

Full Length Research Paper

Anti-inflammatory effect of oleanoic acid 28-O- β -D-glycopyranosyl ester isolated from *Aralia cordata* in activated HMC-1 cells

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Aralia cordata is known in traditional Korean medicine as a remedy for arthritis. As a part of our program to screen medicinal plants for potential anti-inflammatory compounds, oleanoic acid 28-O- β -D-glycopyranosyl ester (OA) was isolated from the roots of *Aralia cordata*. So, the immune modulation molecular mechanism of OA isolated from *A. cordata* was studied. One of the possible mechanisms for its protective activities is by down regulation of the inflammatory responses. Therefore, cells from the human mast cell line (HMC-1) were used to investigate this effect. OA significantly inhibits the way in which phorbol 12-myristate 13-acetate (PMA) plus A23187 induces the production of inflammatory cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-6, IL-8 and intracellular Ca²⁺ levels. In activated HMC-1 cells, phosphorylation of extra-signal response kinase (ERK) 1/2, degradation of I κ B, and activation of NF- κ B decreased after treatment with OA. OA suppressed the expression of TNF- α , IL-6 and IL-8 through a decrease in the intracellular levels of Ca²⁺ and ERK 1/2, as well as activation of NF- κ B. These results indicated that OA exerted a regulatory effect on inflammatory reactions mediated by mast cells.

Key words: Oleanoic acid 28-O- β -D-glycopyranosyl ester (OA), *Aralia cordata*, anti-inflammation effect, NF- κ B, human mast cell.

INTRODUCTION

Mast cells are important effector cells in the pathogenesis of allergic reactions and immune response system. Activated mast cells release pro-inflammatory cytokines and inflammatory mediators such as histamine, leukotrienes, serotonin, prostaglandin (PG)E₂, and prostaglandin (PG)D₂ (Zhu et al., 1999; Royer et al., 2001; Stassen et al., 2001). Cytokines such as interleukin (IL)-6, tumor necrosis factor (TNF)- α , and IL-8 are released in

a coordinate network and play an important role in chronic inflammation. As such, the pattern of cytokine expression largely determines the nature and persistence of the inflammatory response (Barnes and Adcock, 1993). Especially, TNF- α is an autocrine stimulator as well as a potent inducer of other inflammatory cytokines, including IL-1 β , IL-6, IL-8, and granulocyte macrophage-colony stimulating factor (GM-CSF) (Arend and Dayer, 1995; Butler et al., 1995). Cytokines produce their cellular effects by activation of various transcription factors such as AP-1 and NF- κ B. Furthermore, the expressions of many of these cytokines and their receptors are also

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up-regulated by these transcription factors. Calcium acts as a second messenger during cell activation, and the increase of the intracellular Ca^{2+} level is believed to be an essential trigger for mast cell activation (Rasmussen and Goodman, 1977; White et al., 1985). Moreover, it has been reported that the Ca^{2+} -induced activation of NF- κ B and increased Ca^{2+} levels induce the release of biological mediators, including TNF- α , IL-6, and IL-8 (Jeong et al., 2002). Moreover, NF- κ B activation is required for the expression of many inflammatory proteins such as TNF- α , IL-6, cyclooxygenase (COX-2), and inducible nitric oxide synthase (iNOS) (Nam, 2006). Therefore, the inhibition of NF- κ B can reduce the expression of inflammatory genes, and this is one of the mechanisms by which anti-inflammatory agents exert their anti-inflammatory effects (Newton et al., 1997).

Aralia cordata Thunb. (Araliaceae) is a perennial herb found throughout Korea, China, and Japan. It has a strong scent typical of an aromatic medicinal plant, and its roots are used for the treatment of rheumatism, toothache, lumbago, and fever remedy (Perry, 1980). *A. cordata* was shown to inhibit cytotoxicity and COX-2 activity (Lee et al., 2006). In biological studies, a few essential oils and diterpenes isolated from the roots have been shown to have anti-inflammatory and analgesic effects (Han et al., 1983; Okuyama et al., 1991). In our previous study, ent-pimara-8(14), 15-dien-19-oic acid, isolated from *A. cordata* has a strong anti-inflammatory effect of which was tested in the murine macrophage model (Kang et al., 2008). For more research, as a part of our ongoing screening program to evaluate the anti-inflammatory potential of natural compounds, the in mast cell-mediated inflammatory model of *A. cordata* was investigated through activity guided fractionation. This led to the isolation of a triterpene-type compound (oleanic acid 28-O- β -D- glycopyranosyl ester (OA)) from *A. cordata*, which is an anti-inflammatory compound tested in the human mast cell model. Furthermore, to investigate how OA affects the anti-inflammatory mechanism, phorbol 12-myristate 13-acetate (PMA) plus A23187-induced expression of pro-inflammatory mediators was used by inhibiting I κ B α /NF- κ B signal pathways.

