

Original Article

Oleanolic acid protects against mast cell-mediated allergic responses by suppressing Akt/NF- κ B and STAT1 activation

Yun-Mi Kang^a, Minho Lee^{b,*}, Hyo-Jin An^{a,*}^a Department of Pharmacology, College of Korean Medicine, Sangji University, 83 Sangjidae-gil, Wonju-si, Gangwon-do 26339, Republic of Korea^b Department of Life Science, Dongguk University-Seoul, Ilsandong-gu, Goyang-si, Gyeonggi-do 10326, Republic of Korea

ARTICLE INFO

Keywords:

Oleanolic acid
Allergic responses
Mast cell
Anaphylaxis
NF- κ B
STAT1

ABSTRACT

Background: Oleanolic acid (OA) is an active compound found in a variety of medicinal herbs and plants. Though OA has been widely attributed with a variety of biological activities, studies focused on its anti-allergic inflammation properties are insufficient.

Purpose: Given the rapid increase in allergic diseases and the lack of fundamental treatment options, this study aimed to find a safe and effective therapy for allergic disorders.

Methods: We evaluated the inhibitory effect of OA on allergic inflammatory response and the possible mechanisms underlying the effect using phorbol-12-myristate 13-acetate plus calcium ionophore A23187 (PMACI)-stimulated human mast cell (HMC)-1, and a mouse model of compound 48/80-induced anaphylactic shock.

Results: OA suppressed pro-inflammatory cytokine expressions in PMACI-induced HMC-1 cells by inhibiting activation of the Akt, p38 mitogen-activated protein kinase (MAPK), nuclear factor- κ B (NF- κ B), and signal transducer and activator of transcription (STAT) 1 signaling pathways. Moreover, OA showed a protective effect against compound 48/80-induced anaphylactic shock through inhibition of histamine release and immunoglobulin E level via regulation of NF- κ B and STAT1 activation.

Conclusion: The results showed that OA suppressed mast cell-mediated allergic response by transcriptional regulation. We suggest that OA has potential effect against allergic inflammatory disorders, including anaphylaxis, and might be a useful therapeutic agent for allergic disease.

Introduction

Allergic inflammation is described as a pathophysiological disorder, is known to be a type of hypersensitivity reaction. Type I hypersensitivity diseases are extremely common, and include allergic rhinitis, atopic dermatitis, allergic conjunctivitis, asthma, and anaphylaxis (Descotes and Choquet-Kastylevsky, 2001; Rajan, 2003). They are characterized by the immunoglobulin E (IgE)-mediated hypersensitivity reaction, which is known as an immediate hypersensitivity reaction. IgE is the antibody responsible for allergic reactions, and the allergic signs and symptoms are triggered by cross-linking of IgE bound on high-affinity IgE receptors (Fc ϵ RI) expressed on mast cells, which leads to mast cell degranulation and release of a variety of inflammatory mediators that are either preformed or newly formed.

Mast cells are key effector cells in allergic inflammation, and are characterized by the release of histamine and other mediators.

Activated mast cells release pro-inflammatory cytokines such as tumor necrosis factor (TNF)- α and interleukins (ILs), play an important role in allergic inflammation, and function in inflammatory cell activation and migration as well as allergic inflammation (Hong et al., 2010). Anaphylaxis is a severe systemic allergic reaction caused by the rapid widespread degranulation of mast cells which can result in bronchospasm, cardiovascular collapse, skin rashes, oedema in nose and throat, vomiting and sometimes death (Kamei et al., 2017). The prevalence of allergic disease is increasing worldwide with unprecedented severity and complexity, however, no fundamental treatment option has been proposed so far.

Natural compounds isolated from medicinal herbs and plants are potential sources of therapeutic agents to prevent and treat inflammatory diseases and improve the quality of life for patients with allergic disorders. Oleanolic acid (OA) is a pentacyclic triterpenoid compound (Fig. 1) found in various medicinal herbs and plants,

Abbreviations: DSCG, disodium cromoglycate; HMC-1, human mast cell-1; MAPK, mitogen-activated protein kinase; NF- κ B, nuclear factor- κ B; OA, oleanolic acid; PMACI, phorbol 12-myristate 13-acetate (PMA) + calcium ionophore A23187; STAT1, signal transducer and activator of transcription 1

* Corresponding authors.

E-mail addresses: minholee@dgu.edu (M. Lee), hjan@sj.ac.kr (H.-J. An).

<https://doi.org/10.1016/j.phymed.2020.153340>

Received 21 April 2020; Received in revised form 20 August 2020; Accepted 7 September 2020

Available online 15 October 2020

0944-7113/© 2020 Published by Elsevier GmbH.

