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Effects of fish scale collagen peptide on an experimental ulcerative colitis mouse model

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ABSTRACT

The aim of this study was to understand the effects of fish scale collagen peptide (SC) on an experimental ulcerative colitis (UC) mouse model. SC shortened colon length, increased colon weight/length ratio, and ameliorated histological tissue injury in dextran sulfate sodium (DSS)-induced acute UC mice. SC suppressed inflammation in acute UC by decreasing myeloperoxidase-dependent activation of inflammatory cells such as leukocytes. SC suppressed the activation of nuclear factor-kappa B (NF-κB) in colon and serum monocyte chemotactic protein-1 in the DSS-induced acute UC mouse model. Gelatin, on the other hand, did not suppress clinical symptoms, colon inflammation, and colon fibrosis in DSS-induced acute UC. These results revealed that SC has anti-inflammatory effects in the DSS-induced acute UC model. Our results indicate that SC could be a new functional food for patients with inflammatory bowel disease.

1. Introduction

Since ancient times, we have been consuming collagen in the form of gelatin obtained from cooked meat and fish. Injections of gelatin or collagen affect functions of various parts of the body, including bone [1], cartilage [2], and skin [3]. Collagen is non-toxic and has mild pain-relieving effects for degenerative joint disease in stifle and/or hip joints [4]. Previously, we reported the synergistic effects of oral administration of collagen peptide and glucosamine on cartilage regeneration after experimental damage [5]. Fish scale collagen peptide (SC) and glucosamine have clinical applications in canine and feline orthopedic diseases and spondylitis deformans [6]. Recently, collagen hydrolyzed from beef and pork has been shown to exert protective effects on ethanol-induced gastric ulcer [7,8].

Inflammatory bowel disease (IBD) is a common disorder and refers to a group of conditions characterized by inflammation of the intestinal tract. Crohn's disease (CD) and ulcerative colitis (UC) account for the majority of these conditions [9].

Currently, several medical treatments are available for IBD patients: 5-aminosalicylic acid drugs such as sulfasalazine or balsalazide, immunomodulators such as thiopurines (azathioprine, 6-mercaptopurine), methotrexate, and biologic therapies that target tumor necrosis factor (TNF)-α or interleukin (IL)-6 [9,10]. Despite their beneficial effects, these drugs also cause adverse effects in IBD patients. In addition, some drugs such as 5-aminosalicylic acids drugs are expensive. Immunomodulators and biologic therapies may also increase the risk of serious infection [9]. Because of these complexities, a functional diet for IBD patients is urgently needed. Presumably, some nutritional supplements are beneficial for IBD, including amino acids [11], omega-3 fatty acids [12], dietary fibers [13], and probiotics [14].

To the best of our knowledge, there are no reports on the effects of collagen peptide on IBD patients or experimental IBD models. The aim of this study was to evaluate the protective effects of SC in an experimental UC model. Using DSS-induced acute UC mouse model, we evaluated the effects of SC on clinical symptoms, histological tissue injury, and inflammation. Furthermore, we compared the protective effects of SC with those of gelatin and glycine in the experimental IBD model.

2. Materials and methods

2.1. Reagents

Dextran sulfate sodium (molecular weight 36–50 kDa; reagent grade) was purchased from MP Biomedicals LLC (Solon, OH, USA). SC was provided by Kanda Giko Co. Ltd. (S-collagen, Yonago, Japan). This collagen peptide was prepared from the scales of *Sparidae* sp. and

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Lutianidae sp. The mean molecular weight of the prepared SC was 800 (range, approximately 500–1000). 3% solution of SC has a pH of 4.5.

The major amino acids constituting SC include glycine (33.6% of the dry matter), alanine (12.6%), proline (11.0%), hydroxyproline (8.6%) and glutamic acid (7.2%). Gelatin from porcine was purchased from Wako Pure Chemical (Osaka, Japan). The major amino acids constituting gelatin include glycine (32.7% of the dry matter), proline (15.7%), alanine (12.7%), hydroxyproline (10.3%) and glutamic acid (8.4%).

2.2. Animals and study design

Thirty female C57BL/6J mice (5–6 weeks old) were purchased from CLEA Japan (Osaka, Japan). The animals were maintained under conventional conditions. Mice were used for the experiment after seven days of acclimatization. The use of these animals and the procedures they undergo were approved by the Animal Research Committee of Tottori University.

Mice ($n = 30$) were randomized into 6 groups: the control (+) group, administered only 3% DSS (w/v) ($n = 5$); the control (−) group was administered tap water ($n = 5$); the SC (−) group, administered 3% SC (w/v) dissolved in tap water ($n = 5$); the SC (+) group, administered 3% SC (w/v) and 3% DSS (w/v) dissolved in tap water ($n = 5$); the G (−) group, administered 3% gelatin (w/v) dissolved in tap water ($n = 4$) and the G (+) group, administered 3% gelatin (w/v) and 3% DSS (w/v) dissolved in tap water ($n = 5$). To induce colitis, mice were administered 3% DSS *ad libitum* for 5 days, designated as day 0 to day 5. Blood and colon samples were collected from all groups at day 5 ($n = 5$). The blood was centrifuged ($500 \times g$, 4 °C, 10 min), and the serum was separated promptly and frozen at −80 °C before measurement of serum cytokines.

