Study of CopA3 Peptide Derived from *Copris tripartitus* on Anti-inflammatory Effect

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ABSTRACT

The objective of this study was to evaluate the skin inflammation effect of the synthetic antimicrobial peptide of *Copris tripartitus*. Regulatory mechanisms of cytokines and nitric oxide(NO) involved in immunological activity of RAW 264.7 cells. Tested cells were treated with CopA3 and further cultured for an appropriated time after lipopolyssacharide(LPS) addition. During the entire experimental period, 5, 25, 50, 100 μg/ml of CopA3 had no cytotoxicity. We examined iNOS, COX-2 by western blot. CopA3 showed iNOS and COX-2 inhibition activity 41%, and 59% at 100 μg/ml, respectively. In addition, CopA3 reduced the release of inflammatory cytokines including TNF-α, IL-1β and IL-6. These results suggest that CopA3 may have significant effects on inflammatory factors, and may be a potential anti-inflammatory therapeutic agent.

Material

The synthetic peptides used in this study CopA3 AnyGen (Gwang-ju, Korea) synthesized with a purity more than 98%

Method

1. Cell viability (MTT assay)

: measured by Carmichael[1)] method.

2. NO production

3. ELISA

: TNF-α, IL-6, IL- β production

4. inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2) activity

: Western blotting

Results

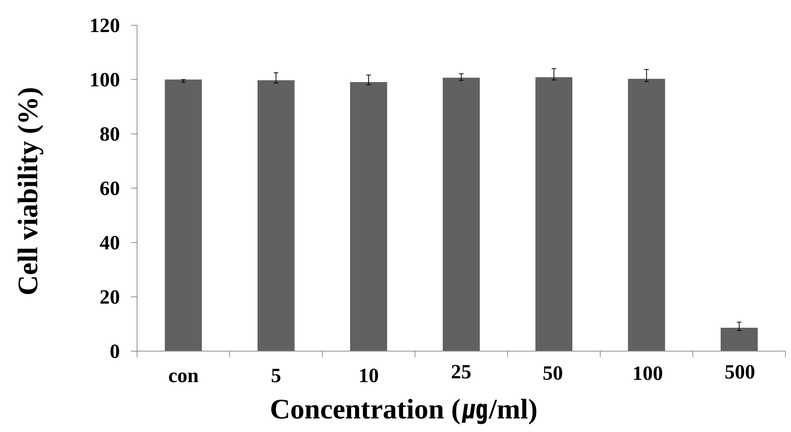

Fig. 1. Cell viability of CopA3 on RAW 264.7 cell. RAW 264.7 cells were treated with 5,10, 25, 50, 100, 500 μg/ml of CopA3 dissolved in media for 1 h prior to the addition of LPS (1 μg/ml), and the cells were further incubated for 24 h. Data represent the mean ± S.D. with eight separate experiments. Data represent the mean ± S.D. with three separate experiments.

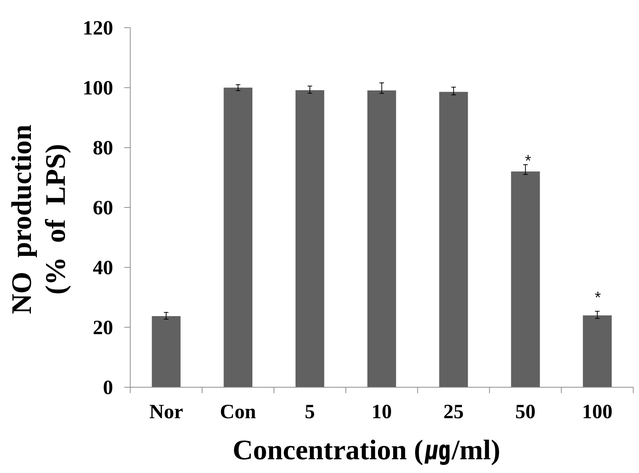

Fig. 2. Inhibitory effects of CopA3 on the production of nitric oxide RAW 264.7 cells. RAW 264.7 cells were cultured with LPS (1 μg/ml) in the presence or absence of CopA3 for 24 h to determine the level of NO. Nor : LPS not induced group, Con : LPS induced group. The data represent the mean ± SD of three separate experiments (significant as compared to control. *$p < 0.05$).

