

Fish Scale Collagen Peptide Protects Colon Inflammation an Experimental Ulcerative Colitis Mouse Model

Kazuo Azuma ¹⁺, Tomohiro Osaki ¹, Norihiko Itoh ¹, Ichiro Arifuku ², and Yoshiharu Okamoto ¹

¹ Faculty of Agriculture, Tottori University, 4-101 Koyama-minami Tottori, 680-8533, Japan

² Department of Applied Biotechnology, Food Developing Laboratory, Tottori Institute of Industrial Technology, Sakaiminato, Tottori 684-0041, Japan

Abstract. The aim of this study was to understand the effects of fish scale collagen peptide (SC) on an ulcerative colitis (UC) mouse model. SC suppressed shortened colon length, decreased 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- κ B (NF- κ B) in colon and serum monocyte chemoattractant 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 the DSS-induced acute UC model. 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.

Keywords: fish scale collagen peptide, functional food, inflammatory bowel disease, nuclear factor- κ B, monocyte chemoattractant protein-1

1. Introduction

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]. Recently, collagen hydrolyzed from beef and pork has been shown to exert protective effects on ethanol-induced gastric ulcer [5] [6].

Presumably, some nutritional supplements are beneficial for inflammatory bowel disease (IBD), including amino acids [7], omega-3 fatty acids [8], dietary fibers [9] and probiotics [10]. 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 fish scale collagen peptide (SC) in an experimental ulcerative colitis (UC) model. Using DSS-induced acute UC mouse model, we evaluated the effects of SC on histological tissue injury and inflammation.

2. Materials and Methods

2.1. Regents

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 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.

⁺ Corresponding author. Tel.: +81-857-31-5433; fax: +81-857-31-5433
E-mail address: kazu-azuma@muses.tottori-u.ac.jp

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).

2.2. Animals and Study Design

Twenty female C57BL/6J mice (5-6 weeks old) were purchased from CLEA Japan (Osaka, Japan). The use of these animals and the procedures they undergo were approved by the Animal Research Committee of Tottori University.

Mice (n = 20) were randomized into 4 groups: the control (+) group, administered only 3% DSS (w/v) (n = 5); the control (-) group, administered tap water (n = 5); the SC (+) group, administered 3% SC (w/v) and 3% DSS (w/v) dissolved in tap water (n = 5) 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 days 0 to day 5. Blood and colon samples were collected from all groups at day 5 (n = 5).

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 μ m) were prepared from each sample for histological observation after hematoxylin-eosin (HE) staining. Each section was examined microscopically, and histological scoring was performed as described by Ohkawara et al [11]. 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 \times 100 magnification using 3 mice from each group. The mean scores for 30 fields were considered the histological score for each group.

2.4. Immunohistochemical Detection of Myeloperoxidase (MPO) and Nuclear Factor- κ B (NF- κ B) in the Colon

MPO-positive cells in the submucosal layer were counted as described previously with slight modifications [12]. Briefly, MPO-positive cells were counted in 10 fields at \times 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. We evaluated the effects of SC on NF- κ B activation in the inflammatory colon, according to previously described methods [13]. To determine the positive area of submucosal layers of the colon, we analyzed the NF- κ B-positive areas of colonic sections through quantitative digital morphometry, according to the protocol described by Azuma et al [13]. 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.

2.5. Statistical Analysis

The data are expressed as the mean \pm 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. Histological Changes

In the SC (+) group, colon lengths were significantly longer than those of the control (+) and G (+) groups (p $<$ 0.01; Table 1). Colon weight/length ratio (mg/cm) of the SC (+) group was significantly decreased compared with the control (+) group (p $<$ 0.01; Table 1).

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 edema were observed (Fig. 1 (b) and (d)). In the SC (+) group, some erosions were observed; however, shortening or

destruction of crypt was markedly suppressed, and edema was slightly suppressed (Fig. 1 (c)). In addition, the severity of tissue damage was evaluated through histological scoring of HE stained sections. Histological scores of the SC (+) group was significantly lower than those of the control (+) and G (+) groups ($p < 0.01$, Table 1).