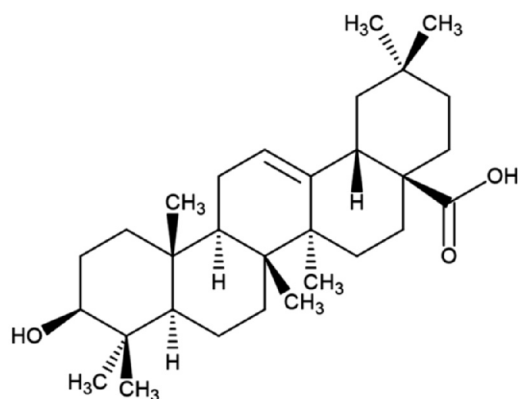


Fig. 1. Chemical structure of OA.

including *Olea europaea* and *Syzygium aromaticum*. OA exists in nature as a free acid or as an aglycone of triterpenoid saponins, and it is often ubiquitously found with its isomer, ursolic acid, betulinic acid, and moronic acid (Ayeleso et al., 2017). OA is well known for its diverse pharmacological activities, including its hepatoprotective (Wang et al., 2010), anti-oxidant (Sultana and Ata, 2008), anti-inflammatory (Giner-Larza et al., 2001), anti-asthmatic (Lee et al., 2010), anti-tumor (Petronelli et al., 2009), immunomodulatory (Martin et al., 2012), anti-diabetic (Li et al., 2015), anti-hypertensive (Somova et al., 2003), and anti-parasitic (Sifaoui et al., 2017) properties. Despite the fact that OA has been widely investigated and attributed with a variety of biological activities, the effect on acute allergic reactions on type 1 hypersensitivity such as anaphylaxis, are insufficient. In this study, we aimed to investigate the effects of OA on mast cell-mediated allergic diseases, using human mast cell lines (HMC-1), and a compound 48/80-induced anaphylaxis mice model.

Materials and methods

Chemicals and reagents

For the present study, OA (O5504, $\geq 97\%$), phorbol 12-myristate 13-acetate (PMA), calcium ionophore A23187 (Calcimycin), 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT), dimethyl sulfoxide (DMSO), dexamethasone (D4902) and all other chemicals were purchased from Millipore Sigma (Billerica, MA, USA). Iscove's modified Dulbecco's medium (IMDM), fetal bovine serum (FBS), penicillin, and streptomycin were obtained from Life Technologies Inc. (Grand Island, NY, USA). Primary antibodies against p-IKK α/β , NF- κ B p65, p-Akt, p-STAT1 (Tyr701) and p-STAT1 (Ser727), and STAT1 were obtained from Cell Signaling Technology, Inc. (Danvers, MA, USA). Primary antibodies for IKK α/β , p-I κ B- α , I κ B- α , Akt1/2/3, PARP, α -tubulin, and β -actin were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Horseradish peroxidase-conjugated secondary antibodies were purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA, USA). The histamine enzyme-linked immunosorbent assay (ELISA) kit was obtained from Enzo life Sciences, Inc. (Farmingdale, NY, USA). The ELISA kits for TNF- α , IL-6, and IL-1 β were obtained from R&D Systems, Inc. (Minneapolis, MN, USA). SYBR Premix Ex Taq was purchased from Takara Bio, Inc. (Shiga, Japan). TNF- α , IL-6, IL-1 β , and GAPDH oligonucleotide primers were purchased from Bioneer Corporation (Daejeon, South Korea).

Cell culture and sample treatment

HMC-1 cells were provided by Professor Jong-Sik Jin (Chonbuk University, Republic of Korea), and were grown at 37 °C in IMDM supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin

(100 μ g/ml) in a humidified atmosphere with 5% CO₂. HMC-1 cells were seeded at a density of 1×10^6 cells per well, treated with OA for 30 min at 37 °C in humidified air with 5% CO₂, and then stimulated with 40 nM of PMA and 1 μ M of A23187 (PMACI) at 37 °C. The OA was dissolved in DMSO. The cells were treated with DMSO as a control.

Cytokine, histamine, and IgE assays

Culture media were collected at 24 h post-treatment with OA and stored at -80 °C. Blood was collected from each mouse at the end of the experiment. Serum was obtained by centrifugation at $1700 \times g$ for 30 min and stored at -80 °C until analysis. The levels of TNF- α , IL-6, IL-1 β , histamine, and IgE were measured using ELISA kits according to the manufacturer's protocol.