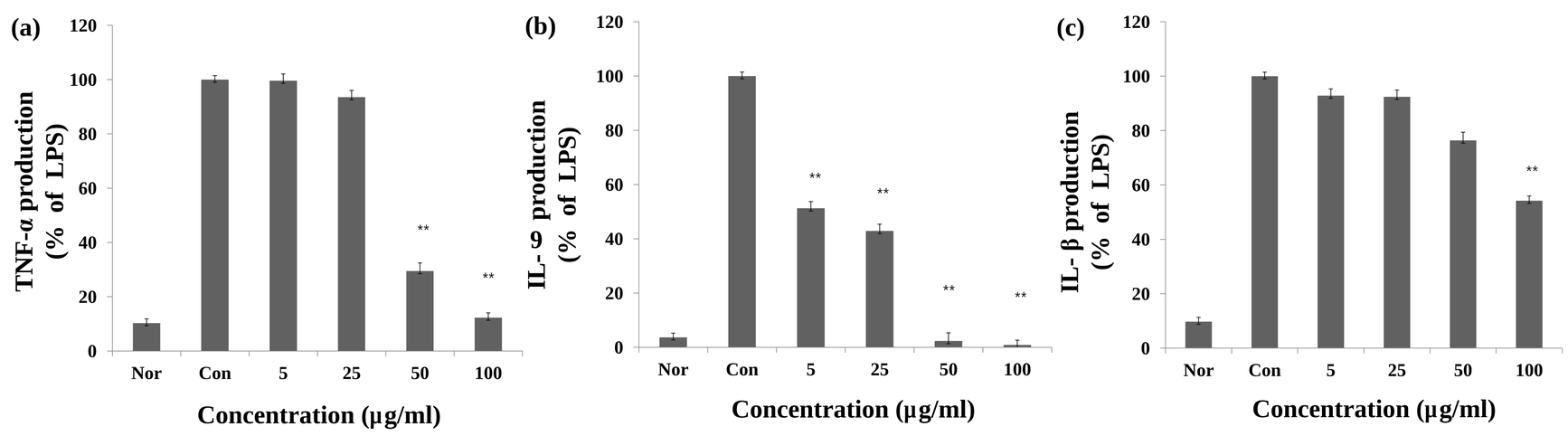

Fig. 3. Effects of CopA3 on the production of cytokines stimulated by LPS. Production of TNF-α (a) IL-6 (b), IL-1β (c) were measured in the medium of RAW 264.7 cells cultured with LPS (1 μg/ml) in the presence or absence of CopA3 for 24 h. The amount of TNF-α was measured by immunoassay as described in materials and methods. Nor : LPS not induced group, Con : LPS induced group. Data represent the mean ± S.D. with three separate experiments. One-way ANOVA was used for comparisons of multiple group means followed by t-test (significant as compared to control. *$p < 0.05$, **$p < 0.01$).

