for a period of 1 week prior to the commencement of the experiment.

2.2. Chemicals

DSS (MW 36000–40000 Da, CAS No. 9011-18-1) was purchased from MP Biomedicals, USA. Endonuclease-III (End-III), formamidopyrimidine DNA glycosylase (FPG), SYBR Green I (CAS No. 163795-75-3), hexadecyltrimethylammonium bromide (CAS No. 57-09-0), o-dianisidine dihydrochloride (CAS No. 20325-40-0), 1,1,3,3-tetramethoxy propane (CAS No. 102-52-3), 2-thiobarbituric acid (CAS No. 504-17-6), 5,5′-dithiobis (2-nitro-benzoic acid) (CAS No. 69-78-3), bovine serum albumin (CAS No. 9048-46-8), hematoxylin and eosin (H & E) and trizma (CAS No. 77-86-1) were purchased from Sigma–Aldrich Chemicals, Saint Louis, MO, USA. Reduced glutathione (GSH), dimethylsulphoxide (DMSO), normal melting point agarose (NMPA), low melting point agarose (LMPA), triton X-100, ethylenediamine-tetraacetic acid (EDTA), Hank's balanced salt solution (HBSS), MacConkey agar, violet red bile glucose (VRBG) agar, brain heart infusion (BHI) agar, de Man-Rogosa-Sharpe agar (MRSA) and Thiosulfate–citrate–bile salts–sucrose (TCBS) agar were obtained from HiMedia Laboratories Ltd., Mumbai, India.

2.3. Experimental design

UC was induced in mice using 3% w/v DSS dissolved in drinking water for various time periods. Animals were randomized into various groups, each group consisting of 8 animals. Group 1 received normal drinking water and served as a control group. Group 2 received DSS for 7 days (1 cycle) and the animals were sacrificed on the 8th day. Group 3 received DSS for 2 cycles with 14 days remission period between each cycle and the animals were sacrificed at the end of the 2nd cycle. Similarly, group 4 received DSS for 3 cycles with 14 days remission period between each cycle. Animals were sacrificed at the end of the 3rd cycle. To assess the extent of colitis, weight loss, stool consistency and rectal bleeding were monitored daily in order to calculate the disease activity index (DAI) as mentioned [21]. One cycle consisted of 7 days of DSS-treated water followed by 14 days of normal drinking water.

2.4. Bacterial translocation assay

The bacterial translocation assay was performed as described [13,22] with some modifications. The liver was removed aseptically and homogenized in ice-cold phosphate buffer saline. Tissue homogenates were seeded onto various media, such as MacConkey agar (for coliform), violet red bile glucose (VRBG) agar (for *Enterobacteriaceae*), brain heart infusion (BHI) agar (for fastidious and facultative aerobic microorganisms), de Man-Rogosa-Sharpe agar (MRSA) (for *Lactobacilli*) and Thiosulfate–citrate–bile salts–sucrose (TCBS) agar (for hydrogen sulfide producing bacteria). The cultures were incubated at 35 °C for 24–48 h. The presence of bacteria was calculated on the basis of colony forming unit (cfu)/g of liver for each bacterial species. Finally, the total bacterial load was calculated by adding up the individual species count to obtain the total cfu/g of liver.

2.5. Measurement of plasma alanine transaminase (ALT) and aspartate transaminase (AST) levels

Plasma ALT and AST levels were measured using commercially available kits (AST kit, ALT kit, Merck, Mumbai, India).

2.6. Measurement of triglyceride level

Liver triglyceride level was assessed using commercially available spectrophotometric kit (ACCUREX Biomedical Pvt Ltd., Mumbai, India).

2.7. Measurement of myeloperoxidase (MPO) activity

MPO activity was determined as an indicator of polymorphonuclear leucocyte accumulation as described [23] with some modifications. The liver tissue was homogenized in ice cold 50 mmol/l potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide. The homogenate was frozen and thawed three times, centrifuged and the level of MPO in supernatant was measured using o-dianisidine. The rate of change in