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The Methanol Extract from the Leaves of *Morus alba* and its Compounds Inhibit LPS-induced NO Production and Inflammatory Cytokine

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Objectives

We examined crude drug that originated from Mulberry (*Morus alba* L., Family; Morceae) leaves containing many nutritional components, which are the best food source for silk worms. The anti-inflammatory effects of mulberry leaves using lipopolysaccaride -stimulated microglia BV-2 and macrophage Raw 264.7 cells were investigated.

Materials and Methods

1. Materials - Plant : Leaves of Morus alba (MML), its compound (ML-3), cell line : BV-2, Raw 264.7 cells
2. Methods -NO production, Griess reaction, iNOS expression (Western blot), IL-1β expression (RT-PCR)

Results and Discussion

In the present study, we found that methanol extract obtained from mulberry leaves (MML) and its compound (ML-3) inhibited the NO production in LPS-stimulated BV-2 and Raw 264.7 cells in a dose-dependant mammer. Among numerous substances, No produced by expression of inducible nitric xoide synthase (iNOS) is an important indicator of inflammation and neuronal cell death. Also iNOS expression can be induced by selected immunological stimuli such as LPS, interferon-γ, tumor necrosis factor-α and interleukin-1β, and this enzyme produces NO continuously. Activation of BV-2 cells and Raw 264.7 cells with lipopolysaccharide (LPS), interferon-γ, tumor necrosis factor-α and interleukin 1β (IL-1β) induced NO production followed by cell death. After treatment with LPS in the presence of MML, ML-3, MML and ML-3 decreased the NO production as well as death of activated cells in a dose-dependant manner. Also these have no toxicity in the BV-2 and Raw 264.7 cells at the experimental concentration. MML, ML-3-mediated inhibition of NO production was accompanied by suppression of inducible nitric oxide synthase (iNOS) protein induction and interleukin 1β (IL-1β) protein expression. Taken together, our results indicate that MML and ML-3 inhibits NO production of microglia and macrophage by suppressing iNOS expression and these inhibitory effects of MML, ML-3 on NO production are exerted through

inhibition of IL-1β-mediated iNOS expression.

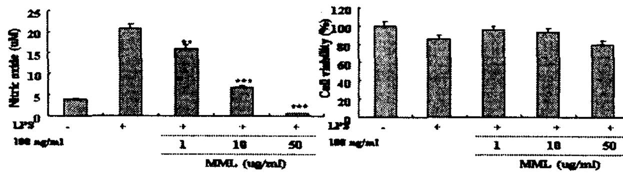

Fig 1. Effect of MML on NO production in LPS-stimulated BV-2 cells. Nitrate was measured using Griess reaction at 24h after treatment with LPS (100ng/ml) in the presence or absence of MML. All data were presented as the mean S.E.M. Asterisks indicated statistically significant differences from treatment with LPS alone (**;$p<0.01$: ***:$p<0.005$)

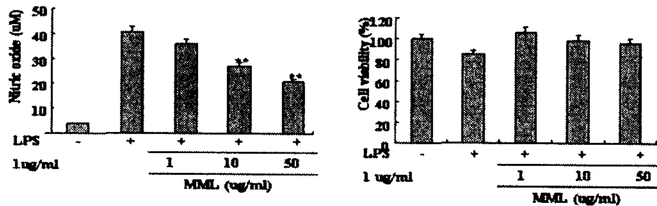

Fig 2. Effect of MML on NO production in LPS-stimulated Raw 264.7 cells. Nitrate was measured using Griess reaction at 24h after treatment with LPS (100ng/ml) in the presence or absence of MML. All data were presented as the mean ± S.E.M. Asterisks indicated statistically significant differences from treatment with LPS alone (**;$p<0.01$)

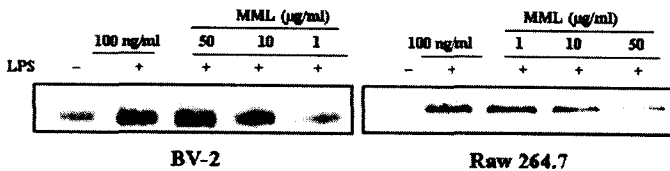

Fig 3. Effect of MML on iNOS induced by LPS in BV-2 cell and Raw 264.7 cells. iNOS protein was detected using western blot analysis at 6h after treatment with LPS 100ng/ml in the presence or absence of MML.

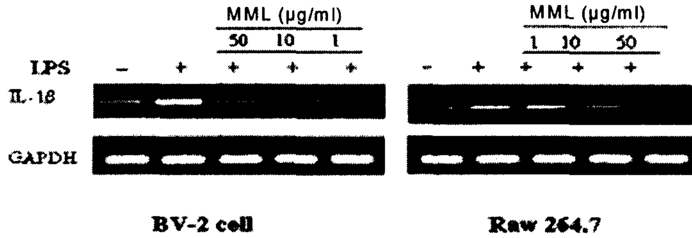

Fig 4. Effect of MML on IL-1β in LPS-stimulated BV-2 cell and Raw 264.7 cells. Expression of mRNA was detected using RT-PCR at 4h after treatment with LPS (100ng/ml) in the presence or absence of MML.

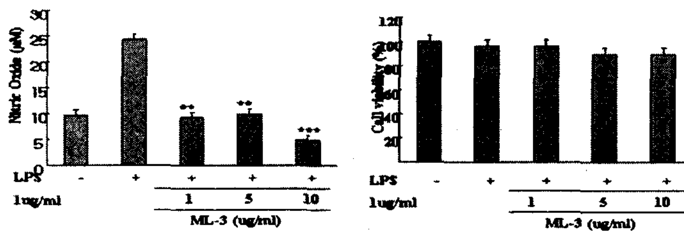

Fig 5. Effect of ML-3 on NO production in LPS-stimulated BV-2 cells. Nitrate was measured using Griess reaction at 24h after treatment with LPS (100ng/ml) in the presence or absence of M-3L. All data were presented as the mean ± S.E.M. Asterisks indicated statistically significant differences from treatment with LPS alone (**;p<0.01: ***:p<0.005)

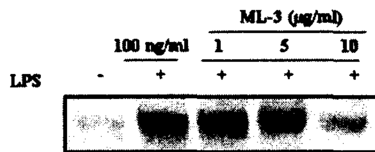

Fig 6. Effect of ML-3 on iNOS induced by LPS in BV-2 cells. iNOS protein was detected using western blot analysis at 6h after treatment with LPS 100ng/ml in the presence or absence of ML-3.

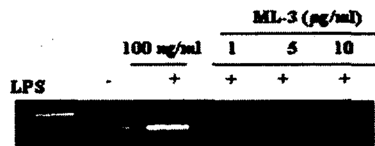

Fig 7. Effect of MML on IL-1 in LPS-stimulated BV-2 cell and Raw 264.7 cells. Expression of mRNA was detected using RT-PCR at 4h after treatment with LPS (100ng/ml) in the presence or absence of MML.

<u>**References**</u>

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