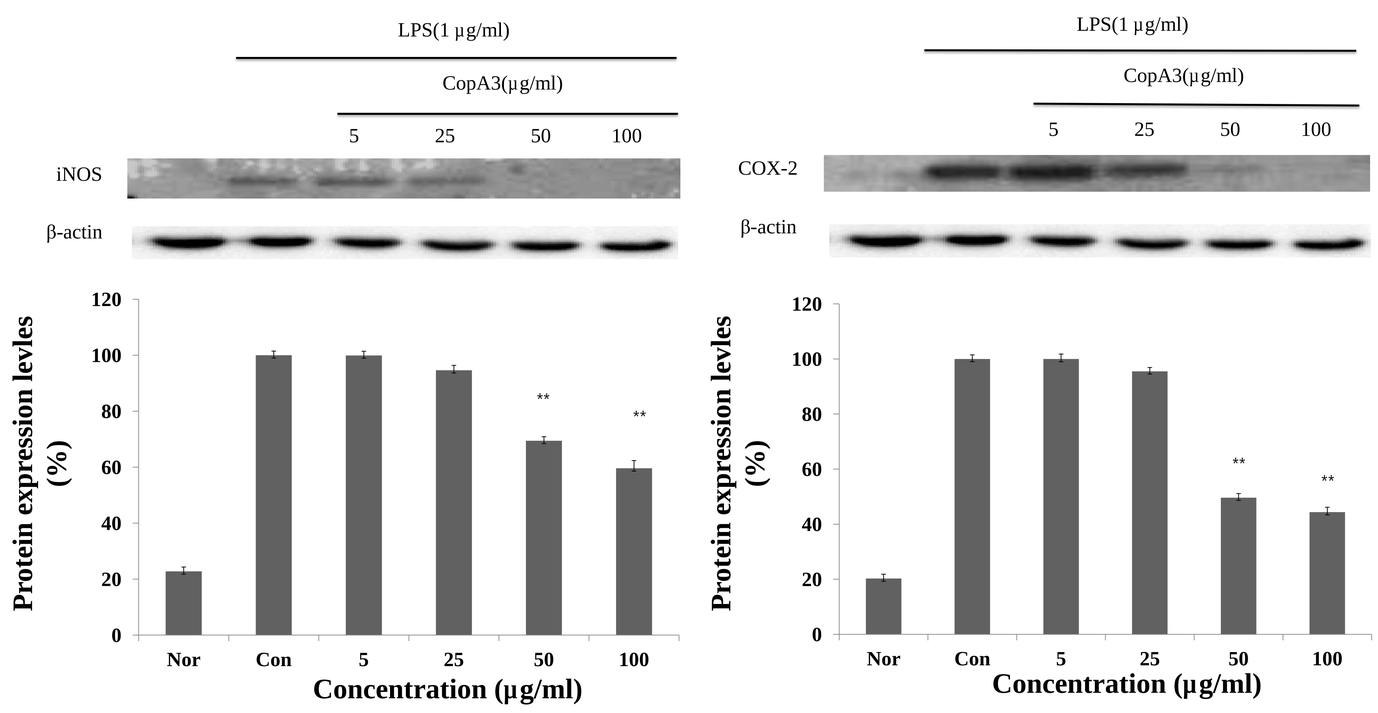

Fig. 4. Inhibitory effects of CopA3 on the protein levels of iNOS and COX-2 in RAW 264.7 cells. RAW 264.7 cells (5 x 10^5 cells/ml) were pre-incubated for 24hr, and the cells were stimulated with lipopolysaccharide (1 μg/ml) in the presence of complex extracts sample(5, 25, 50, 100 μg/ml) for 24 hr. Nor : LPS not induced group, Con : LPS induced group. Data represent the mean ± S.D. with three separate experiments. One-way ANOVA was used for comparisons of multiple group means followed by t-test (significant as compared to control. *$p < 0.05$, **$p < 0.01$).

Conclusion

1. 1. The CopA3 caused no significant cellular toxicity to macrophages and significantly restricted the creation of NO, TNF-α, IL-6, IL-1β, iNOS and COX-2 in the LPS-induced macrophages at the Concentration of 100 ug/ml.
2. 2. CopA3 decreased nitric oxide(NO) production activity dose dependently, especially at 100 μg/ml of 83%.
3. CopA3 inhibited the production of NO, TNF-α, IL-6, IL-1β as well as the expressions of iNOS, COX-2 in the LPS-induced macrophages.
4. The results above indicate that CopA3 significantly reduces the effect of inflammatory cytokines.

Reference

1) Carmichael J, DGraff WG, Gazdar AF, Minna JD and Mitchell JB (1987) Evaluation of a tetrazolium based semiautomated colorimetric assay : assessment of chemosensitivity testing. *Cancer Res.* **47(4)**, 936-942.