SUPPLEMENTARY MATERIAL

Anti-inflammatory effect of fucoxanthin on dextran sulfate sodium-induced colitis in mice

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No potential conflict of interest was reported by the authors.

The anti-inflammatory activities of fucoxanthin, a marine carotenoid derived from the macroalgae and microalgae, have been demonstrated in the previous studies. However, the effect of fucoxanthin on ulcerative colitis (UC), an inflammatory bowel disease, was still unclear. In this study, we evaluated the in vivo anti-inflammatory effect of fucoxanthin on dextran sulfate sodium(DSS)-induced colitis in mice. Fucoxanthin at the doses of 50 and 100 mg/kg/day significantly protected against DSS-induced gradual loss of body weight, exhibited inhibitory effects on the DSS-induced increase of disease activity index and colon shortening. Moreover, fucoxanthin treatment resulted in a marked amelioration of the histological damage in the colon, and reduced the colonic PGE2 levels in colitic mice. In addition, the DSS-induced overexpressions of inflammation-related molecules including COX-2 and NF-κB were significantly decreased in fucoxanthin-treated mice. These finding suggested that the use of fucoxanthin provides a new and attractive alternative to control UC.

Keywords: fucoxanthin; ulcerative colitis; dextran sulfate sodium; anti-inflammatory effect
Experimental

Induction of colitis and fucoxanthin administration

Eight-week-old female C57BL/6J mice were obtained from the Laboratory Animal Center of Hubei Province (Wuhan, China). After acclimation for 1 week, all mice were randomly divided into 5 groups of 10 animals each: (1) normal control; (2) fucoxanthin 100 mg/kg/day group; (3) dextran sulfate sodium (DSS)-induced colitis model control group; (4) DSS + fucoxanthin 50 mg/kg/day group; (5) DSS + fucoxanthin 100 mg/kg/day group; (6) positive control group. Mice in 2, 4 and 5 groups orally administered with fucoxanthin obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA) dissolved in soybean oil at doses of 50 mg/kg/day or 100 mg/kg/day respectively. In positive control group, salazosulfapyridine (SASP) at a dosage of 500 mg/kg/day was orally administered. Other mice were orally administered with the same volume of soybean oil. After pretreatment with the corresponding agents or vehicles for 1 week, all mice, with the exception of those in group 1 and 2, were fed 3.5% (w/v) DSS (mol wt. 36,000-50,000, MP Biomedicals, Aurora, USA) mixed with drinking water for 7 days to induce colitis. Mice in the normal control and fucoxanthin 100 mg/kg/day groups were given normal drinking water. The study protocol was approved by the Laboratory Animal Ethical Committee of Three Gorges University (authorization number 2017493).

Assessment of the colitis severity

To evaluate the severity of experimental colitis, mice were daily monitored for body weight changes calculated as % of the initial weight. The disease activity index (DAI), a combined score of body weight loss, stool consistency and fecal occult blood, was calculated as described previously (De Fazio et al. 2016) at 1 hour after the last administration, then all animals were sacrificed, and the lengths of the colons were measured.

Histological examination

Samples of the distal colon from each animal were stained with hematoxylin and eosin (H&E) for the histological examination. The colonic damage was evaluated using a histological scoring system (Yan et al. 2015) in a blinded fashion.

Colonic PGE_2 levels

PGE_2 levels in the supernatant of homogenate from the distal colon tissues were measured according to the ELISA kit protocol (R&D Systems, Minneapolis, USA).
Real-time PCR

Total RNA was extracted from colon tissue using a TRIzol Reagent. After the photometrical determination of RNA concentration and purity, cDNA was generated from total RNA using the All-in-One™ First-Strand cDNA Synthesis kit (GeneCopoeia, USA). Primers were as follows: COX-2: 5’- ACG CTT CTC CCT GAA GCC GTA C -3’ and 5’- GTA GAG GGC TTT CAA TTC TGC AGC C -3’; NF-κB p65: 5’- GCT TTG CAA ACC TGG GAATA -3’ and 5’- TCC GCC TTC TGC TTG TAG AT -3’; β-actin: 5’- GAT TAC TGC TCT GGC TCC TAG C -3’ and 5’- GAC TCA TCG TAC TCC TGC TTG C -3’. Real-time PCR was performed using an Applied Biosystems StepOnePlus™ Real-Time PCR System. Levels of TLR4 COX-2 and NF-κBp65 mRNA were normalized against those of β-actin mRNA.

Immunofluorescence

The protein expression levels of COX-2 and NF-κB p65 were detected by immunofluorescence. For immunohistochemical staining, the distal colon samples were embedded in paraffin, cut into 5μm-thick sections. The sections were deparaffinized, rehydrated, blocked, then incubated with anti-NF-κB p65 or anti-COX-2 (Cell Signaling, USA) primary antibodies overnight at 4°C. After washes, sections were incubated with the corresponding secondary antibodies (Cell Signaling, USA), and stained with 4’,6-diamino-2-phenyl indole (DAPI) (Sigma, St. Louis, USA) to visualize nuclei. Images were obtained using an Olympus fluorescent microscope (Olympus, Center Valley, USA).

Statistical analysis

Results are expressed as mean ± standard deviation (SD). The statistical significance of difference between groups was performed using one-way ANOVA followed by Bonferroni’s post-hoc test. Statistically significant differences were considered when \( p < 0.05 \).
Figure S1. Effect of fucoxanthin on body weight changes, DAI scores and colon lengths in DSS-induced colitic mice (n=10). *p<0.05, **p<0.01, compared with the normal control group; # p<0.05, ## p<0.01, compared with the DSS control group.
Figure S2. Effect of fucoxanthin on the colonic inflammation in DSS-induced colitic mice (n=10). (a) Representative HE-stained sections of colon tissues; (b) Histology scores; (c) Colonic PGE$_2$ levels. **$p<0.01$, compared with the normal control group; $^#p<0.05, ^{##}p<0.01$, compared with the DSS control group.
Figure S3. Effect of fucoxanthin on the mRNA and protein expressions of COX-2 and NF-κB in DSS-induced colitic mice (n=10). (a) The mRNA expressions of COX-2;
(b) The mRNA expressions of NF-κB; (c) Immunofluorescence assay. # $p<0.05$, ## $p<0.01$, compared with the DSS control group.

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