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Anti-inflammatory effect of glucose-lysine Maillard reaction products on intestinal inflammation model *in vivo*

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ABSTRACT

Inflammatory bowel diseases (IBDs) are chronic disorders that are characterized by intestinal epithelial inflammation and injury. Currently, the most employed therapies are antibiotics and anti-inflammatory drugs; however, the side effects limit long-term effectiveness. We evaluated the impact of glucose-lysine Maillard reaction products (Glc-Lys MRPs) on colitis, induced in rats by an administration of 5% dextran sulfate sodium (DSS) in drinking water. Glc-Lys MRPs ameliorate DSS-induced colitis, as determined by a decrease in disease index activity, colon weight/length ratio, nitric oxide levels in serum, recovery of body weight loss, colon length and serum lysozyme levels. Furthermore, Glc-Lys MRPs increase the glutathione content and the activity of glutathione peroxidase, superoxide dismutase and catalase, and inhibit lipid peroxidation and myeloperoxidase activity in colon tissues. In particular, Glc-Lys MRPs suppress the mRNA level of the inflammatory cytokines and nuclear factor-κB in colon tissues. This study suggests the potential of Glc-Lys MRPs in preventing or treating IBDs.

1. Introduction

Inflammatory bowel diseases (IBDs), such as Crohn's disease and ulcerative colitis, are chronic relapsing disorders of the gastrointestinal tract that are characterized pathologically by intestinal epithelial inflammation and injury [1]. The representative symptoms of IBDs are diarrhea, occult bleeding, abdominal pain, body weight loss and anemia [2]. Ulcerative colitis is histologically characterized by a continuous inflammation of colonic lamina propria with crypt abscesses, distortion and loss, ulceration, and infiltration of neutrophils, monocytes and lymphocytes [2]. Crohn's disease is a relapsing inflammatory disease, mainly affecting the gastrointestinal tract, and frequently presenting with abdominal pain, fever and clinical signs of bowel obstruction or diarrhea with passage of blood and/or mucus [1]. IBDs are considered an autoimmune disease, and its pathogenesis depends on complex interactions between genetic and environmental factors and innate and adaptive immune mechanisms, which are still not entirely clear [3].

Dextran sulfate sodium (DSS) has been used to induce colitis in animals, and the mouse model of DSS-induced colitis mimics the morphological and pathological features of human disease [4]. The DSS-induced colitis model exhibits high reproducibility for colitis lesions, which are induced primarily in the left colon in a similar fashion to human colitis [5]. DSS is a heparin-like sulfated polysaccharide containing 17% sulfur with up to three sulfate groups in a glucose molecule [6]. Depending on the concentration and the duration and frequency of DSS administration, animals develop acute or chronic colitis [7].

The current treatments for IBDs focus on ameliorating disease symptoms and are associated with several disadvantages. Although antibiotic therapy is commonly used, it may adversely affect the balance of gut microflora and cause antibiotic resistance. Moreover, immunosuppressant and anti-inflammatory drugs such as corticosteroids have many undesirable side effects [1,8]. Finding new, and effective treatments for IBDs are thus becoming an increased focus of research.

The Maillard reaction, which is well known as a non-enzymatic browning reaction, mainly occurs from a reaction between the amino group of proteins and the carbonyl group of sugars during food processing and storage. This reaction forms Maillard reaction products (MRPs) with many different physical and chemical properties [9,10]. Many studies have reported that MRPs have anti-oxidant, anti-mutagenic, carcinogenic and anti-bacterial activities [11]. However, there are few studies on the anti-inflammatory effect of MRPs. Fructose-tyrosine MRPs exhibited anti-inflammatory activity in astrocytes and BV-2

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cells [11]. Kitts, Chen and Jing [12] studied the anti-inflammatory effect of sugar- (glucose, fructose or ribose) amino acid (lysine or glycine) MRPs using Caco-2 cells as an intestinal epithelial cell model.

The effect of glucose-lysine MRPs (Glc-Lys MRPs) in the regulation of the intestinal barrier and its anti-oxidant and anti-inflammatory response in DSS-induced colitis is unknown. The objective of this study is to evaluate the impact of Glc-Lys MRPs on DSS-induced colitis in rats. We hypothesized that Glc-Lys MRPs have an important role in maintaining the integrity of the intestinal mucosal barrier and enhancing the anti-inflammatory response in colitis, which implies a novel means of preventing or treating IBDs. We evaluated the disease activity index (DAI), biochemical inflammatory markers, histopathological status, anti-oxidant enzyme activities and levels of inflammatory cytokines in a DSS-induced animal model with the treatment of Glc-Lys MRPs.

2. Materials and methods

2.1. Chemicals

Catalase (CAT), 5, 5-dithiobis-(2-nitrobenzoic acid) (DTNB), hexadecyltrimethylammonium bromide (HTAB), hydrogen peroxide (H_2O_2), glucose, lipopolysaccharide (LPS), lysine, lysozyme hydrogen peroxide (H_2O_2), glutathione (GSH), glutathione peroxidase (GPx), *Micrococcus lysodeikticus*, myeloperoxidase, nitric oxide (NO), *o*-dianisidine, phosphate buffered saline (PBS) and superoxide dismutase (SOD) were purchased from Sigma (St. Louis, MO, USA). Dextran sulfate sodium (DSS) was purchased from MP Biomedicals, LLC (Santa Ana, California, USA). All chemicals were of the highest possible grade.