Table 1. Histological changes of the colon

	control (-)	control (+)	SC (+)	G (+)
Colon length (cm)	7.3 \pm 0.1	5.0 \pm 0.1	5.6 \pm 0.1**	5.0 \pm 0.1
Colon weight/length ratio (mg/cm)	25.0 \pm 0.5	42.8 \pm 1.6	35.9 \pm 1.2**	39.8 \pm 1.7
Histological score	0.0 \pm 0.0	3.7 \pm 0.2	2.1 \pm 0.2**	3.6 \pm 0.2

Data represent the mean \pm SE in each group (n = 5). **: $p < 0.01$ compared with the control (+) and G (+) groups.

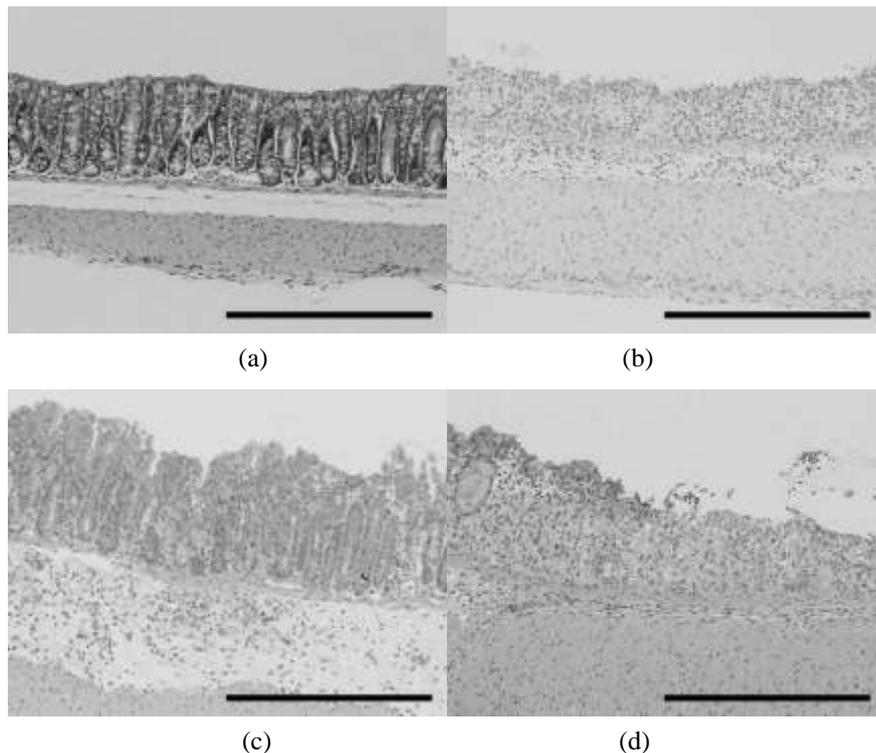


Fig. 1. Effect of SC on histopathological changes on the colon. Data presented are for 1 mouse each from control (-) (a), control (+) (b), SC (+) (c) and G (+) (d) groups. Bar = 200 μ m.

3.2. Immunohistological Detections

In the SC (+) group, counts of MPO-positive cells were significantly fewer than those of the control (+) and G (+) groups ($p < 0.01$, Table 2). The percentage of NF- κ B-positive areas in epithelial cells is also shown in Table 2. In the SC (+) group, the score was significantly lower than those of the control (+) and G (+) groups ($p < 0.01$).

Table 2. Immunohistochemical detections and serum MCP-1 level

	control (-)	control (+)	SC (+)	G (+)
MPO positive cells (cells/fields)	0.0 \pm 0.0	11.0 \pm 0.6	7.8 \pm 0.4**	14.6 \pm 0.9
Positive area of NF- κ B (%/field)	1.8 \pm 0.3	10.7 \pm 0.9	4.0 \pm 0.7**	14.4 \pm 0.9
Serum MCP-1 level (pg/ml)	25.6 \pm 5.4	55.5 \pm 4.5	24.2 \pm 5.9†	41.8 \pm 9.6

Data represent the mean \pm SE in each group (n = 5). **: $p < 0.01$ compared with the control (+) and G (+) groups, †: $p < 0.05$ compared with the control (+) group.

3.3. Serum MCP-1 levels

The results are shown in Table 2. In the SC (+) group, serum MCP-1 concentration was significantly lower than that in the control (+) group ($p < 0.05$). In the G (+) group, the serum concentration of MCP-1 was slightly less than that in the control (+) group.