MATERIALS AND METHODS

Reagents

PMA, calcium ionophore A23187 (Calcymycin; C29H37N3O6), Fluo-3/AM and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were from Sigma Chemical Co. (St. Louis, MO, USA). In addition, Iscove's modified Dulbecco's medium (IMDM) was purchased from Gibco BRL (Grand Island, NY, USA); the anti-human TNF- α , IL-6, IL-8, and GM-CSF antibodies, the biotinylated anti-human TNF- α , IL-6, and IL-8 antibodies, and recombinant human TNF- α , IL-6, and IL-8, were purchased from BD PharMingen (San Diego, CA, USA); the COX-2, p65 NF- κ B, I κ B, and p-I κ B antibodies were from Santa Cruz Biotechnology (Santacruz, CA, USA).

Plants

The aerial parts of *A. cordata* were collected in Daejeon, Korea, in November 2004, and identified by one of the authors (KiHwan Bae). A voucher specimen (CNU 1499) was been deposited in the herbarium at the College of Pharmacy, Chungnam National University, Daejeon, Korea.

Isolation of oleanic acid 28-O- β -D- glycopyranosyl ester (OA)

OA was isolated from the dried aerial parts of *A. cordata* as described previously (Lee et al., 2006). Briefly, the dried aerial parts of *A. cordata* (4 kg) were extracted three times with ethanol under reflux for 3 days, filtered and concentrated to yield an ethanol extract (300 g). The ethanol extract was suspended in H_2O and then partitioned successively with hexane, ethylacetate, and butanol to yield a hexane-soluble fraction (85 g), an ethylacetate-soluble fraction (63 g), and a butanol-soluble fraction (82 g), respectively. The hexane fraction (85 g) was subjected to silica gel column chromatography eluted with gradient CHCl_3 -MeOH (100:1 to 1:2) to afford some fractions (Fraction 5 to 9). Repeated silica gel column chromatography of fraction 9 using hexane-EtOAc (2:1) gave compound OA (15 mg). Column chromatography was performed by using silica gel (Kieselgel 70 to 230 mesh and 230 to 400 mesh, Merck), and thin layer chromatography (TLC) was performed on pre-coated silica gel 60 F254 (0.25 mm, Merck). The structure of this compound was confirmed by comparison of spectral data with those reported in the literature (Figure 1) (Lee et al., 2006).

Cell culture

Human leukemic mast cell line (HMC-1 cells) was kindly provided by Dr. Kim SH (Kyungpook National University, Daegu, Korea). The cells were grown in IMDM and supplemented with 100 U/ml of penicillin, 100 $\mu\text{g}/\text{ml}$ of streptomycin, and 10% fetal bovine serum at 37°C in 5% CO_2 with 95% humidity. The HMC-1 cells were pretreated with OA (1, 10 and 100 μM). Next, the cells were stimulated with 50 nM of PMA plus 1 μM of A23187 and they incubated at 37°C for an indicated time.

MTT assay for cell viability

For the MTT colorimetric assay of cell survival, the method of Kang et al. (2008) was used with minor modifications. First, cell aliquots (3×10^5) were seeded in microplate wells and incubated with 20 μl of an MTT solution (5 mg/ml) for 4 h at 37°C under 5% CO_2 and 95% air. Next, 100 μl of dimethyl sulfoxide was added to extract the MTT formazan, and an automatic microplate reader was used to read the absorbance of each well at 540 nm.

Cytokines assay

The HMC-1 cells were pretreated with various concentrations of OA (1 to 100 μM) for 1 h before PMA plus A23187 stimulation. Enzyme-linked immunosorbent assay (ELISA) method was used to assay the culture supernatants for TNF- α , IL-6, and IL-8 protein levels. To measure cytokine concentration, a modified ELISA method was used. First, a sandwich ELISA was conducted for TNF- α , IL-6, and IL-8 in duplicate in 96-well ELISA plates (Nunc, Denmark). Second, the supernatant was decanted into a new microcentrifuge tube, and the amount of cytokines was determined by ELISA. ELISA plates (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) were coated overnight at 4°C with anti-human TNF- α , IL-6, and IL-8

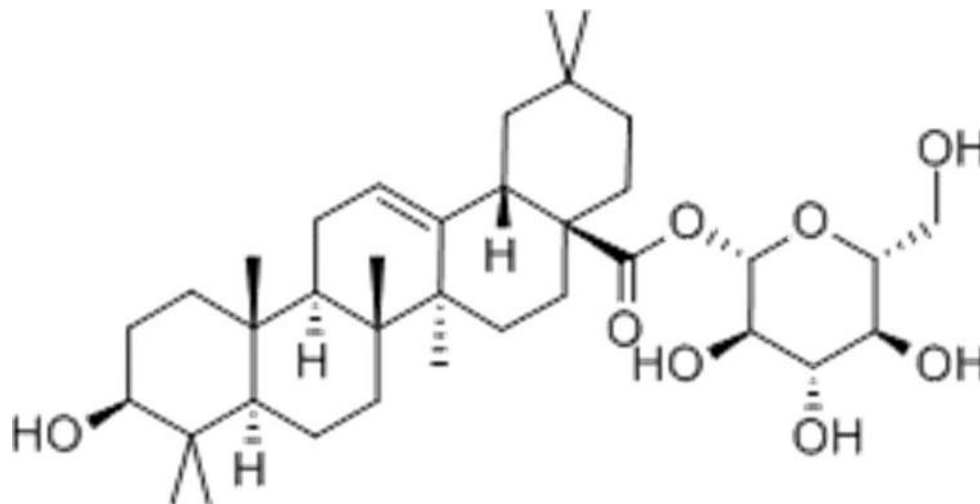


Figure 1. Chemical structure of oleanolic acid 28-O- β -D- glycopyranosyl ester (OA)