2.2. Preparation of Glc-Lys MRPs

Glucose (1 mol/L) was mixed in a 1:1 M ratio with lysine (1 mol/L) in distilled deionized water. The mixture was immediately placed in a screw-capped glass tube and boiled at 100 °C for 30 min in a shaking bath. After cooling for 30 min, glucose-lysine (Glc-Lys) MRPs were lyophilized and then stored at − 80 °C until further use. A positive control of fresh Glc-Lys was made with a mixture of Glc and Lys, immediately lypolized, and then stored at − 70 °C until further use.

2.3. Animals

Six- to seven-week-old male Wistar rats with a body weight of 150 ± 10 g were purchased from Samtako Bio Korea Co. (Gyeonggi-do, Korea). They were allowed free access to a standard diet and tap water. Rats were housed in stainless steel cages in a room kept at 22 ± 1 °C with a 12-h light/12-h dark cycle (lights on from 07:00 to 19:00). Animals were acclimatized to the laboratory setting for at least 1 week before the start of the experimental protocols. All animal handling was performed according to the guidance of the Committee for Ethical Usage of Experimental Animals of Korea University.

2.4. In vivo colitis model

The *in vivo* colitis model was designed with slight modifications according to Hayashi, Narumi, Tsuji, Tsubokawa, Nakaya, Wakayama, Zuka, Ohshima, Yamagishi and Okada [2], Liu, Beaumont, Walker, Chaumontet, Andriamihaja, Matsumoto, Khodorova, Lan, Gaudichon and Benamouzig [13] and Nagib, Tadros, ELSayed and Khalifa [8]. Before colitis induction, rats were treated daily with oral administration of saline alone or Glc-Lys MRPs (210 or 2100 mg/kg body weight (B.W.)) or Glc-Lys without MRPs (2100 mg/kg B.W.) for 7 d. Then, the experimental colitis model was induced by an *ad libitum* administration of 5% (wt/vol) DSS (molecular weight 36,000 to 50,000 kDa) in drinking water for 7 d. During this colitis induction period, rats also received daily oral administrations of Glc-Lys MRPs or Glc-Lys without MRPs.

Rats were randomly allocated into 5 groups: control (saline) (n = 6); 5% DSS, 5% DSS + saline group (n = 6); 5% DSS + MRPL, 5% DSS + 42.1 mg of Glc-Lys MRPs (210 mg/kg B.W.) (n = 6); 5% DSS + MRPH, 5% DSS + 421 mg of Glc-Lys MRPs (2100 mg/kg B.W.) (n = 6); and, 5% DSS + GL, and 5% DSS + 421 mg of Glc-Lys without MRPs (2100 mg/kg B.W.) (n = 6). During the experimental period, food and water consumption were not significantly different in each group (data not shown). Rats were sacrificed 18 h after the last day of the induction period. A blood sample was taken from the vena cava caudalis for nitric oxide (NO) and lysozyme assays in serum. Immediately after blood collection, the colon was excised and flushed by gentle washing with ice-cold PBS 3 times before measuring its length and weight. The distal segments of the colon were collected for histopathological studies. Adjacent segments were treated with Trizol reagent and immediately frozen in liquid nitrogen and kept at − 80 °C for cytokine genes expression analysis. A section of the remaining colon was taken for the measurement of myeloperoxidase activity (MPO), glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) content. A histopathological section was fixed immediately in 10% formalin. The other sections were frozen immediately in liquid nitrogen and stored at − 80 °C for biochemical analysis.

2.5. Disease activity index (DAI)

The consistency and condition of the stools of the rats were measured on the last day of the 7 consecutive days of DSS treatment using the DAI scoring system described by Nagib, Tadros, ELSayed and Khalifa [8]. The scores were determined as follows: stool consistency (0 and 1: normal, 2 and 3: loose stool, 4: diarrhea) and stool bleeding (0: negative, 1: ± , 2: +, 3: + +, 4: gross). The DAI score was calculated using the following formula:

DAI = (stool consistency score + stool bleeding score)/2.

2.6. Measurement of NO and lysozyme levels in serum

Blood was collected from the vena cava and left in an ice box for 10 min. After centrifugation at 12,000 RPM for 10 min, the supernatant was collected. Serum was stored at − 80 °C for further study. The analysis of NO levels in serum was determined by measuring nitrite (NO_2^-) levels using a colorimetric method based on the Griess reaction with some modification [14]. A serum lysozyme assay was done by measuring the lysis of suspension of *Micrococcus lysodeikticus* according to a slightly modified method of Nudo and Catap [15]. Lysozyme activity was expressed as mg lysozyme/g protein. Protein content was measured with a BCA protein kit using BSA as the standard.

2.7. Measurement of glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (MDA) assay in colon tissue

Colon tissue was homogenized in ice-cold PBS, divided into aliquots, and frozen at − 80 °C until analysis. Spectrophotometric analysis of GSH content was done with a slightly modified method according to Owens and Belcher [16] and Nagib, Tadros, ELSayed and Khalifa [8] based on the reaction of DTNB with GSH. The GSH content was expressed as nM/mg protein. GPx and CAT and lipid peroxidation content were determined by our previous methods [17,18]. SOD activity was determined with a slightly modified method according to Kovaceva, Platenik, Vejrazka and Stipek [19]. Lipid peroxidation was measured by estimating the level of thiobarbituric acid reactive substances (TBARS) as determined by MDA, and the MDA content was expressed as nM/mg protein.