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Feeding with olive oil attenuates inflammation in dextran sulfate sodium-induced colitis in rat☆

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Abstract

Chronic inflammation of long-term ulcerative colitis contributes to an increased risk of colon cancer. Few studies address whether extra-virgin olive oil (EVOO) intake suppresses inflammation, cell proliferation and signal transducers and activators of transcription (STAT) in the experimental colitis model. The aim of this study was to assess whether a 5% EVOO suppressed inflammation, increased cell proliferation and the expressions of STAT3 and STAT3 phosphorylation (pSTAT3) in dextran sulfate sodium (DSS)-induced colitis. Rats were administered DSS via drinking water (weight percentage: 4%) for 1 week with a 1-week recovery period for three cycles. Rats were divided into three groups: control group, standard diet without DSS; DSS group, standard diet+DSS; and DSS+EVOO group, EVOO diet (weight percentage: 5%)+DSS. Rats were sacrificed 5 weeks after DSS was first administered, and colonic damage was histologically and biochemically evaluated. As a result, chronic feeding of 5% EVOO attenuated inflammation. This was evaluated using a disease activity index, body weight loss and a histological score. Enhanced expressions of STAT3, pSTAT3, COX-2 and iNOS by DSS was attenuated by EVOO. In addition, EVOO attenuated increases in cell proliferation (PCNA) caused by DSS and recovered decreases in apoptosis (cleaved caspase-3). In conclusion, the study indicated that chronic feeding of 5% EVOO inhibited chronic inflammation in DSS-induced colitis in rats and also attenuated cell proliferation and recovered apoptosis in DSS colitis.

Keywords: Apoptosis; Colon cancer; Inflammatory bowel disease; Ulcerative colitis; STAT3; pSTAT3

1. Introduction

Inflammatory bowel disease (IBD) includes two major chronic intestinal disorders: ulcerative colitis (UC) and Crohn's disease (CD). The prevalence of IBD is increasing in developed countries, and one of the most serious complications of IBD is colorectal cancer (CRC) [1]. The risk of colitis-associated CRC is increased after long disease duration, especially in patients with chronic active disease [2]. Although many studies suggested that chronic inflammation might be positively correlated to the development of IBD-related carcinogenesis, the mechanisms of the IBD-related carcinogenesis process are not completely understood [3].

Many epidemiological studies indicate that dietary fat intake was a promoting factor for the incidence of CRCs [4,5]. These studies are supported by several animal studies showing that a chronic high-fat diet enhanced carcinogenesis of CRCs [6–11]. In contrast, chronic feeding of several types of fat, including fish oil and olive oil, diminish carcinogenesis of CRCs, which has been demonstrated by epidemiological studies [12,13] and *in vivo* and/or *in vitro* studies [8,14–16]. Our previous study indicated that dietary olive oil intake inhibited formation of aberrant crypt foci and mucosal arachidonate concentrations in azoxymethane (AOM)-induced CRCs in rats [8]. Other report has indicated that hydroxytyrosol (HT) isolated from olive oil inhibited cancer cell proliferation by inducing cell apoptosis and cell cycle arrest [17].

There have been some studies that investigated the effects of dietary fat, such as n-3, n-6 and n-9 polyunsaturated fatty acids, in IBD [18–26]. However, the effect of n-9 polyunsaturated fatty acids, such as olive oil, in experimental colitis is not very clear.

Cytokine signaling pathways involving transcription factors of the signal transducers and activators of transcription (STAT) family play a key role in the pathogenesis of IBD. STAT proteins are latent cytoplasmic transcription factors that induce transcription upon phosphorylation, dimerization and nuclear translocation. STAT3 and phospho-STAT3 (pSTAT3) levels were significantly increased in UC patients compared with controls [27].

Inflammation-related enzymes, including cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), are indicated to be key predisposing factors to CRCs [23], and COX-2 and iNOS expressions was closely associated with the development of cancers [28,29].

☆ Conflicts of interest: The authors declare that there are no conflicts of interest.

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Dextran sulfate sodium (DSS), with or without AOM, induced colon carcinogenesis in rodents [23,30,31], and the feeding of DSS enhanced cell proliferation [32]. Equability between cell proliferation and apoptosis was closely correlated to colon inflammation and carcinogenesis, as demonstrated in UC in humans and the experimental colitis model [33–35].

The aim of the present study was to demonstrate whether feeding with olive oil to rats suppressed inflammation, cell proliferation, STAT3 and pSTAT3 induced by oral administration of DSS for 1 week at a time followed by a 1-week recovery period and repeated three times.

