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Telmisartan treatment targets inflammatory cytokines to suppress the pathogenesis of acute colitis induced by dextran sulphate sodium

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ABSTRACT

The renin angiotensin system (RAS) is essential for the regulation of cardiovascular and renal functions to maintain the fluid and electrolyte homeostasis. Recent studies have demonstrated a locally expressed RAS in various tissues of mammals, which is having pathophysiological roles in those organ system. Interestingly, local RAS has important role during the inflammatory bowel disease pathogenesis. Further to delineate its role and also to identify the potential effects of telmisartan, an angiotensin receptor blocker, we have used a mouse model of acute colitis induced by dextran sulphate sodium. We have used 0.01 and 5 mg/kg body weight doses of telmisartan and administered as enema to facilitate the on-site action and to reduce the systemic adverse effects. Telmisartan high dose treatment significantly reduced the disease activity index score when compared with the colitis control mice. In addition, oxidative stress and endoplasmic reticulum stress markers expression were also significantly reduced when compared with the colitis control mice. Subsequent experiments were carried out to investigate some of the mechanisms underlying its anti-inflammatory effects and identified that the mRNA levels of pro-inflammatory cytokines such as tumour necrosis factor α, interleukin 1β, interleukin 6 and monocyte chemoattractant protein 1 as well as cellular DNA damage were significantly suppressed when compared with the colitis control mice. Similarly the apoptosis marker proteins such as cleaved caspase 3 and 7 levels were down-regulated and anti-apoptotic protein Bcl2 level was significantly upregulated by telmisartan treatment. These results indicate that blockade of RAS by telmisartan can be an effective therapeutic option against acute colitis.

1. Introduction

Inflammatory bowel diseases (IBD) are persistent, perennial diseases, which constitute pertinent clinical problems in day-to-day medical practice. In spite of vast advances in recognition of factors underlying these diseases, the origination and development of non-specific colitis and similar inflammatory gastrointestinal diseases lasts uncertain [1]. With the prevalence of IBD and its associated risk for development of colorectal cancer, it is important to prevent and treat IBD. But, because of the complexity of etiology and potentially serious adverse effects, options for its treatment are relatively limited [2]. Although IBD are multifactorial diseases, involving genetic, immunological and environmental factors, the onset is characterised by an autoimmune inflammation that causes excessive production of pro-inflammatory cytokines to damage intestinal mucosa [3].

The renin angiotensin system (RAS) is classically known for controlling the blood pressure however, recently it is also considered as an inflammation regulator. Apart from the systemic RAS, tissue intrinsic RASs have been identified in various tissues and reported

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to be important for maintenance of local homeostasis [4]. Recent studies suggest that the inflammatory cytokines and RAS can cross-regulate each other, resulting in self-sustaining and/or self-amplifying positive feedback loops [5]. In vitro experiments have shown that the activation of angiotensin II type I receptor will lead to nuclear factor (NF)-κB activation, and further increases the production of inflammatory mediators such as tumour necrosis factor (TNF)-α, transforming growth factor (TGF)-β1, interleukin 1β and monocyte chemoattractant protein (MCP)-1 [6]. Blockade of this signaling using either an angiotensin receptor blocker or angiotensin converting enzyme inhibitor could be effective against the colon inflammatory conditions. A study with enalapril suggested that it can hamper the NF-κB signaling pathway, hinder the stimulation of intestinal epithelial cells and macrophages, and ameliorate experimental colitis by down-regulation of IκBα activation [7]. Similarly angiotensin II type I receptor knock down in mice effectively protected from the pathogenesis of acute colitis triggered by dextran sodium sulphate (DSS) [8]. Various angiotensin receptor blockers are under screening for their action against the pathogenesis of IBD. A recent study using telmisartan as reported its effectiveness in protecting the rats from DSS-induced colitis. They have focused on inflammation and oxidative stress in DSS-induced colitis rats using telmisartan at a dose of 10 mg/kg [9]. Here we have used two different lower doses of telmisartan (administered intra rectally as enema) in a mouse model of acute colitis created by DSS administration in drinking water and focused on oxidative and endoplasmic reticulum (ER) stresses, inflammation and DNA damage.

2. Materials and methods

2.1. Drugs and chemicals

Dextran sulphate sodium (MW 36,000-50,000) was purchased from Wako, Japan. Telmisartan was provided by Boeringer Ingelheim (Ingelheim am Rhein, Germany). All other chemicals used were purchased from Sigma, Japan until mentioned otherwise.

