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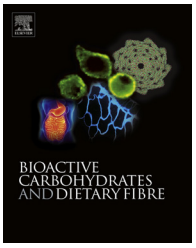

Anti-inflammatory effects of cellulose nanofiber made from pear in inflammatory bowel disease model

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ABSTRACT

The aim of this study was to evaluate the effects of cellulose nanofibers from Japanese pear (P-CNF) on acute IBD by using a mouse model of this disorder. Furthermore, using this mouse model, we compared the effects between P-CNF and cellulose nanofibers from wood (W-CNF).

P-CNF suppressed shortening of the colon length and improved the histological tissue injury in the mice. It also suppressed the activation of nuclear factor-kappa B and fibrosis of the colon, as well as the myeloperoxidase activation of inflammatory cells such as leukocytes. On the other hand, W-CNF did not improve the histological tissue injury, or suppress shortening of the colon length, colon inflammation, and fibrosis in the mice. These results revealed that P-CNF has anti-inflammatory effects in the experimental IBD mouse model. Our results indicate that P-CNF could be a potential source of new dietary fiber for patients with IBD.

1. Introduction

Traditionally, dietary fibers are defined as the portions of plant foods that are resistant to digestion by human digestive enzymes (Anderson et al., 2009). The intake of dietary fibers can provide many health benefits to humans, including reducing the risks for coronary heart disease (Liu et al. 1999), stroke (Steffen et al. 2003), hypertension (Whelton et al. 2005), diabetes (Montonen, Knekt, Jarvinen, Aromaa, & Reunanen, 2003), obesity (Lairon et al. 2005), and certain gastrointestinal disorders (Petruzziello, Iacopini, Bulajic, Shah, & Costamagna, 2006). Dietary fibers can also influence intestinal functions through both their direct (non-fermentative) and indirect (their metabolic products, fermentative) effects on the colon (Rose, DeMeo, Keshavarzian, & Hamaker, 2007).

Inflammatory bowel disease (IBD) includes both ulcerative colitis (UC) and Crohn's disease (CD), and is characterized by a chronic inflammation of the gut (Morrison, Headon, & Gibson,

Abbreviations: P-CNF, cellulose nanofiber from Japanese pear; W-CNF, cellulose nanofiber from wood; DSS, dextran sodium sulfate; UC, ulcerative colitis; IBD, inflammatory bowel disease; CD, Crohn's disease; MPO, myeloperoxidase

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2009). The incidence of IBD has increased steadily in some areas of the world over the past 40 years (Loftus, 2004). This may be due to changes in dietary habits, particularly in the consumption of diets that are low in fiber content (Rose et al. 2007). The goal of IBD treatment is to induce and maintain remission (Pithadia & Jain, 2011). However, drugs can have both beneficial and adverse effects in patients with IBD (Morrison et al., 2009).

Previously, Abe, Iwamoto, & Yano (2007) described an efficient method for isolating cellulose nanofibers, with a uniform width of approximately 15 nm, from wood. Their nanofiber preparation method could also be used to isolate cellulose nanofibers from any natural plant such as flax, sugarcane bagasse, and wheat straw (Abe & Yano, 2009, 2010). These cellulose nanofibers contain the matrix substances of the cell wall such as lignin and hemicelluloses. Recently, Ifuku, Adachi, Morimoto, & Saimoto (2011) isolated natural cellulose nanofibers from parenchyma cells of pears and apples. The structure of the cellulose nanofibers from pear was uniform, with an average width of approximately 18 nm and a high aspect ratio (ratio between length and thickness). The parenchyma cells of fruits and vegetables consist of cellulose nanofiber networks, which are embedded in matrix substances such as hemicellulose polysaccharides, the pectic matrix, and phenolic polymer lignin in the primary cell wall (Mccann, Wells, & Roberts, 1990). These cellulose nanofibers are expected to be available as new dietary fibers.

In this study, we evaluated the suppressive and anti-inflammatory effects of cellulose nanofibers from Japanese pear (P-CNF) by using an acute IBD mouse model. Furthermore, we used this mouse model to compare the effects between P-CNF and cellulose nanofibers from wood (W-CNF).

