

The anti-inflammatory effect of Xuanwei ham derived peptides in the dextran sulfate sodium-induced C57BL/6 mice model

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Objectives: Including Crohn's disease and ulcerative colitis, inflammatory bowel disease (IBD) is regarded as a chronic inflammatory disease and becomes the major metabolic disorder to influences intestinal health. The long terms intake of drugs would accompany by side effects of gastrointestinal irritation, and immune suppression along with the increasing risks of cardiovascular and gastrointestinal bleeding (Guha & Majumder, 2018). Recently, the anti-inflammatory efficacy of Xuanwei peptides (XP) was demonstrated in RAW264.7 macrophage cells with the involvement of the PI3K/AKT signal pathway (Fu et al, 2021). Till now, few studies have ever focused on the anti-inflammatory efficacy of dry-cured ham peptides *in vivo*.

Materials and Methods:

1. Animal treatments Thirty-two healthy male C57BL/6 mice (ten-week-old) were purchased from the Model Animal Research Center of Nanjing University (2018-0027). All mice were randomly separated into four groups: Normal control (NC), Dextran sulphate sodium induced group (DSS), XP-1 (100 mg/kg), XP-2 (300 mg/kg).
2. Serum and colon biochemical analysis For the serum sample, the inflammatory biomarkers were measured according to the commercial ELISA kit (Neobioscience Biological Technology, Shenzhen, China).
3. Western Blot The proteins in mice colon were extracted by RIPA lysis and then homogenized under 4 °C. The primary antibodies include rabbit anti-Claudin (1:1000), rabbit anti-TLR-4 (1:1000), rabbit anti-COX-2 (1:2000, Abclonal, Wuhan, Hubei, China), rabbit anti- β -actin (1:5000, Sigma, St Louis, MO, USA) were investigated here.

Results and Discussion:

1. XP attenuated DSS-induced colitis symptoms In the NC group, the bodyweight of mice was increased gradually from 23.2 g to 26.4 g at the end of 20 days. Compared with NC, the supplement of DSS induced body weight loss from day 2 in DSS, XP-1, and XP-2 treatments. Starting from Day 10, the bodyweight of mice was increased gradually as the evacuation of DSS. Known from colon length, the DSS treatment shorted the colon from 8.25 cm (NC) to 7.25 cm (DSS), whereas the XP treatments had a longer colon length of 8.63 cm and 8.92 cm, respectively.
2. Analysis of inflammatory cytokines The two doses of XP significantly reduced TNF- α production compared to the DSS group ($p < 0.05$). Similarly, IL-6, MCP-1 concentrations in XP groups were all reduced than DSS, whereas the dose of XP-2 (300 mg/kg) showed no differences in IL-1 β secretion compared with DSS ($p > 0.05$).
3. The proteins expressions The expression of COX-2 in the DSS group was increased significantly than in the NC, and the XP supplementation suppressed COX-2 expression ($p < 0.05$). In addition, the DSS induced inhibition on Claudin expression than NC, and the supplement of XP exhibited an improving effect on that. As for the TLR-4, the DSS also induced a suppressing expression than NC ($p < 0.05$), whereas the XP had a relieving effect on it ($p < 0.05$). In general, the supplement of XP had a significant effect on the key proteins, where the COX-2 and TLR-4 were suppressed and Claudin was improved.

Conclusions: The supplement of XP attenuated IBD symptoms and pro-inflammatory cytokines secretion along with resuming the tight-junction protein expressions in DSS-induced colitis mice. Generally, the XP might be regarded as a potential ingredient for restoring the inflammatory level in colon health and developed as a multi-targeted functional formula for IBD patients.

Key words: Dry-cured ham, Peptides, Anti-inflammatory, Cytokines