Table 1. Primer sequences for the RT-PCR.

cDNA	Primer sequence
TNF- α forward	5'-CAC CAG CTG GTT ATC TCT CAG CTC-3'
reverse	5'-CGG GAC GTG GAG CTG GCC GAG GAG-3'
IL-6 forward	5'-GAT GGA TGC TTC CAA TCT GGA T-3'
reverse	5'-AGT TCT CCA TAG AGA ACA ACA TA -3'
IL-8 forward	5'-CGA TGT CAG TGC ATA AAG ACA-3'
reverse	5'-TGA ATT CTC AGC CCT CTT CAA AAA-3'
β -actin forward	5'-CAA AAG GGT CAT CAT CTC TG-3'
reverse	5'-CCT GCT TCA CCA CCT TCT TG -3'

monoclonal antibodies antibody diluted in coating buffer (0.1 M carbonate, pH 9.5) and then was washed four times with PBS containing 0.05% Tween 20. The nonspecific protein binding sites were blocked with assay diluent (PBS containing 10% fetal bovine serum, pH 7.0) for at least 1 h. After washing the plates again, a sample or recombinant was added for TNF- α , IL-6, and IL-8 standards. After incubation for 2 h, a working detector (biotinylated anti-human TNF- α , IL-6, and IL-8 monoclonal antibodies and streptavidin-horseradish peroxidase reagent) was added and incubated for 1 h. Accordingly, substrate solution (tetramethylbenzidine) was added to the wells and incubated for 30 min in the dark before the reaction was stopped with stop solution (2 N H₃PO₄). The absorbance was read at 450 nm. All subsequent steps took place at room temperature, and all standards and samples were assayed in duplicate.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis

Using a GeneAll^R RiboEx RNA extraction kit (GeneAll Biotechnology, Republic of Korea), the total RNA was isolated from the HMC-1 cells in accordance with the manufacturer's specification. The concentration of total RNA in the final eluate was determined by spectrophotometry. The total RNA (2.0 μ g) was heated at 65°C

for 10 min and then chilled on ice. Next a cDNA synthesis kit (iNtRON Biotech, Republic of Korea) was used to reverse-transcribed each sample to cDNA for 90 min at 37°C. Primer sequences for glyceraldehyde phosphate dehydrogenase (GAPDH), TNF- α , IL-6, and IL-8 were performed as described in Table 1. The PCR products increased as the concentration of RNA increased. Finally, the products were electrophoresed on a 2.0% agarose gel and visualized by staining with ethidium bromide.

Fluorescent measurements of the intracellular Ca²⁺ level

The intracellular Ca²⁺ values were obtained from a single cell by using Fluo-3/AM, a fluorescent Ca²⁺-sensitive indicator. The cells were incubated with 4 μ M Fluo-3/AM at 37°C for 30 min, and then washed with PBS. The culture medium was then added and incubation continued at 37°C for 10 min; the cells were then viewed using a confocal laser scanning microscope (Olympus, Japan). The Fluo-3-loaded cells were illuminated with the 488-nm line of an argon laser, and the emitted fluorescence was observed through a 20 \times water-immersion objective and by setting the confocal pinhole to 2 μ m. The fluorescence intensity was detected using one of the two photomultipliers. For improved analysis of the spatial distribution of fluorescence, three successive frames were collected for each cell. The intracellular Ca²⁺ level was evaluated on the basis

of fluorescence intensity. (Grynkiewicz et al., 1985)

Preparation of cytoplasmic and nuclear extracts

To prepare the nuclear and cytoplasmic extracts, cells were washed twice in cold PBS, incubated on ice for 10 min in lysis buffer [25 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 mM CaCl₂, 1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 10 μM aprotinin], and centrifuged at 14,000 rpm for 10 min at 4°C. To prepare the nuclear extracts, the cells were washed in 1 ml of ice-cold PBS, resuspended in 400 μl of ice-cold hypotonic buffer [10 mM hydroxyethyl piperazineethanesulfonic/potassium hydroxide (HEPES/KOH), 10 mM potassium chloride (KCl), 2 mM MgCl₂, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM dithiothreitol, and 0.5 mM phenylmethylsulfonyl fluoride (pH 7.9)], left on ice for 10 min, vortex-mixed, and centrifuged at 15,000 g for 30 s. The nuclear pellets were gently resuspended in 50 μl of ice-cold saline buffer [50 mM HEPES/KOH, 50 mM KCl, 1 mM dithiothreitol, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol, and 0.5 mM phenylmethylsulfonyl fluoride (pH 7.9)], left on ice for 20 min, vortex-mixed, and centrifuged at 15,000 g for 5 min at 4°C. After conducting the centrifugation (15,000 ×g for 15 min at 4°C), the aliquots of supernatant containing the nuclear proteins in liquid nitrogen were froze and stored them at -70°C until ready for analysis. Finally, PRO-MEASURE™ Protein Measurement Solution (iNtRON Biotech, Republic of Korea) was used to measure the protein concentrations.