4. Discussion

SC improved shortening of colon length, increased colon weight/length ratio, and ameliorated histological tissue injury in DSS-induced acute UC of mice model. MPO is a known marker of oxidative stress, and high MPO activity was reported in a DSS-induced UC model [14] [15]. Significantly fewer MPO-positive cells were observed in the SC (+) group than in the control (+) and G (+) groups. A plausible explanation is that SC suppresses the MPO-dependent activation of inflammatory cells such as leukocytes and decreases inflammation. Gelatin, however, did not suppress clinical symptoms or colon inflammation in this DSS-induced acute UC mouse model.

This is consistent with the observation that NF- κ B activity in the colon increases during active episodes of IBD [16]. MCP-1 plays an important role in the pathogenesis of colitis and recruits immune and enterochromaffin cells in the experimental model. Absence of MCP-1 was associated with significant reduction of inflammation in an experimental colitis model [17]. Ju et al. demonstrated that pro-inflammatory cytokines induce the expression of MCP-1 via p38MAPK and NF- κ B signalling [18]. Our data indicate that SC decreases colon NF- κ B activation and serum MCP-1 concentration. These results confirm that SC suppresses the expression of MCP-1 in the serum by suppressing NF- κ B activation.

Several food-based collagen peptides are detectable in the human peripheral blood after ingestion of collagen peptides. Iwai et al. identified such peptides, and Pro-Hyp, Pro-Hyp-Gly, Ala-Hyp, Ala-Hyp-Gly, Ser-Hyp, Ser-Hyp-Gly, Leu-Hyp, Ile-Hyp, and Phe-Hyp are the most commonly found food-derived collagen peptides [19]. Among these peptides, Pro-Hyp is a major food-derived collagen peptide, and it remains in circulation for a few hours [19] [20]. To the best of our knowledge, the relationship between specific collagen peptides and their protective action against IBD is not well characterized. Further studies evaluating the blood peptide profiles in experimental IBD models and IBD patients are required to decipher the differences in the plasma profile after ingestion of SC and gelatin.

In conclusion, our results revealed that SC inhibits colonic inflammation and reduces tissue injury in DSS-induced acute UC mice. SC inhibits mucosal inflammation by suppressing MPO activation of inflammatory cells and activation of NF- κ B in colon and serum MCP-1. These findings suggest that SC has potential applications as a new functional food for IBD patients.

5. Acknowledgements

This work was supported by a Tottori prefecture-financed aid project for beauty & health products (2012–2013). The authors thank to Kandagiko Co. Ltd. (Yonago, Japan) for providing test materials.

6. References

- [1] Y. Nomura, K. Oohashi, M. Watanabe and S. Kasugai. Increase in bone mineral density through oral administration of shark gelatin to ovariectomized rats. *Nutrition*. 2005, 21 (11-12): 1120-1126.
- [2] S. Nakatani, H. Mano, C. Sampei, J. Shimizu and M. Wada. Chondroprotective effect of the bioactive peptide prolyl-hydroxyproline in mouse articular cartilage in vitro and in vivo. *Osteoarthritis Cartilage*. 2009, 17 (12): 1620-1627.
- [3] M. Tanaka, Y. Koyama and Y. Nomura. Effects of collagen peptide ingestion on UV-B-induced skin damage. *Biosci. Biotechnol. Biochem*. 2009, 73 (4): 930-932.
- [4] C.L. Deal and R.W. Moskowitz. (1999) Nutraceuticals as therapeutic agents in osteoarthritis. The role of glucosamine, chondroitinsulfate, and collagen hydrolysate. *Rheu. Dis. Clin. North. Am*. 1999, 25 (2): 379–395.
- [5] G.A. Castro, V.C. Sgarbieri, J.E. Carvalho, S.V. Tinti and A. Possenti. (2007) Protective effect of collagen derivatives on the ulcerative lesions caused by oral administration of ethanol. *J. Med. Food*. 2007, 10 (1): 154-158.
- [6] G.A. Castro, J.E. Carvalho, S.V. Tinti, A. Possenti, V.C. Sgarbieri. Anti-ulcerogenic effect of a whey protein

isolate and collagen hydrolysates against ethanol ulcerative lesions on oral administration to rats. *J. Med. Food.* 2009, 13 (1): 83-90.