2.2. Animals

Female C57Bl/6J mice were maintained and housed in the animal facility of Niigata University of Pharmacy and Applied Life Sciences, Niigata city, Japan. The animals were kept under temperature and humidity controlled conditions with a light dark cycle of 12–12 h. They were given food pellets (Oriental Yeast Co., Ltd, Tokyo, Japan) and drinking water ad libitum. Animal experiments were done during the light phase of the cycle and as per the regulations of the Committee on Bioethics for Animal Experiments of Niigata University of Pharmacy and Applied Life Sciences, Niigata city, Japan.

2.3. Experimental design

The mice were divided into four groups of equal numbers ($n = 8$). First group served as normal control, received plain drinking water. Remaining all the groups of mice received 3% DSS in drinking water. Second group served as DSS control received 1% carboxy methyl cellulose (CMC) suspension transrectally. Third and fourth groups were given telmisartan suspension in 1% CMC (0.01 and 5 mg/kg body weight respectively via rectal administration) once daily for six consecutive days and served as treatment groups.

2.4. Body weight change and disease activity index

Body weights of the mice were measured daily from the day before the start of the protocol and continued till the end of the study. The clinical disease activity index (DAI) was calculated as per the method described previously [10], which is the total of the individual scores for loss of body weight (0 – none; 1 – 1–5%; 2 – 5–10%; 3 – 10–20% and 4 – over 20%), stool consistency (0 – well-formed pellets; 2 – loose stools; and 4 – diarrhoea), and presence or absence of faecal blood (0 – negative haemoccult; 2 – positive haemoccult and 4 – gross bleeding).

2.5. Colonic damage analysis

On the final day of experiment, colons were dissected and examined for its length change, which is measured without including the last 1 cm. To evaluate the colon damage by light microscopy, the samples from the distal colon were fixed in 10% formalin, paraffin-embedded and cut into 5 μm thick transverse sections on glass slides. They were then deparaffinised, and processed for haematoxylin and eosin (H&E) staining [11]. In the specimens were viewed under a light microscope (CIA-102, Olympus, Tokyo, Japan).

2.6. Western blotting analysis

The colon tissue samples were homogenized under cold condition using lysis buffer and their protein concentrations were measured following bicinchoninic acid method. Using sodium dodecyl sulphate polyacrylamide gel electrophoresis, the proteins were separated and identified with the following polyclonal antibodies; p22phox, p67phox, 3-nitrotyrosine, glucose regulated protein (GRP)-78, growth arrest and DNA damage inducible gene (GADD)-153, Bcl2, cleaved caspase-7, cleaved caspase-12 and β-tubulin (Purchased from either Cell Signaling technology or Santa Cruz Biotechnology). The separated proteins were transferred to nitrocellulose membranes and then the membranes were blocked with 5% skim milk or 5% BSA in tris buffered saline with tween20. After an hour of incubation with the primary antibody, their binding sites were identified with the appropriate peroxidase conjugated secondary antibody purchased from Santa Cruz Biotechnology, and chemiluminescence developing agents purchased from Amersham Biosciences, Buckinghamshire, United Kingdom. Each sample was also analysed for its β-tubulin content. After scanning the films, the band density quantification was done with Scion Image Program (Epson GT-X700, Tokyo, Japan). Finally, the densitometric data were normalized with β-tubulin [12].

2.7. DNA damage analysis

DNA damage analysis was performed using alkaline comet assay as per the previously reported method [13,14]. In short, immediately after harvesting, the colon tissue was washed with ice-cold phosphate buffered saline and made into cells using a cell dissociation sieve-tissue grinder kit. Approximately, 1×10^5 cells were mixed with 1.5 ml of 0.8% low melting agarose in normal saline at 38 °C and poured on a frosted microscopic slide. Once after the gel formation, the slide was kept in lysis buffer for 1 h. Then electrophoresis was carried out in alkaline buffer at room temperature for 20 min to unwind the DNA strands. After washing with a neutralizing buffer, the slide was stained with SYBR green II and viewed under a fluorescence microscope (BH2-RFCA, Olympus, Japan). The comet images were analysed with a digital image analysing software "casp". Tail moment (TM) and tail length (TL) were obtained from the images and used for the DNA damage analysis.