The anti-inflammatory effects of dry-curing ham derived bioactive peptides

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Introduction: During the fermentation of dry-cured hams, a large amount of peptides are released from proteins along with bioactive function. In our previous study, the Xuanwei ham peptides (XHP) exhibited excellent free radical scavenging activities, presenting the potential of its inflammatory regulatory activity (Xing et al., 2018). In current study, the RAW264.7 macrophage cells were cultured *in vitro* and treated with different dose of XHP. The secretion of inflammatory cytokines was measured to investigate the anti-inflammatory effects of XHP.

Materials and methods:

1. The cytotoxicity testing

   The cytotoxicity of XHP in RAW264.7 cells was measured by CCK-8 assay (Lee & Cho, 2021).

2. Enzyme-Linked Immunosorbent Assay

   The experiment was separated with normal control, positive control (PC) and XHP treated groups. The cytokines concentration in different groups was analyzed by QuantiCyto ELISA kit. The OD values were measured at 450 nm, and the concentration of cytokines was proportional to the standard curve values. Similarly, the secretion of TNF-α, IL-8 and IL-6 was measured with the same method (Qian, Zhao, & Yang, 2020).

3. Real-time PCR analysis

   Total RNA was extracted by the RNAprep pure cell Kit. RT-PCR analysis was carried out using ChamQ SYBR qPCR Master Mix on QuantStudio™Real-Time PCR system (Thermo Fisher Scientific, Waltham, MA, U.S.A).

Results:

1. The cytotoxicity of XHP

   At the concentration of 0.2-2.0 mg/mL, the cytotoxicity of XHP was measured by CCK-8 method. Compared to the control group, the cell viability showed no significant differences in 0.2-1.0 mg/mL treatments, whereas a higher concentration of XHP (2.0 mg/mL) posed to inhibit the cell activity. Thus, the dose of XHP was checked without cytotoxicity among 0.2-1.0 mg/mL in RAW264.7 cells.

2. The anti-inflammatory effect of XHP

   In current study, the lipopolysaccharide (LPS) induced inflammatory condition was established in RAW 264.7 cells along with the incubation of XHP. Compared with positive control (PC, LPS induced group), the concentration of nitric oxide (NO) was decreased in XHP treated group. Among the concentration of 0.2-1.0 mg/mL, the treatment of XHP also inhibited the secretion of TNF-α, IL-8 and IL-6 in RAW264.7 cells.

3. The isolation of XHP

   XHP were separated to four fractions by dextran gel chromatography and the peak 2 had a higher content than the other three peaks. From the inflammatory testing of purified fractions, the peak 4 had a stronger effect to suppress the secretion of inflammatory cytokines than other three peaks. Compared with PC, bioactive peptides in peak 4 reduced mRNA expression of IL-8 and IL-6 significantly.

Conclusions: The cellular NO level was shown to be reduced along with the inflammatory cytokines were inhibited in XHP treatment. These results suggested that XHP exhibited remarkable anti-inflammatory effect in RAW264.7 cells.

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Literature:

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