Anti-inflammatory activities of cellulose nanofibers made from adlay and seaweed in an inflammatory bowel-disease model

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Abstract. Inflammatory bowel disease (IBD) is one of the common diseases all over the world. In this study, we investigated the anti-inflammatory effects of cellulose nanofibers made from adlay (A-CNF) and seaweed (S-CNF) on colon inflammation using the mouse model of IBD. A-CNF and S-CNF improved the histological tissue injury in mice. A-CNF and S-CNF also suppressed activation of nuclear factor-kappa B in the colon. Furthermore, A-CNF and S-CNF suppressed myeloperoxidase activities of inflammatory cells such as leukocytes. On the other hand, cellulose nanofibers made from wood did not improve the histological tissue injury and colon inflammation in mice. These results revealed that A-CNF and S-CNF have suppressive effects on colon inflammation in an experimental IBD mouse model. Furthermore, our results indicate that A-CNF and S-CNF may be a potential source of dietary fiber for patients with IBD.

Keywords: dietary fiber, cellulose nanofiber, inflammatory bowel disease, adlay, seaweed

1. Introduction

Inflammatory bowel disease (IBD) is characterized by chronic inflammation of the gut [1]. Incidences of IBD have increased and it may be because of changes in dietary habits in recent years, particularly diets with low fiber content [2]. Recently, Abe *et al.* (2007) described an efficient method for isolation of cellulose nanofibers with a uniform width of approximately 15 nm from wood [3]. This nanofiber preparation method can be used to isolate cellulose nanofibers from any natural plant such as flax, sugarcane bagasse, wheat straw, and pear [4]-[6] Recently, we reported that nanofibrillated chitin has anti-inflammatory effects on IBD mouse model [7], [8]. Furthermore, we also reported the anti-inflammatory effects of cellulose nanofiber made from pear in IBD mouse model [9]. This result indicate that nanofibrillation confers beneficial and new aspects to materials. In this study, we evaluated cellulose nanofibers made from adlay and seaweed as new types of dietary fibers [10].

2. Materials and Methods

2.1. Preparation of cellulose nanofibers

Cellulose nanofibers from adlay (*Coix lacryma-jobi*) chaff (A-CNF) and Hijiki seaweed (*Saragassum fusiforme*) (S-CNF) were prepared by methods described previously [6] with some modifications. Briefly, dried Hijiki and adlay chaff were soaked in water and roughly crushed in a domestic blender. The suspensions were passed through a grinder (MKCA6-3; Masuko Aangyo Co. Ltd., Saitama, Japan) set at 1500 rpm. Grinding was performed with a clearance gauge of −1.5 (corresponding to a 0.15 mm shift) from the zero position, which was determined by the point of slight contact between the grinding stones. The samples were placed in a pressure-tight glass vessel and hydrothermally treated at 150 ℃ for 120 min in a

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high-pressure cooker (VS-2416; Koyo Engineering Corp., Saitama, Japan) to break down the matrix substances such as hemicellulose polysaccharides, the pectin matrix, and phenolic polymer lignin embedded in the cellulose nanofibers of the cell wall. The thermally treated wet samples were then passed through the Star Burst system (Star Burst Mini, HJP-25001S; Sugino Machine Co., Ltd.) equipped with a ball-collision chamber. The slurry was ejected from a small nozzle with a diameter of 100 μm under high pressure (245 MPa) and collided with a ceramic ball with a diameter of 12.7 mm. The suspensions were passed through 10 (adlay chaff) or 5 (Hijiki seawood) mechanical treatments. Concentrations of A-CNF and S-CNF homogeneous slurries were 3 and 1 wt%, respectively. Before the oral administration experiment, A-CNF and S-CNF were diluted to 0.1 w% of homogeneous slurries in water. Each 0.1 w% diluted A-CNF and S-CNF were used for the oral administration experiment. Isolation of cellulose nanofibers from wood (W-CNF) was performed according to a previous report (Abe et al., 2007). Wood powder from Radiata Pine (*Pinus radiata D. Don*) was used for this study. Before the oral administration experiment, A-CNF, S-CNF and E-CNF were diluted to 0.1 w% of homogeneous slurries in water.

2.2. Animals and reagents

Twenty-five C57BL/6 mice (female, 5 weeks old) were purchased from CLEA Japan (Osaka, Japan). The animals were maintained under conventional conditions. Mice were used in experiments after 7 days of acclimation. Animal procedures were approved by the Animal Research Committee of Tottori University. DSS (molecular weight: 36–50 kDa; reagent grade) was purchased from MP Biomedicals LLC (Solon, OH, USA).