Western blot analysis

HMC-1 cells (5 × 10⁶ cells/well) were stimulated with PMA (50 nM) plus A23187 (1 μM). Then, the cell lysates were prepared in a sample buffer containing sodium dodecyl sulfate (SDS). The samples were heated at 95°C for 5 min and briefly cooled on ice. Following the centrifugation at 15,000 ×g for 5 min, the proteins in the cell lysates were then separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose paper. The membrane was then blocked with 5% skim milk in PBS-tween-20 for 1 h at room temperature and then incubated with anti-NF-κB (p65), IκB and p-IκB. After washing the blot in PBS-tween-20 three times, the blot was incubated with a secondary antibody for 1 h and then visualized the antibody-specific proteins by means of an enhanced chemiluminescence detection system in accordance with the recommended procedure (Amersham Corp., Newark, NJ, USA).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's t-test for multiple comparisons. The data from the experiments are presented as means ± standard error of mean (SEM).

RESULTS

Effects of OA on cytotoxicity in HMC-1 cells

The cytotoxicity of OA was evaluated using MTT assay, and OA was found not to affect HMC-1 cells viability at concentrations of 1, 10, and 100 μM. But the cytotoxicity of OA was evaluated at concentration of 100 μM (Figure 2).

Effects of OA on the pro-inflammatory cytokine expression

To evaluate the effect of OA on the production of pro-inflammatory cytokines, OA (1, 10, and 100 μM) was pretreated before stimulation with PMA (50 nM) plus A23187 (1 μM) for 8 h, and was analyzed using ELISA. As shown in Figure 3A, TNF-α, IL-6, and IL-8 were considerably increased after stimulation with PMA plus A23187 in HMC-1. Pretreatment of OA (1, 10 and 100 μM) significantly inhibited these increases in a concentration-dependent manner. Next, the pro-inflammatory cytokine gene expression was analyzed using RT-PCR. The enhanced TNF-α, IL-6, and IL-8 mRNA expression induced by PMA plus A23187 was also inhibited by pretreatment of OA (Figure 3B). Especially, pretreatment of OA (10 and 100 μM) inhibited PMA plus A23187-induced gene expression of TNF-α, IL-6, and IL-8.

Effects of OA on intracellular Ca²⁺ levels

The effect of OA on the intracellular level of Ca²⁺ was also investigated using a confocal laser microscope to detect the fluorescence signal emitted by the individual cells. The treatment of cells with PMA plus A23187 for 30 min resulted in a considerable increase in the intracellular Ca²⁺ levels, but pretreatment of the cells with OA (100 μM) inhibited this increase (Figure 4).

Effects of OA on activation of extra-signal response kinase (ERK)

In order to elucidate the mechanisms underlying the effects of OA, we examined the possible effects of OA on activation of MAP Kinases (MAPKs). The stimulation of HMC-1 cells with PMA plus A23187 resulted in an increased phosphorylation of all the three types of MAPKs, p38, JNK, and ERK activation, 15 to 30 min of post treatment (data not shown). As shown in Figure 5, OA attenuated PMA plus A23187-induced ERK 1/2, but did not affect the phosphorylation of JNK 1/2, and p38 MAPK.

Effects of OA on activation of NF-κB

To evaluate the mechanism of effect of OA on the gene expression pro-inflammatory cytokine, the effect of OA on the NF-κB activation was examined. Expression of these pro-inflammatory cytokines is regulated by a transcription factor, p65 NF-κB (Azzolina et al., 2003). Stimulation of HMC-1 cells with PMA plus A23187 induced the degradation and phosphorylation of IκBα and nuclear translocation of NF-κB (p65) after 2 h of incubation (Figure 6). OA inhibited the PMA plus A23187-induced degradation and phosphorylation of IκBα and nuclear translocation of