- [7] M. Coëffier, R. Marion-Letellier and P. Déhelotte. Potential for amino acids supplementation during inflammatory bowel diseases. *Inflamm. Bow. Dis.* 2010, 16 (3): 518–524.
- [8] N. Rajendran and D. Kumar. Role of diet in the management of inflammatory bowel disease. *World J. Gastroenterol.* 2010, 16 (12): 1442-1448.
- [9] M.E. Rodríguez-Cabezas, J. Gálvez, M.D. Lorente, A. Concha, D. Camuesco, S. Azzouz, A. Osuna, L. Redondo and A. Zarzuelo. Dietary fiber down-regulates colonic tumor necrosis factor alpha and nitric oxide production in trinitrobenzenesulfonic acid-induced colitic rats. *J. Nutr.* 2002, 132 (11): 3263-3271.
- [10] C. Vanderpool, F. Yan and D.B. Polk. Mechanisms of probiotic action: Implications for therapeutic applications in inflammatory bowel diseases. *Inflamm. Bow. Dis.* 2008, 14 (11): 1585-1596.
- [11] T. Ohkawara, J. Nishihira, H. Takeda, K. Miyashita, K. Kato, M. Kato, T. Sugiyama and M. Asaka. Geranylgeranylacetone protects mice from dextran sulfate sodium-induced colitis. *Scand. J. Gastroenterol.* 2005, 40 (9): 1049–1057.
- [12] K. Azuma, T. Osaki, T. Wakuda, S. Ifuku, H. Saimoto, T. Tsuka, T. Imagawa, Y. Okamoto Y and S. Minami. Beneficial and preventive effect of chitin nanofibrils in a dextran sulfate sodium-induced acute ulcerative colitis model. *Carbohydr. Polym.* 2012, 87 (2): 1399-1403.
- [13] K. Azuma, T. Osaki, S. Ifuku, H. Saimoto, T. Tsuka, T. Imagawa, Y. Okamoto Y and S. Minami. α -Chitin nanofibrils improve inflammatory and fibrosis responses in mice with inflammatory bowel disease. *Carbohydr. Polym.* 2012, 90 (1): 197-200.
- [14] Y. Naito, T. Takagi and T. Yoshikawa. Neutrophil-dependent oxidative stress in ulcerative colitis. *J. Clin. Biochem. Nutr.* 2007, 41 (1): 18-26.
- [15] R.K. Schindhelm, L.P. van der Zwan, T. Teerlink and P.G. Scheffer. Myeloperoxidase: a useful biomarker for cardiovascular disease risk stratification? *Clin. Chem.* 2009, 55 (8): 1462-1470.
- [16] T. Zarubin and J. Han. Activation and signaling of the p38 MAP kinase pathway. *Cell Res.* 2005, 15 (1): 11-18.
- [17] W.I. Khan, Y. Motomura, H. Wang, R.T. El-Sharkawy, E.F. Verdu, M. Verma-Gandhu, B.J. Rollins and S.M. Collins. Critical role of MCP-1 in the pathogenesis of experimental colitis in the context of immune and enterochromaffin cells. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 2006, 291 (5): G803-G811.
- [18] Y. Ju, J. Hua, K. Sakamoto, H. Ogawa and I. Nagaoka. Modulation of TNF-alpha-induced endothelial cell activation by glucosamine, a naturally occurring amino monosaccharide. *Int. J. Mol. Med.* 2008, 22 (6): 809-815.
- [19] K. Iwai, T. Hasegawa, Y. Taguchi, F. Morimatsu, K. Sato, Y. Nakamura, A. Higashi, Y. Kido, Y. Nakabo and K. Ohtsuki. Identification of food-derived collagen peptides in human blood after oral ingestion of gelatin hydrolysates. *J. Agr. Food. Chem.* 2005, 53 (16): 6531-6536.
- [20] H. Ohara, H. Matsumoto, K. Ito, K. Iwai and K. Sato. Comparison of quantity and structures of hydroxyproline-containing peptides in human blood after oral ingestion of gelatin hydrolysates from different sources. *J. Agr. Food. Chem.* 2007, 55 (4): 1532-1535.