Western blot analysis

Cells and segments liver and dorsal tissue were suspended in PRO-PREP™ protein extraction solution (Intron Biotechnology, Inc., Seoul, Korea) and incubated for 20 min at 4 °C. Cell debris was removed via microcentrifugation at $11,000 \times g$ for 30 min at 4 °C, followed by rapid freezing of the supernatant. The protein concentration was determined using Bio-Rad protein assay reagent (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the manufacturer's protocol. Cellular proteins from the treated and untreated cell extracts were electro-blotted onto a polyvinylidene fluoride membrane following separation via 8–12% SDS-PAGE. The membrane was incubated for 1 h with blocking solution (5% skim milk) at room temperature, followed by overnight incubation with the primary antibodies (1:1000) at 4 °C. The blots were washed three times with Tween 20/Tris-buffered saline (T/TBS) and incubated with horseradish peroxidase-conjugated secondary antibody (1:2000) for 2 h at room temperature. The blots were washed three times with T/TBS, and then developed via enhanced chemiluminescence (GE Healthcare Life Sciences, Chalfont, UK). Densitometric analysis was performed using Bio-Rad Quantity One software version 4.3.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNA was isolated from the cells, or liver tissues using an Easy Blue kit (Intron Biotechnology, Inc., Seoul, Korea) according to the manufacturer's protocol. Total RNA was quantified using an Epoch micro-volume spectrophotometer system (BioTek Instruments, Inc., Winooski, VT, USA). cDNA was obtained using isolated total RNA (2 μ g), d(T)16 primer, and Avian Myeloblastosis Virus reverse transcriptase with genomic DNA remover. The relative gene expression was quantified using RT-qPCR analysis (Real Time PCR System 7500; Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with SYBR Premix Ex Taq. Fold changes of gene expression were calculated using the comparative quantification cycle method. The Cq values of target genes TNF- α , IL-6, and IL-1 β were normalized to that of GAPDH using the ABI Gene Express 2.0 program (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Compound 48/80-induced anaphylactic shock model

A total of 24 male ICR mice (6 weeks old; 20–25 g body weight) were obtained from Charles River Laboratories (Harlan Laboratories, Inc., Wilmington, MA, USA) and maintained under constant conditions at a temperature of 20–25 °C, humidity of 40–60%, and a 12 h light/dark cycle. The mice were randomly assigned to one of four groups ($n = 6$ per group). The ICR mice were injected intraperitoneally (i.p.) with phosphate-buffered saline (PBS) or compound 48/80 (8 mg/kg dissolved in PBS) as described previously (Choi et al., 2006). OA, disodium cromoglycate (DSCG; Sigma-Aldrich; EMD Millipore, Billerica,