2. Materials and methods

2.1. Experimental animals and diets

Six-week-old male Sprague–Dawley rats (Japan CLEA Inc., Tokyo, Japan) were housed in an air-conditioned room that was maintained at 24°C–25°C, with constant humidity and an alternating 12-h light/dark cycle. All rats had access to food and water *ad libitum*. The base diet was standard laboratory diet AIN 76 (NOSAN, Kanagawa, Japan). Five percent extra-virgin olive oil (EVOO) (Ajinomoto, Tokyo, Japan) was added to diet AIN (weight percentage: 5%). EVOO contained 125±25 mg/kg total phenolics. HT in EVOO was 7.9±2.7 mg/kg (0.40±0.14 mg/kg in 5% EVOO), and tyrosol in EVOO was 15±4.1 (0.75±0.21 mg/kg in 5% EVOO). The diet was kept at 4°C and was exchanged with a fresh one every few days. Body weights, drink intake, diet consumption and survival were monitored during the experimental period.

All experiments involving animals were approved by the Animal Ethics Committee of Saga Medical School.

2.2. Induction of chronic inflammation

DSS (MW: 36,000–50,000, MP Biomedicals, LLC, Illkirch, France) was administered to rats via drinking water (weight percentage: 4%) for 1 week, which was followed by a 1-week recovery period. The same administration method was repeated for three times. Rats were divided into three groups at random as shown in Fig. 1: control group, standard diet and no DSS (n=12); DSS group, standard diet+DSS (n=17); and DSS +EVOO group, olive oil diet+DSS (n=12). Activity of rat DSS-induced colitis during the experimental period was evaluated using a disease activity index (DAI) [36]. This index consisted of three scales: presence of rectal bleeding, weight loss and stool consistency.

2.2.1. Macroscopic and histological evaluation. On the final day of the experimental period, the rats were sacrificed under halothane anesthesia. The entire colon was carefully removed and opened longitudinally. Samples were photographed and measured and then segmented (proximal, middle, distal) for pathological evaluation. Samples were then fixed in 4% buffered formaldehyde (pH 7.4–7.5), dehydrated by increasing the concentration of ethanol and embedded in paraffin. Paraffin sections (4 μm thick) were sliced and stained with hematoxylin and eosin (H&E) in accordance with the standard procedures for the histological evaluation of colonic damage. The histological study was representative of at least five animals per group. Evaluation was undertaken by a pathologist (A.N.) who was unaware of the experimental protocol. A histological score [37] and the efficacy of treatment were analyzed on H&E-stained tissue using a standard microscope (Olympus, Tokyo, Japan). A histological score reflecting infiltration of inflammatory cells and epithelial structure was given on a scale of 0 to 6: 0=no signs of damage; 1=few inflammatory cells, no signs of epithelial degeneration; 2=mild inflammation, few signs of epithelial degeneration; 3=moderate inflammation, few epithelial ulcerations; 4=moderate to severe inflammation, ulcerations in more than 25% of the tissue section; 5=moderate to severe inflammation, large ulcerations of more than 50% of the tissue section; 6=severe inflammation and ulcerations of more than 75% of the tissue section.

2.3. Collection of colon tissue samples and Western blotting analysis

The colon mucosal layer was harvested by gently scraping the epithelium using a glass slide. Mucosal scrapings were then immediately washed twice with ice-cold phosphate-buffered saline (PBS; pH 7.4) and centrifuged at 1,000*g* for 5 min at 4°C. The pellet was resuspended with 2 vol of buffer A and lysed at 4°C for 30 min. Buffer A consisted of 250 mM sucrose (Sigma), 20 mM *N*-(2-hydroxyethyl)piperazine-*N*-(2-ethanesulfonic acid) (Sigma), KOH (pH 7.5), 10 mM KCl, 1.5 mM $MgCl_2$, 1 mM ethylenediamine tetraacetate, 1 mM ethyleneglycoltetraacetic acid, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 10 g/ml aprotinin, 10 g/ml leptin and 1.8 mg/ml iodoacetamide.