2.3. Histological evaluation of colitis

The length (cm) and weight (mg) of the colon were measured, and tissue samples were obtained from each colon. Colon tissues were fixed in 10% buffered formalin. Thin sections (3 μm) were prepared from each sample for histological observation after hematoxylin-eosin staining. Each section was examined microscopically, and histological scoring was performed as described by Ohkawara et al. [15]. In brief, tissue damage was classified using 6 grades: 0: normal mucosa; 1: infiltration of inflammatory cells; 2: shortening of the crypt by less than half of the height; 3: shortening of the crypt by more than half of the height; 4: crypt loss; and 5: destruction of epithelial cells. Histological scoring was performed in 10 fields at ×100 magnification using 3 mice from each group. The mean scores for 30 fields were considered the histological score for each group.

2.4. Myeloperoxidase (MPO) staining

MPO, a marker of leukocyte invasion into tissue, was stained as described previously [16]. MPO-positive cells in the submucosal layer were counted as described previously with slight modifications [17]. Briefly, MPO-positive cells were counted in 10 fields at ×400 magnification using 3 mice for each group. The mean scores for 30 fields were considered the number of MPO-positive cells for each group.

2.5. Immunohistochemical detection of nuclear factor-κ B (NF-κB) in the colon

NF-κB is activated in the inflamed colonic mucosa in IBD [18,19]. We evaluated the effects of SC on NF-κB activation in the inflammatory colon, according to previously described methods [20]. To determine the area of fibrosis of the mucosal and submucosal layers of the colon, we analyzed the NF-κB–positive areas of colonic sections through quantitative digital morphometry, according to the protocol described by Azuma et al [20]. In brief, 10 randomly chosen high-power fields (×200 magnification) for each cross section were photographed with a digital camera attached to an Olympus microscope system (Olympus Corporation, Tokyo, Japan). The color wavelengths of the copied image were transformed into digital readings using Lumina Vision software (Mitani Corporation, Tokyo, Japan), allowing for quantification of the various color wavelengths with pixels as the unit of measure. The original image was used for comparison, and the color spectra were analyzed; those corresponding to the NF-κB–positive areas were quantified. The percentage of NF-κB–positive areas in the mucosal and submucosal layers was calculated by dividing the total pixel area of NF-κB–positive areas by the total pixel area corresponding to the total colonic tissue in the field of view. The colons of 3 mice were analyzed from each group. The mean score from 30 fields was considered to represent the extent of fibrosis for each group.

2.6. Masson's trichrome staining

In DSS-induced UC, fibrosis of the mucosal and submucosal layers of the colon is observed in both the acute and chronic phases [21]. To determine the area of fibrosis of the mucosal and submucosal layers of the colon, we used quantitative digital morphometric analyses of the extracellular matrix (ECM) of the colonic sections, and Masson's trichrome (MT) staining was performed according to the protocol described above for the imaging analyses of NF-κB. The mean score from 30 fields was considered to represent the extent of fibrosis in each group.

2.7. Measurements of serum monocyte chemotactic protein-1 concentrations

Serum monocyte chemotactic protein-1 (MCP-1/CCL2) was quantified by a sandwich enzyme-linked immunosorbent assay (ELISA) by using a commercial mouse MCP-1 ELISA kit (Quantikine®, R&D Systems Inc., Minneapolis, USA) according to the manufacturer's protocol.

2.8. Statistical analysis

The data are expressed as the mean ± SE. Statistical analyses were performed using one-way ANOVA followed by Tukey–Kramer's test or Steel–Dwass test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Effects of SC on colon length and colon weight/length in experimental UC mice

According to the induction of colitis, the shorten of colon length and the increase of the colon weight/length ratio were observed [22]. In the SC (+) group (5.6 ± 0.1 cm), colon lengths were significantly longer than those of the control (+) (5.0 ± 0.1 cm) and G (+) (5.0 ± 0.1 cm) groups ($p < 0.01$; Table 1). Colon weight/length ratio (mg/cm) of the SC (+) group (35.9 ± 1.2 mg/cm) was significantly decreased compared with the control (+) (42.8 ± 1.6 mg/cm) group ($p < 0.01$; Table 1).

3.2. Effects of SC on histological changes in experimental UC mice

The damage to intestinal mucosa was microscopically evaluated and was graded through histological scoring. In the control (+) and G (+) groups, erosions, shortening, or destruction of crypt and