2.3. Animals and reagents

Mice (n = 25) were randomized into 5 groups: the control (−) group was administered tap water (n = 5), the control (+) group was administered 3% DSS dissolved in tap water (n = 5), the A-CNF (+) group was administered A-CNF and 3% DSS dissolved in tap water (n = 5), the S-CNF (+) group was administered S-CNF and 3% DSS dissolved in tap water (n = 5), and the wood cellulose nanofiber (W-CNF) (+) group was administered W-CNF and 3% DSS dissolved in tap water (n = 5). To elicit colitis, mice were administered with 3% DSS ad libitum for 5 days. A-CNF, S-CNF, and W-CNF were diluted to 0.1 w% in water. The A-CNF (+), S-CNF (+), and W-CNF (+) groups, 0.1 w% diluted A-CNF, S-CNF, and W-CNF were also administered ad libitum for 5 days. Colon sampling was performed at day 5 in all groups.

2.4. Histological evaluation of colitis

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 us [7]. Histological scoring was performed in 10 fields at ×100 magnification using 3 mice in each group. The mean score for 30 fields was considered as the histological score for each group. Counting of MPO-positive cells in the submucosal layer was performed as described previously [7]. Immunohistochemical detection of NF-κB was performed by methods described previously [8]. Quantitative digital morphometric analyses of NF-κB-positive areas of colonic sections were performed according to methods described previously [8].

2.5. Statistical analysis

The data are expressed as the mean ± S.E. Statistical analyses were performed using Steel-Dwass test. A *p*-value of <0.05 was considered statistically significant.

3. Results

3.1. Effects of A-CNF and S-CNF on histological changes in IBD model mice

The damage of the intestinal mucosa was microscopically evaluated by histological scoring. In control (+) and W-CNF (+) groups, we observed erosions, shorting or destruction of the crypts, and edema. In A-CNF (+) and S-CNF (+) groups, we observed some erosion and marked suppression of shorting or destruction of the crypts, and slight suppression of edema. The results of the histological scoring are shown in Figure 1. In the control (+) group, the histological scores were significantly higher than those in the non-treated control

(control (−)) group were (p < 0.01). In addition, histological scores of the A-CNF (+) and S-CNF (+) groups were significantly lower than those of the control (+) and W-CNF (+) groups were (p < 0.01).
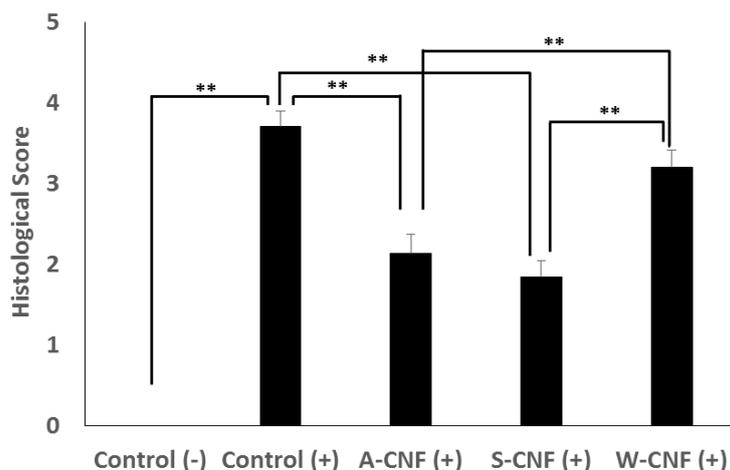

Fig. 1: Effects of A-CNF and S-CNF on histological changes in IBD model mice. Data represent the mean ±S.E. in each group. Statistical analysis was performed with the Steel-Dwass test. **p < 0.01.

3.2. Effects of A-CNF and S-CNF on colon MPO-positive cells in IBD mouse model

The results of the numbers of MPO-positive cells are shown in Figure 2. In the control (+) group, the numbers of MPO-positive cells were significantly higher than those of the non-treated control (control (−)) group were (p < 0.01). In A-CNF (+) and S-CNF (+) groups, the numbers of MPO-positive cells were significantly lower than those of the control (+) and W-CNF (+) groups were (p < 0.01).
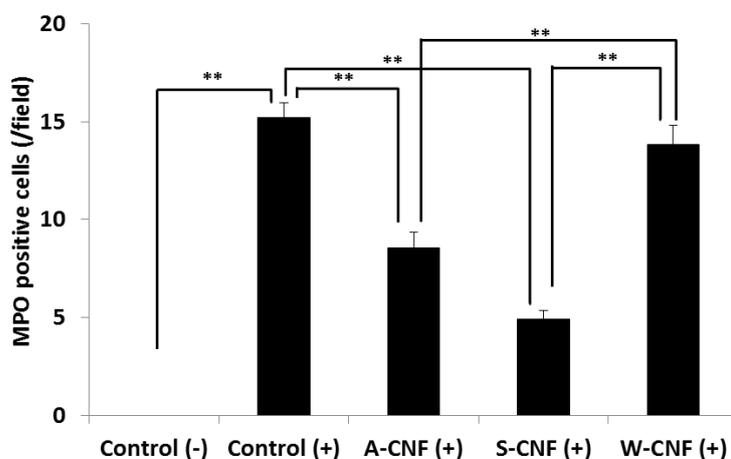