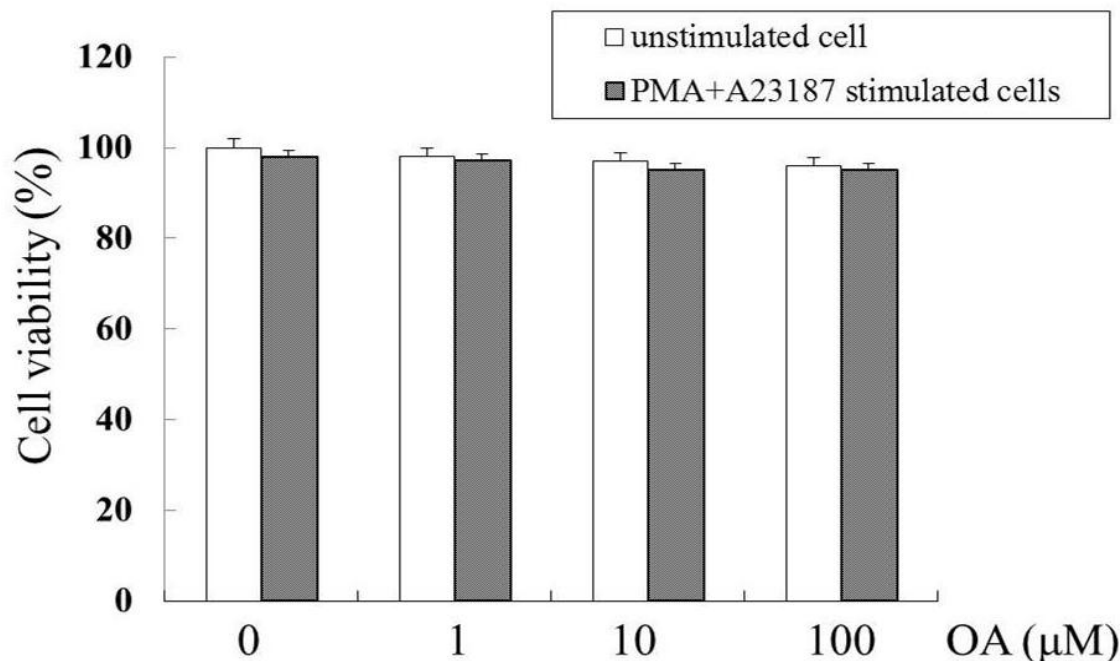


Figure 2. Effects of OA on cell viability in HMC-1 cells. Cell viability was evaluated using the MTT assay.

NF-κB (p65).

DISCUSSION

In recent studies, naturally occurring chemical substances derived from plants have been of interest as therapeutic interventions in several inflammatory diseases. They serve as template molecules for the development of new drugs (Calixto et al., 2004). These molecules interfere with three key processes involved in the inflammatory process. In this study, OA isolated from *A. cordata* exerted anti-inflammatory effects. In a study on this plant, twelve compounds, including three triterpenes, one saponin, four sterols, and one cerebroside, were isolated and evaluated for their biological activity (Dang et al., 2005). This led to the isolation of a saponin compound, OA, which has a sugar moiety in the oleanoic acid. Oleanolic acid, a natural pentacyclic triterpene widely found in a variety of plants, has been shown to display numerous biological properties with therapeutic potential (Dzubak et al., 2006; Liu, 2005; Martinez-Gonzalez et al., 2008). Recent reports have demonstrated anti-inflammatory effects of oleanoic acid by the modulation of high mobility group box 1 (HMGB1)-dependent pro-inflammatory responses (Yang et al., 2012). But, no report has been issued on anti-inflammatory effect of OA, which has a sugar moiety in the oleanoic acid. In our system, calcium ionophore A23187 and PKC activator PMA were used as inflammatory-stimulator. The calcium ionophore A23187 induces the release of intracellular

free Ca^{2+} , which regulates granule-plasma membrane fusion and eicosanoid synthesis. PMA activates PKC, thereby inducing pro-inflammatory cytokine production (Tanifuji et al., 2010; Westcott et al., 1996; Jung et al., 2009). In this study, it has been demonstrated that OA inhibits PMA plus A23187-mediated secretion of TNF- α , IL-6 and IL-8 in HMC-1 cells. OA was also found to significantly reduce mRNA levels of the pro-inflammatory cytokines TNF- α , IL-6, and IL-8 (Figure 3B).

Especially, mast cell contains potent mediator, these have potential contributions to the processes of inflammation and therefore play important roles in inflammation (Bradding and Holgate, 1999). Mast cell-derived pro-inflammatory cytokines, such as TNF- α , IL-6 and IL-8, have critical biological roles to play in allergic inflammation. These cytokines are released as prestored cytokines, but can also be newly synthesized upon mast cell activation (Hide et al., 1997). The reduced level of pro-inflammatory cytokines released from mast cells is a key indicator of reduced inflammatory symptoms. These reports indicated the anti-inflammatory effect of OA as a potent inhibitor of mast cell activation. So, we demonstrated that OA suppressed the expression of TNF- α , IL-6 and IL-8 in PMA plus A23187-induced HMC-1 cells, as these cytokines have powerful inflammatory effects and are released by activated mast cells.