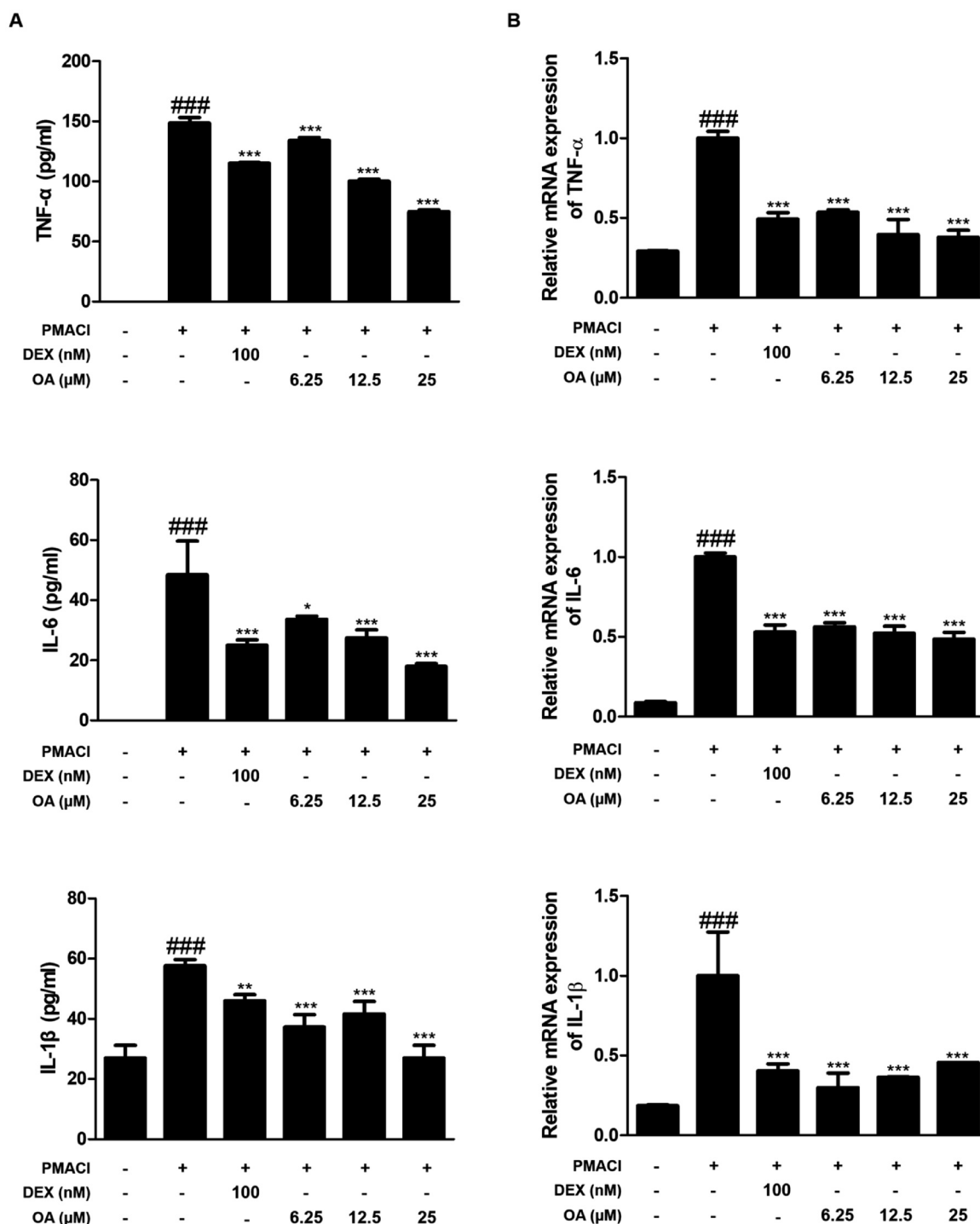


Fig. 2. Effect of OA on the pro-inflammatory cytokines in PMACI-stimulated HMC-1 cells.

(A) HMC-1 cells were treated with of OA for 30 min prior to the addition of PMACI, and the cells were further incubated for 24 h against determination of TNF-α, IL-6, and IL-1β production. Cytokines production was measured using ELISA kit. (B) The mRNA level of TNF-α, IL-6 and IL-1β were determined by qRT-PCR. Dexamethasone (DEX) was used as a positive control. Values represent mean $\pm$ S.D. of three independent experiments. ### $p < 0.001$ vs. the control group; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. PMACI-treated group.

MA, USA), or PBS was dissolved in saline and injected i.p. at doses of 25 mg/kg DSCG and 20 mg/kg OA for 1 h prior to the compound 48/80 injection. Survival was monitored for 1 h following the induction of anaphylactic shock. Survival data were analyzed using the Kaplan-Meier method and log rank test. Following the assessment of animal survival, blood was collected from the heart of each mouse to measure serum cytokine production. The blood was allowed to clot for 1 h at room temperature and then centrifuged for 20 min at $3000 \times g$ at $4^\circ C$

to obtain serum. Following collection of blood samples from the mice, the mice were sacrificed by cervical dislocation. All procedures were performed in accordance with university guidelines and approved by the Ethical Committee for Animal Care and the Use of Laboratory Animals, Korean Medicine, Sangji University (Wonju, Korea; approval no. 2015-11).