2. Materials and methods

2.1. Animals and reagents

Thirty C57BL/6 mice (females, 5 weeks old) were purchased from CLEA Japan (Osaka, Japan). The animals were maintained under conventional conditions and used for the experiment after 7 days of acclimation. The use of these mice and the procedures they underwent were approved by the Animal Research Committee of Tottori University. Dextran sulfate sodium (molecular mass 36–50 kDa; reagent grade) was purchased from MP Biomedicals LLC (Solon, OH, USA).

2.2. Preparation of cellulose nanofibers

The cellulose nanofibers from Japanese pear were generally prepared by referring to and modifying our previous experiments (Ifuku et al., 2011). Approximately 210 g of mature Nijuseiki pear pulp was roughly crushed in a domestic blender to render pear juice (PJ). Another 210 g of fruit pulp was added into a pressure tight glass vessel and hydrothermally treated at 140 °C for 60 min in a high-pressure cooker (VS-2416; Koy Engineering Corp., Saitama, Japan), to break down the matrix substances such as hemicellulose polysaccharides, the pectic matrix, and phenolic polymer lignin embedded in the cellulose nanofibers of the cell wall (hydrothermally treated pear juice: PJ-htt). The pH of the sample before and after hydrothermal treatment was 4.8. This suspension was cooled to room temperature, and then filtered and washed thoroughly with distilled water to remove the water-soluble parts. The treated wet sample was dispersed in water with 5.7 wt% concentration and passed three times through a grinder set at 1500 rpm. The individualized nanofibers were approximately 20 nm wide. The average diameter of the isolated nanofibers was estimated by FE-SEM image analysis (JSM-6700F; JEOL, Ltd.,). This P-CNF homogeneous slurry, with a concentration of 5.7 wt%, was used for the oral administration experiment.

The isolation of cellulose nanofibers from wood was performed according to previously reported methods (Wise, Murphy and d'Addieco 1946) with slight modification (Abe et al., 2007). Wood powder from radiata pine (*Pinus radiata* D. Don) was used for this study. Solvent extraction was first performed in a Soxhlet apparatus for 6 h, using a 2:1 mixture of toluene:ethanol. The lignin in the sample was then removed using an acidified sodium chlorite solution at 70 °C for 1 h, and the process was repeated until the product became white. The sample was further treated in 6 wt% KOH overnight at room temperature, and then at the same concentration for 2 h at 80 °C, in order to leach the hemicelluloses. Finally, the slurry of 1 wt% purified cellulose was passed through a grinder (MKCA6-3; Masuko Sangyo Co., Ltd., Saitama, Japan) set at 1500 rpm. The grinding was performed with a clearance gauge of −1.5 (corresponding to a 0.15 mm shift) from the zero position, which was determined as the point of slight contact between the grinding stones. The final concentration of the slurry was 1 wt%.

2.3. Experimental design

Mice ($n=30$) were randomized into the following six groups: the control(−) group was administered tap water ($n=5$); the control(+) group was administered only 3% dextran sodium sulfate (DSS; $n=5$); the P-CNF(+) group was administered P-CNF and 3% DSS dissolved in tap water ($n=5$); the PJ(+) group was administered PJ and 3% DSS dissolved in tap water ($n=5$); the PJ-htt(+) group was administered PJ-htt and 3% DSS dissolved in tap water ($n=5$); and the W-CNF(+) group was administered W-CNF and 3% DSS dissolved in tap water ($n=5$). To elicit UC, the mice were administered 3% DSS ad libitum for 5 days; from day 0 to day 5. The P-CNF, PJ, PJ-htt, and W-CNF samples were diluted 10 times in water. For the P-CNF(+), PJ(+), PJ-htt(+), and W-CNF(+) groups, the diluted P-CNF, PJ, PJ-htt, and W-CNF water were also administered ad libitum from day 0 to day 5 with 3% DSS. Colon samplings were done at day 5 in all groups.

2.4. Histological evaluation of ulcerative colitis

The length (cm) and weight (mg) of the colon were measured, and tissue was obtained from each colon. The colon tissues were fixed in 10% buffered formalin. Thin sections (3 μm) were made 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. (2005). 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