Calcium acts as a secondary messenger in the intracellular level (Rasmussen and Goodman, 1977). Increase in levels of intracellular Ca^{2+} has been proposed as an essential trigger for mast cell activation (White et al., 1986). A depletion of intracellular Ca^{2+} levels inhibited

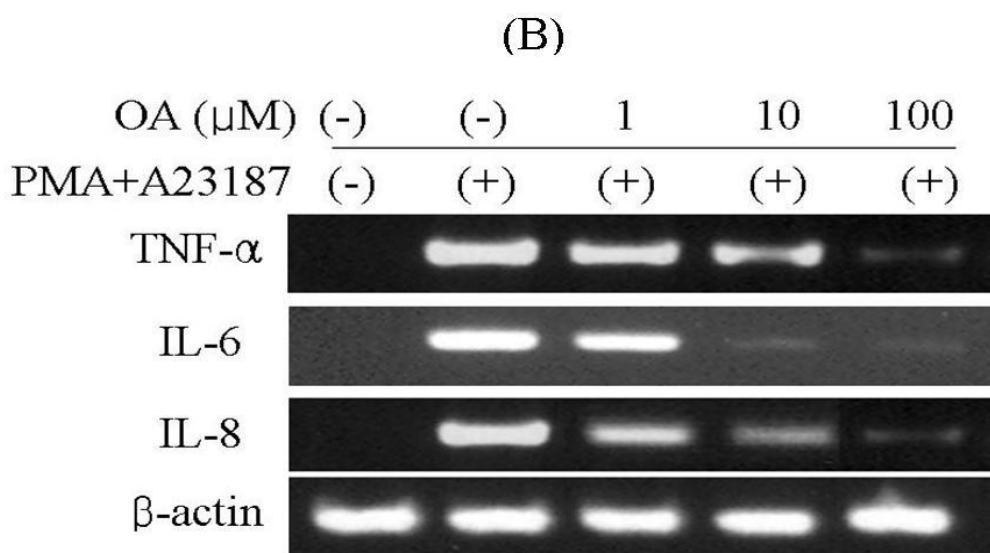
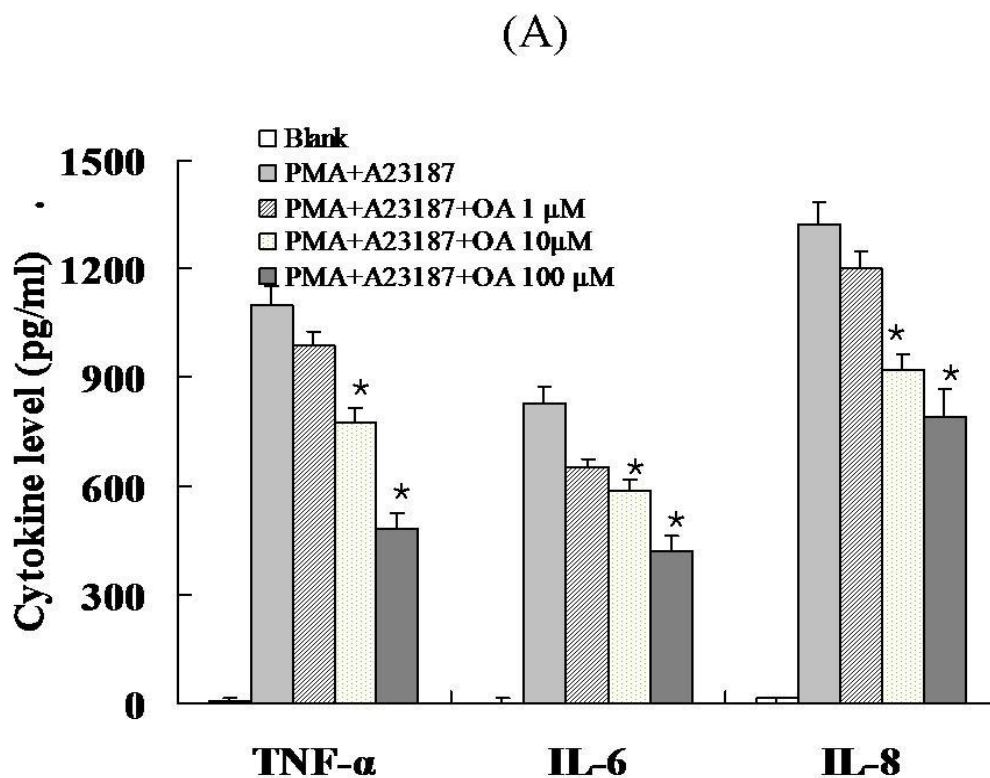


Figure 3. Effect of OA on production and gene expression of pro-inflammatory cytokines in PMA plus A23187-induced HMC-1 cells. (A) Cells were pretreated with OA (1, 10 and 100 μM) for 1 h prior to PMA (50 nM) plus A23187 (1 μM) stimulation for 8 h. The protein levels of TNF-α, IL-6 and IL-8 were determined by ELISA. Each bar represents the means ± S.E.M. of three independent experiments. **p* < 0.05, compared with PMA +A23187-stimulated values. (B) Cells were pretreated with OA (1, 10 and 100 μM) for 1 h prior to PMA (50 nM) + A23187 (1 μM) stimulation for 6 h. The mRNA expression level of TNF-α, IL-6 and IL-8 was determined by RT-PCR.

pro-inflammatory cytokine expression via MAPKs activation and NF-κB signaling pathway (Crossthwaite et al., 2002; An and Kim, 2005). From the results of this

study, the inhibitory effects of OA on the expression of TNF-α, IL-6, and IL-8 levels was suggest to be mediated by the reduction of intracellular Ca²⁺ in HMC-1 cells.