The homogenate was centrifuged at 1000*g* for 10 min at 4°C to remove nuclei before the supernatant was centrifuged at 10,000*g* for 15 min at 4°C to remove mitochondria. The supernatants of the 10,000*g* spin were further centrifuged at 1,000,000*g* for 1 h at 4°C. The resulting supernatant was the soluble cytosolic fraction, and the pellet was the membranous fraction. The supernatant and pellet fractions of the resuspended solution in buffer A were divided into multiple samples and frozen at −80°C for analysis. The amounts of STAT3, pSTAT3, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, COX-2, iNOS, proliferating cell nuclear antigen (PCNA) and cleaved caspase-3 in the frozen samples were determined by Western blotting using a previously described method with modification [9]. Equal quantities of protein were electrophoresed in a sodium dodecyl sulfate polyacrylamide gel and were then electroblotted onto a polyvinylidene difluoride membrane. After being blocked with PBS containing 0.1% Tween 20 (Sigma) and 5% skim milk at room temperature for 1 h, the membrane was incubated with rabbit monoclonal anti-STAT3 (Cell Signaling Technology, Beverly, MA, USA) at a dilution of 1:8000; rabbit monoclonal anti-pSTAT3 (Cell Signaling Technology) (1:1000); mouse monoclonal anti-TNF-α (Sigma-Aldrich, Saint Louis, MO, USA) (1:1000); rabbit polyclonal anti-IL-1β (Santa Cruz Biotechnology, Santa Cruz, CA, USA)(1:4000); mouse monoclonal anti-IL-6(R&D Systems, Minneapolis, MN, USA) (1:4000); rabbit polyclonal anti-COX-2 (Cell Signaling Technology) (1:1000); rabbit polyclonal anti-iNOS (Cayman, Ann Arbor, MI, USA) (1:1000); rabbit polyclonal anti-PCNA (Santa Cruz Biotechnology, Santa Cruz, CA, USA) (1:2000); rabbit polyclonal anti-cleaved caspase-3 (Cell Signaling Technology) (1:4000) and rabbit polyclonal anti-β-actin antibody (Cell Signaling Technology) (1:2000) was used as a loading control, respectively, at 4°C overnight. The antigen–antibody complex was detected with a peroxidase-conjugated secondary antibodies anti-rabbit for STAT3, pSTAT3, IL-1β, COX-2, iNOS, PCNA and cleaved caspase-3 (Cell Signaling Technology) at a dilution of 1:1000 or anti-mouse for TNF-α and IL-6(Cell Signaling Technology) (1:2000). Detection of chemiluminescence was performed using ECL Western blotting detection reagents (GE Healthcare, Buckinghamshire, UK). Densitometric assessment of the bands on the autoradiogram was done using Multi Gauge Version 3.0 (Fujifilm, Tokyo, Japan). Band intensities were quantified by measurements of absolute integrated optical intensities, estimating bands in each lane profile. Results were expressed as ratios to β-actin densitometry units.

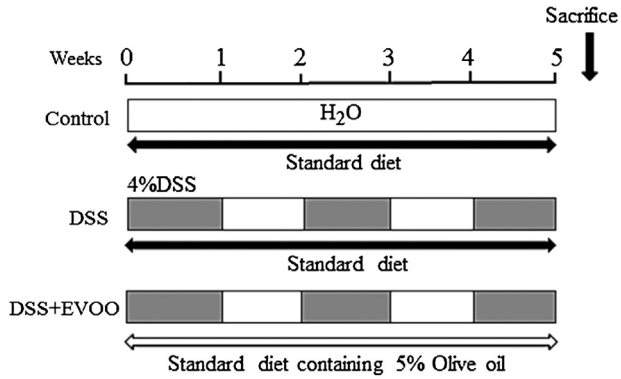

Fig. 1. Experiment design. DSS was administered to rats via drinking water (weight percentage: 4%) for a 1-week period followed by a 1-week recovery period. This cycle was repeated three times. Rats were divided into three groups at random as shown: control group, standard diet and no DSS; DSS group, standard diet+DSS; and DSS+EVOO group, 5% olive oil diet+DSS.

2.4. Statistical analysis

All data are expressed as the means±standard error of the mean (S.E.M.). Comparisons were done using analysis of variance (ANOVA) followed by Tukey, or repeated-measure ANOVA was used (SPSS ver. 18.0; SPSS, Chicago, IL, USA). A P value of <.05 was considered statistically significant.

3. Results

3.1. Effect of olive oil diet on DAI score in DSS-induced colitis

DAI score (Fig. 2) refers to external signs of colitis, indicating that DSS drinking induced symptoms of colitis at the beginning of week 3 and the DAI score increased in a time-dependent manner. Increases in the DAI score induced by DSS drinking were significantly attenuated by feeding with 5% olive oil (P<.01).

3.2. Total DSS drinking, diet consumption and body weight in DSS-induced colitis

DSS drinking was the same in the two groups (data not shown). Regarding diet consumption, the DSS+EVOO group significantly took diet more compared with control and DSS groups (data not shown).