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N-acetylated Chitooligosaccharides Inhibit TNF-α-induced E-selectin Expression in Endothelial Cells via the JNK-NF-κB Pathways

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1 Introduction

Chitooligosaccharides (COS) have been shown to regulate various cellular and biological functions. However, the effect of COS on inflammatory responses of the cells remain unclear. Based on the previous study [1], we used a facile fractionation method to recover the chitooligosaccharides of different molecular weights after the nitrous acid degradation of chitosan. In the present study, we further explored the anti-inflammatory properties of highly N-acetylated chitooligosaccharides (NACOS) for their potential medical applications. The adhesion of circulating leukocytes to endothelial cells (ECs) and their subsequent extravasation are critical for inflammatory responses. Expression of certain inflammatory genes, including E-selectin, in ECs is regulated at sites of leukocyte recruitment. Transient increase in E-selectin expression by the cytokine tumor necrosis factor-α (TNF-α) is shown. Our study aimed at elucidation of the role of NACOS in the TNF-α-induced E-selectin expression in ECs. The mechanisms underlying the effect of NACOS on E-selectin expression in response to TNF-α were investigated.

2 Materials and Methods

To analyze the effects of NACOS on TNF-α-induced E-selectin expression, human umbilical vein ECs were pre-treated with NACOS with varied concentrations (50, 200, 500 μg/mL) for 1 day before activation by TNF-α (5 ng/mL) for 4 hours. The expression of E-selectin mRNA was determined using Northern blot analysis. The phosphorylation of different mitogen-activated protein kinases (MAPKs), i.e. extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK, were examined by Western blot analysis. ECs were transiently transfected with the promoter constructs containing the promoter regions of E-selectin (–540 bp) and the reporter gene luciferase, and the effects of NACOS on the E-selectin promoter activity induced by TNF-α were examined by luciferase assay. The chromatin immunoprecipitation (ChIP) assay was performed to investigate the in vivo regulation of nuclear factor-κB (NF-κB) binding to the promoter regions of E-selectin in these ECs by using antibodies against p65 subunit of NF-κB and the promoter-specific primers.

3 Results

ECs treated with NACOS at varied concentrations (50, 200, 500 μg/mL) significantly inhibited the TNF-α-induced E-selectin mRNA expression in a dose-dependent manner. TNF-α induced the phosphorylation of ERK, JNK, and p38 MAPK in ECs, reaching a maximal level within 15 minutes after TNF-α stimulation. Pre-treatment of ECs with NACOS (200 μg/mL) for 15 minutes inhibited the TNF-α activation of p38, but it had no effect on the TNF-α activation of ERK and JNK. However, ECs treated with COS for 6 hours significant inhibited the TNF-α-induced JNK activation, but not ERK and p38. TNF-α induced promoter activity of E-selectin in ECs. ECs co-transfected with dominant negative mutants of JNK, but not ERK and p38 MAPK, significantly inhibited the TNF-α-induced E-selectin promoter activity. Pre-treatment of transfected ECs with COS (200 μg/mL) significantly inhibited the TNF-α-induced E-selectin promoter activity. ChIP assay demonstrated

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that COS inhibited the in vivo binding of NF-κB to the promoter regions of E-selectin induced by TNF-α.

4 Conclusion

This study demonstrates that NACOS inhibites TNF-α-induced E-selectin expression in ECs, and that this inhibitory effect is mediated through the JNK/NF-κB signaling pathways. Our findings provide a molecular basis for the mechanism by which NACOS inhibit TNF-α-induced adhesion molecule expression in ECs and hence may be able to serve for medical and pharmaceutical application against inflammation.

References

Lin, C.W. and Lin J.C. (2003): *Biomacromolecules* **4**, 1691-1697.