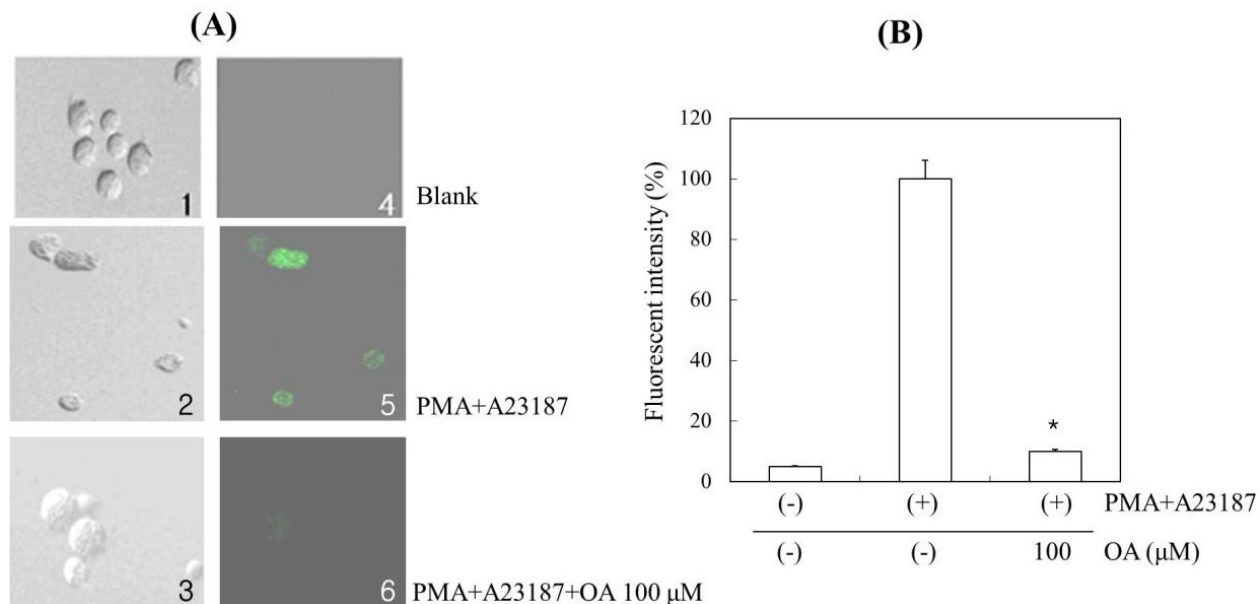


Figure 4. Effect of OA on intracellular calcium levels. Cells were pretreated with OA for 1 h before stimulation with PMA (50 nM) + A23187 (1 μ M). (A) Fluorescent images (1 to 3) were analyzed using confocal microscopy (4 to 6). Confocal images of HMC-1 cells were stained with fluo-3/AM. (B) The intensity of intracellular calcium was measured in three separate experiments. * $P < 0.05$ compared with PMA + A23187-stimulated values.

