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Short communications

Protective effects of galacturonic acid-rich vinegar brewed from Japanese pear in a dextran sodium sulfate-induced acute colitis model

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ABSTRACT

The effects of pear vinegar (PV), which was specially brewed for enhanced galacturonic acid content, on the DSS-induced ulcerative colitis (UC) mouse model were evaluated. PV improved clinical symptoms, colon inflammation, and histological tissue injury in the DSS-induced acute UC mouse model. Moreover, PV suppressed inflammation due to acute UC by suppressing the myeloperoxidase (MPO)-mediated activation of inflammatory cells such as leukocytes and decreasing the serum concentration of IL-6. Our results demonstrated the protective action of PV in the DSS-induced acute UC mouse model. On the other hand, commercial apple vinegar did not show a protective effect in the DSS-induced acute UC mouse model. Our findings indicate that PV may act as a new functional food for inflammatory bowel disease patients.

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1. Introduction

Inflammatory bowel disease (IBD) is common and refers to a group of conditions characterized by inflammation of the intestinal tract. Crohn's disease (CD) and ulcerative colitis

(UC) account for majority of the IBD cases (Morrison, Headon, & Gibson, 2009).

The intake of certain foods such as fruits is routinely recognized to be beneficial for human health. Fruit intake can reduce the risk factors of colon cancer (Terry et al., 2001). Reif

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Abbreviations: DSS, dextran sodium sulfate; UC, ulcerative colitis; IBD, inflammatory bowel disease; CD, Crohn's disease; IL, interleukin; MPO, myeloperoxidase.

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et al. (1997) reported that a high intake of fruit was negatively associated with the risk for IBD. In addition, Hou, Abraham, and El-Serag (2011) reported that intake of high fiber and fruit was associated with decreased CD.

Another beneficial food is vinegar; kurosu, a traditional Japanese black vinegar, which has anticancer activities against colon cancer *in vitro* and *in vivo* (Nanda et al., 2004; Shimoji et al., 2003, 2004). Shizuma, Ishiwata, Nagano, Mori, and Fukuyama (2011) reported that Kurosu has a protective effect against dextran sodium sulfate (DSS)-induced UC.

Currently, many medical treatments are used for IBD patients, of which some are mentioned below: 5-aminosalicylic acid drugs such as sulfasalazine or balsalazide, immunomodulators such as thiopurines (azathioprine, 6-mercaptopurine), methotrexate, and biological therapies that target tumour necrosis factor (TNF)- α or interleukin (IL)-6 (Morrison et al., 2009; Nakamura, Honda, Mizutani, Akiho, & Harada, 2006). However, these drugs may also have adverse effects in IBD patients. The 5-aminosalicylic acid drugs are expensive. Immunomodulators and biological therapies increase the risk of serious infection (Morrison et al., 2009). Because of these negative effects, the necessity for a functional food for IBD patients is increasing.

Vinegars made from fruit would have the beneficial effects of both fruit and vinegar in our health (Shahidi, McDonald, Chandrasekara, & Zhong, 2008). To the best of our knowledge, the studies which describe the beneficial effects of vinegar made from fruit to experimental IBD model and IBD patients. In this study, we evaluated if galacturonic acid (GalU)-rich vinegar, brewed from Japanese pear, can be a potential functional food for IBD patients. Galacturonic acid (GalU) is the main constituent of pectin (Richard & Hiduth, 2009). Little is known about the bioactivity of GalU *in vivo*. The aim of this study was to reveal the effects of GalU-rich pear vinegar and GalU in the DSS-induced acute colitis model.

2. Materials and methods

2.1. Reagents

DSS (molecular weight, 36–50 kDa; reagent grade) was purchased from MP Biomedicals LLC (Solon, OH, USA). GalU-rich vinegar brewed from Japanese pear (PV) was supplied by AON Chemical Corporation (Tottori, Japan). PV contains acetic acid (4.5 g/dL) and GalU (0.51 g/dL) as a major carboxylic acid. Total amount of minor carboxylic acids, such as lactic acid, succinic acid, and malic acid, was less than 0.2 g/dL. The amount of GalU in PV was determined by HPLC method with external standard as follows: Shimadzu LC-10A; column, Shim-pack

SCR-102H $\times$ 2; mobile phase, *p*-TsOH (5 mM) aq solution; flow rate, 0.8 mL/min; temperature, 40 °C; electric conductivity detector, Shimadzu CCD-6A (Kyoto, Japan). Commercial apple vinegar (AV) was purchased from Mizkan Co. (Ringo su, Aichi, Japan). GalU was not present in AV. GalU (reagent grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals

Fifty-four female C57BL/6 mice (6 weeks old) were purchased from CLEA Japan (Osaka, Japan). The animals were maintained under conventional conditions. The use of these animals and the procedures they underwent were approved by the Animal Research Committee of Tottori University.

2.3. Experiment 1: effects of PV on DSS-induced acute UC mouse model

The mice ($n = 54$) were randomized into 8 groups: the control (+) group ($n = 14$); the control (–) group ($n = 7$); the PV 4.5 (+) group ($n = 7$); the PV 4.5 (–) group ($n = 4$); the PV 9 (+) group ($n = 7$); the PV 9 (–) group ($n = 5$); AV 9 (+) group ($n = 5$), and the AV 9 (–) group ($n = 5$). Control (+) mice were administered 3% (w/v) DSS *ad libitum* for 7 days from day 0 to day 6. Control (–) mice were administered tap water *ad libitum*. PV 4.5 (+), PV 9 (+), and AV 9 (+) mice were administered 3% (w/v) DSS and 4.5% (v/v) PV, 9% (v/v) PV, or 9% (v/v) AV *ad libitum*, respectively, from day 0 to day 6. PV 4.5 (–), PV 9 (–), and AV 9 (–) mice were administered 4.5% (v/v) PV, 9% (v/v) PV, or 9% (v/v) AV *ad libitum*, respectively, from day 0 to day 6. In the PV 4.5 (+) and (–) group, intake of GalU was approximately 100 mg/kg/day. In the PV 9 (+) and (–) groups, intake of GalU was about 200 mg/kg/day. Blood collection and colon sampling were performed on day 6 in all groups.

2.4. Experiment 2: effects of GalU on DSS-induced acute UC mouse model

The mice ($n = 20$) were randomized into 4 groups: the GalU (+) 100 group ($n = 5$); GalU 100 (–) group ($n = 5$); the GalU 200 (+) group ($n = 5$); and the GalU 200 (–) group ($n = 5$). GalU 100 (+) mice were administered 3% (w/v) DSS and 0.066% (w/v) GalU *ad libitum* for 6 days from day 0 to day 6. GalU 100 (–) mice were administered 0.066% (w/v) GalU *ad libitum*. GalU 200 (+) mice were administered 3% (w/v) DSS and 0.132% (w/v) GalU *ad libitum* from day 0 to day 6. GalU 200 (–) mice were administered 0.132% (w/v) GalU *ad libitum* from day 0 to day 6. In the GalU 100 (+) and (–) groups, intake of GalU was approximately 100 mg/kg/day. In the GalU 200 (+)

Table 1 – Scoring of inflammation based on clinical parameters during treatment.

Score	Weight loss (% of initial weight)	Diarrhea score	Visible fecal blood
0	<5%	Normal	Normal
1	5–10%	Slightly loose feces	Slightly bloody
2	10–20%	Loose feces	Bloody
3	>20%	Water diarrhea	Blood in entire colon

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