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MP-2, compound isolated from *Dendropanax morbifera* Lev inhibits LPS-induced NO production by down-regulated NF-kB in RAW 264.7 macrophage cells

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Objectives

To investigate the mechanisms of the anti-inflammatory effect of MP-2, oleifolioside isolated from *Dendropanax morbifera*, we examined production of pro-inflammatory mediators such as nitric oxide (NO) and prostaglandin E_2 (PGE_2) in LPS-induced RAW 264.7 cells. We also assessed expressions of inducible nitric oxide synthase (iNOS) and cyclooxigenase-2 (COX-2), which are involved in immune and inflammatory responses, at the protein and mRNA level using Western blot and RT-PCR. Because nucler transcription factor kB (NF-kB) is involved in the expression of inflammatory mediator gene, activation and translocation of NF-kB were determined by electrophoretic mobility shift assay (EMSA) and immunocytochemical analysis.

Materials and Methods

○ Materials

- Isolation of MP-2 compound from *Dendropanax morbifera*,
- Cell culture : RAW 264.7 macrophage cells
- Lipopolysaccharide (LPS): Sigma
- DAPI and FITC-conjugqted antibody

○ Methods

- MTT cytotoxicity assay
- NO production, PGE_2 and TNF-a ELISA assay
- RT-PCR and Western blot assay
- iNOS promoter assay, DAPI staining
- EMSA and immunocytochemical analysis

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Results

MP-2 significantly inhibited the LPS-induced NO and PGE_2 productions in LPS-stimulated Raw 264.7 macrophage cells. The decrease in quantity of NO product was accompanied by the decrease in the iNOS protein and its mRNA expression levels. In addition, the expression level of COX-2 protein responsible for PGE_2 production was inhibited by MP-2 treatment in a dose-dependent manner. MP-2 suppressed NF-kB DNA binding activity and nuclear translocation of p65, which was accompanied by inhibition of IkB-a phosphorylation and IkB-a degradation. These results suggest that inhibition of NO production by MP-2 occurs via blocking the phosphorylation as well as the degradation of IkB-a protein, thus preventing the translocation and activation of NF-kB in the nucleus.

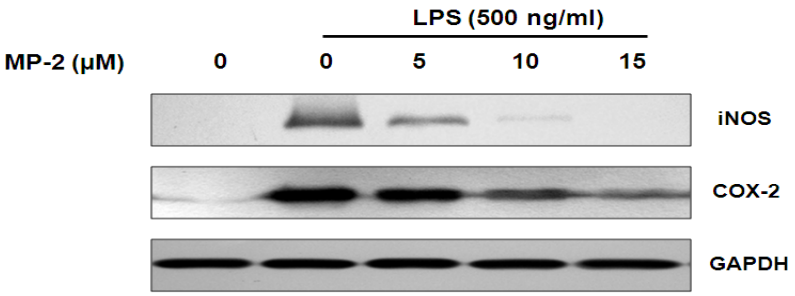

Fig. 1. Effects of MP-2 on iNOS and COX-2 expression in LPS-stimulated RAW 264.7 cells.

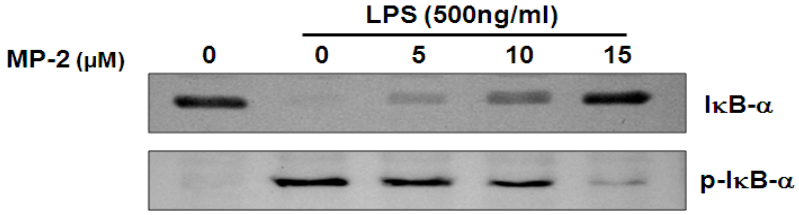

Fig. 2. Effects of MP-2 on degradation and phosphorylation of IkB-a protein in LPS-stimulated RAW 264.7 cells.

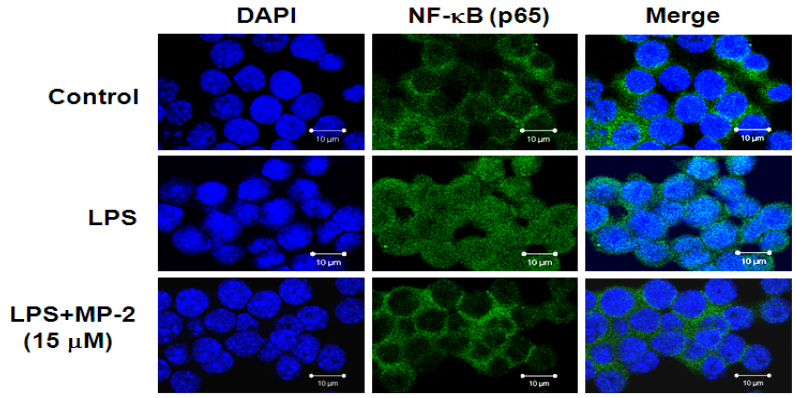

Fig. 3. Effect of MP-2 on NF-kB nuclear translocation in LPS-stimulated RAW 264.7 cells.