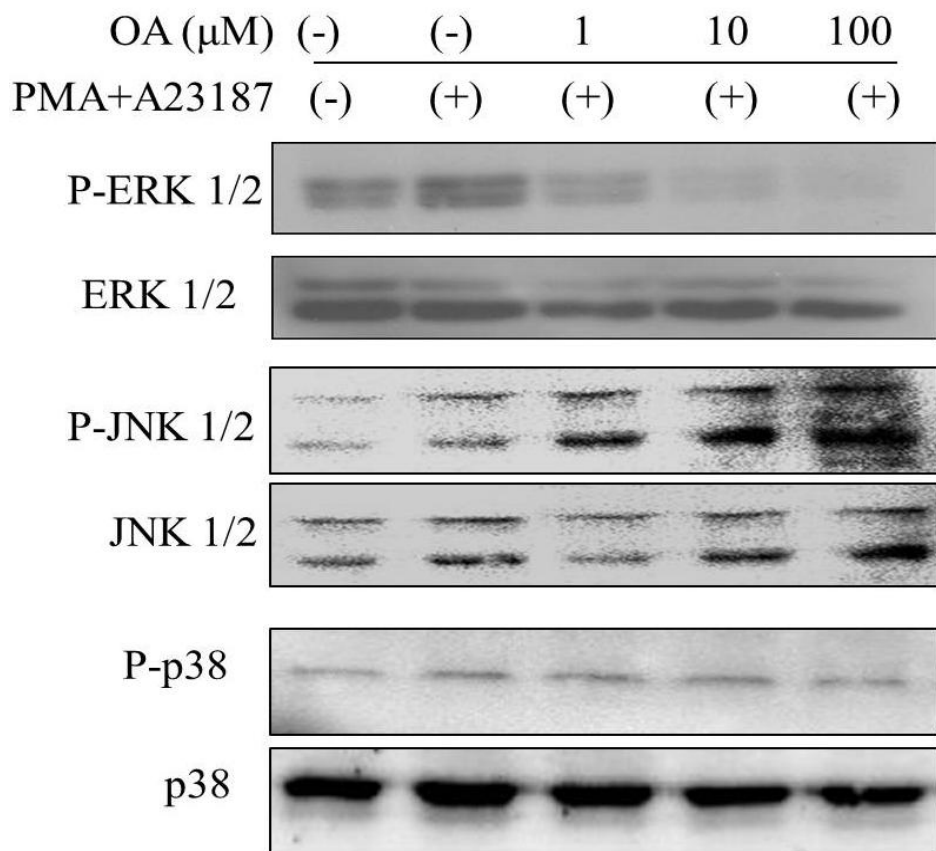


Figure 5. Effect of OA on activation of ERK. After pretreatment with OA for 1 h, HMC-1 cells were stimulated by PMA (50 nM) + A23187 (1 μ M) 30 min for ERK 1/2, JNK 1/2, p38 activation.

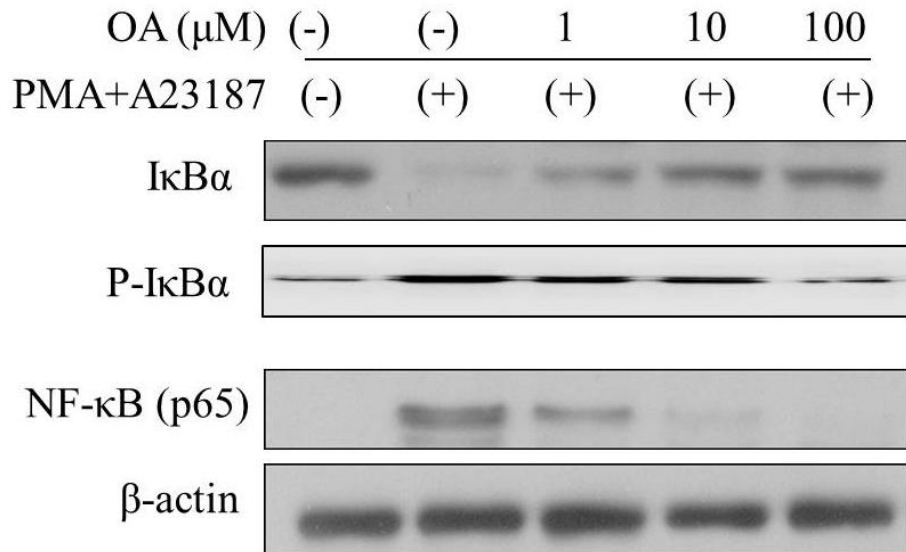


Figure 6. Effect of OA on the activation of NF- κ B in HMC-1 cells. Cells were pre-treated with OA for 1 h prior to PMA (50 nM) + A23187 (1 μ M) stimulation, after I κ B degradation, I κ B phosphorylation and NF- κ B (p65) translocation were assayed by Western blot.

The MAPKs (that is, ERK, JNK, and p38 MAPK) pathways are appropriate targets for pharmacological treatment of inflammatory disorders (Lewis et al., 1998). Also, the suppression of NF- κ B activation has been linked with anti-inflammation. Moreover, *A. cordata* inhibited the activation of MAPK and NF- κ B in LPS-induced macrophage (Kang et al., 2008). So, it was postulated that OA mediated its effects at least partly through suppression of MAPK and NF- κ B (p65) activation. This study showed that OA inhibited phosphorylation of ERK 1/2, but not of p38 MAPK and JNK1/2 (Figure 5). These data suggested that OA inhibited pro-inflammatory cytokine production and intracellular Ca^{2+} release via the inhibition of ERK activation. Although, NF- κ B activation is regulated by MAPKs through multiple mechanisms, accumulating evidence indicates that NF- κ B activation is modulated by MAPKs that induce site-specific phosphorylation of an inhibitory protein called I κ B (Kim et al., 1997). Activation of NF- κ B is dependent on the degradation of I κ B, an endogenous inhibitor that binds to NF- κ B in the cytoplasm (Lee et al., 2006). In these studies, OA was shown to decrease the degradation and phosphorylation of I κ B and nuclear translocation of NF- κ B (p65) in PMA plus A23187-stimulated mast cells. This demonstrated that OA inhibited the PMA and A23187-induced TNF- α , IL-6, IL-8 and intracellular Ca^{2+} release via attenuation of NF- κ B (p65) activation, I κ B α degradation, and I κ B α phosphorylation.

Conclusion

Conclusively, OA regulated the production of TNF- α , IL-6, and IL-8 in PMA plus A23187-stimulated HMC-1 cells. OA

also decreased intracellular Ca^{2+} release and ERK 1/2 phosphorylation. Furthermore, OA inhibited degradation and phosphorylation of I κ B α and activation of NF- κ B (p65). Therefore, the regulation of the NF- κ B (p65) signal pathway by OA isolated from the *A. cordata* in HMC-1 cells is a potentially attractive and characteristic probe for studying mast cell-mediated inflammatory diseases.

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