Fig. 2: Effects of A-CNF and S-CNF on colon MPO-positive cells in IBD mouse model. Data represent the mean ±S.E. in each group. Statistical analysis was performed with the Steel-Dwass test. **p < 0.01.

3.3. Effects of A-CNF and S-CNF on NF-κB expression in the colon epithelium

In the control (+) group, the positive areas of NF-κB were significantly increased compared with those of the non-treated control (control (−)) group (p < 0.01). In A-CNF (+) and S-CNF (+) groups, the positive areas of NF-κB were significantly decreased compared with those of the control (+) and W-CNF (+) groups (p < 0.01) (Figure 3).

4. Discussion

Intake of dietary fiber reduces the risk of developing certain gastrointestinal disorders [11]. In this study, we evaluated the potential of A-CNF and S-CNF as new types of dietary fiber using the IBD mouse model. In A-CNF (+) and S-CNF (+) groups, histological scores were significantly lower than those of the control (+) and W-CNF (+) groups were. Being a marker of oxidative stress, high MPO activities were observed in a

IBD mouse model [12], [13]. In the A-CNF(+) and S-CNF (+) groups, MPO-positive cells were significantly fewer than in the control(+) and W-CNF(+) groups. Therefore, we can infer that P-CNF suppresses the inflammation caused by acute UC by decreasing the MPO activation of inflammatory cells such as leukocytes. On the other hand, W-CNF did not suppress the clinical symptoms and colon inflammation in the IBD mouse model. These data indicated that A-CNF and S-CNF suppressed colon damage in the experimental IBD mouse model. NF-κB is a critical transcription factor needed to express genes associated with proinflammatory responses [14]. It stimulates expression of cyclooxygenase-2, prostaglandin E_2, and pro-inflammatory cytokines (IL-6, TNF-α, and monocyte chemotactic protein-1) [15]. In A-CNF (+) and S-CNF (+) groups, positive areas of NF-κB in colon epithelia were significantly decreased compared with those of the control (+) and W-CNF (+) groups. Our results indicated that A-CNF and S-CNF had anti-inflammatory effects via suppression of NF-κB activation in the IBD mouse model.
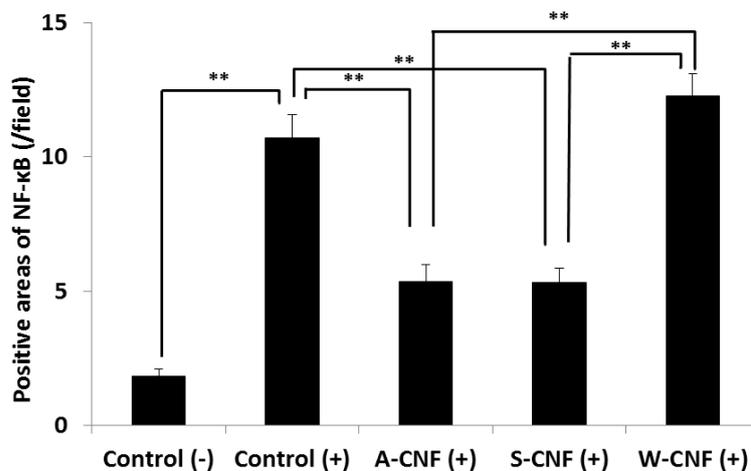

Fig. 3: Effects of A-CNF and S-CNF on NF-κB expression in the colon epithelium. Data represent the mean ± S.E. in each group. Statistical analysis was performed with the Steel-Dwass test. **p < 0.01.

In conclusion, A-CNF and S-CNF suppress shortening of colons and increases the colon weight/length ratio. A-CNF and S-CNF also suppress colon inflammation. Our data indicate that the cellulose nanofibers made from adlay and seaweed have the potential to be considered new types of beneficial dietary fibers for IBD patients.

5. Acknowledgements

6. References

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