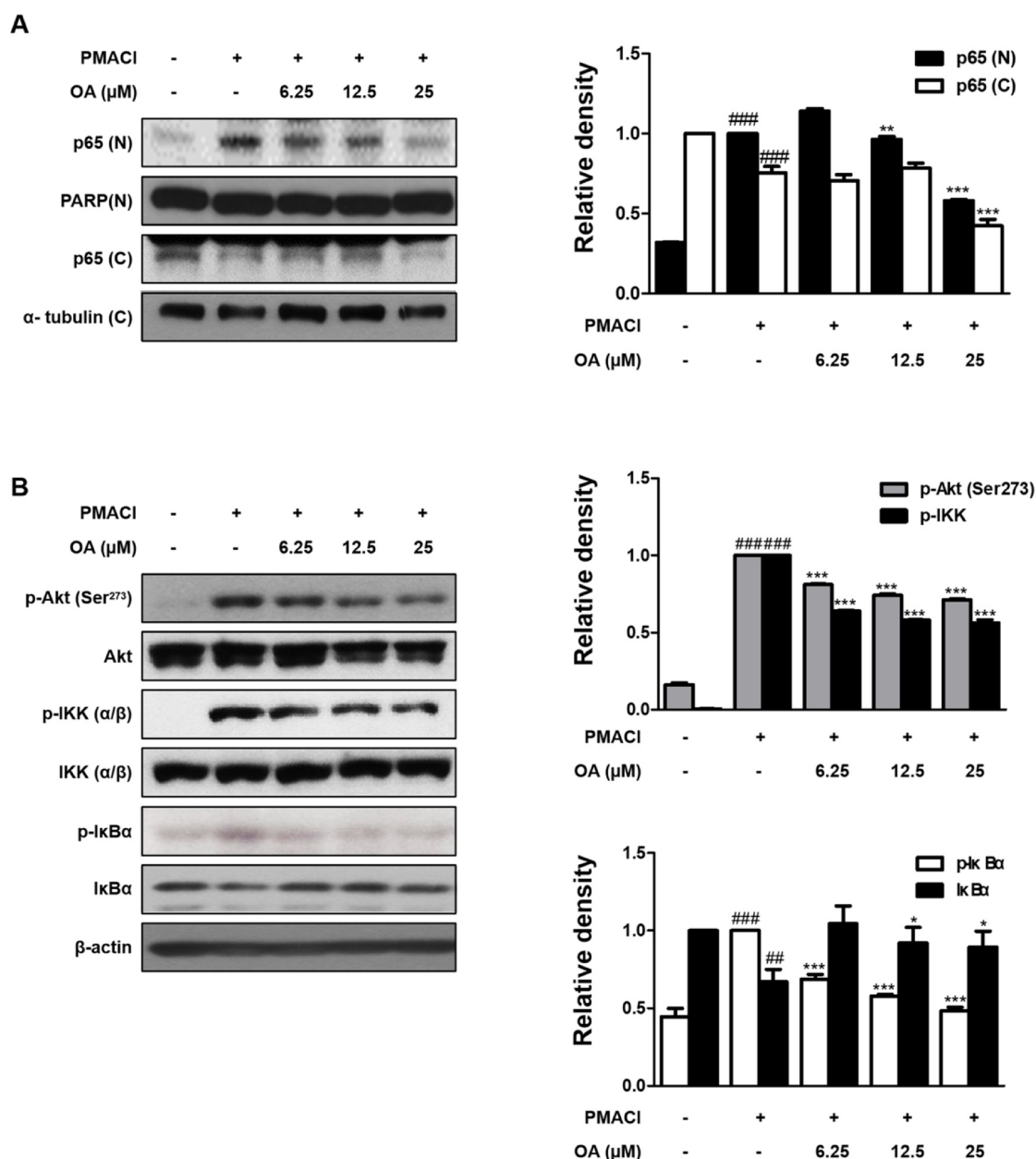


Fig. 3. Effect of OA on PMACI-induced activation of NF- κ B and Akt pathway and in HMC-1 cells.

HMC-1 cells were pre-treated with of OA for 30 min prior to the addition of PMACI. Total proteins were prepared, and the expressions of p65 NF- κ B (A), Akt, IKK, and I κ B (B) were determined by Western blot analysis using specific antibodies. PARP, α -tubulin and β -actin were used as internal control. Densitometric analysis was performed using Bio-Rad Quantity One® Software. The data shown represent mean $\pm$ S.D. of three independent experiments. ### $p < 0.001$ vs. the control group; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. PMACI-treated group. (N): nucleus, (C): cytoplasm.

Statistical analysis

The data are expressed as the mean $\pm$ standard deviation of triplicate experiments. Statistically significant differences were compared using one-way analysis of variance and Dunnett's post hoc test. $p < 0.05$ was considered to indicate a statistically significant difference. Statistical analysis was performed using SPSS statistical analysis software (version 19.0, IBM SPSS, Armonk, NY, USA).

Results

OA suppressed pro-inflammatory cytokines in PMACI-stimulated HMC-1 cells

To determine whether OA inhibits TNF- α , IL-6, and IL-1 β , its effects on the PMACI-induced production and mRNA expression of these mediators were investigated. As shown in Fig. 2A and B, pre-treatment of cells with OA significantly downregulated the PMACI-induced production and mRNA expression of cytokines. Treatment with high concentration of OA exhibited the better effect on the levels than that of dexamethasone, the positive control. The results showed that OA regulates the PMACI-induced expression of TNF- α , IL-6, and IL-1 β via transcriptional inhibition. The possibility that cytotoxicity of OA to

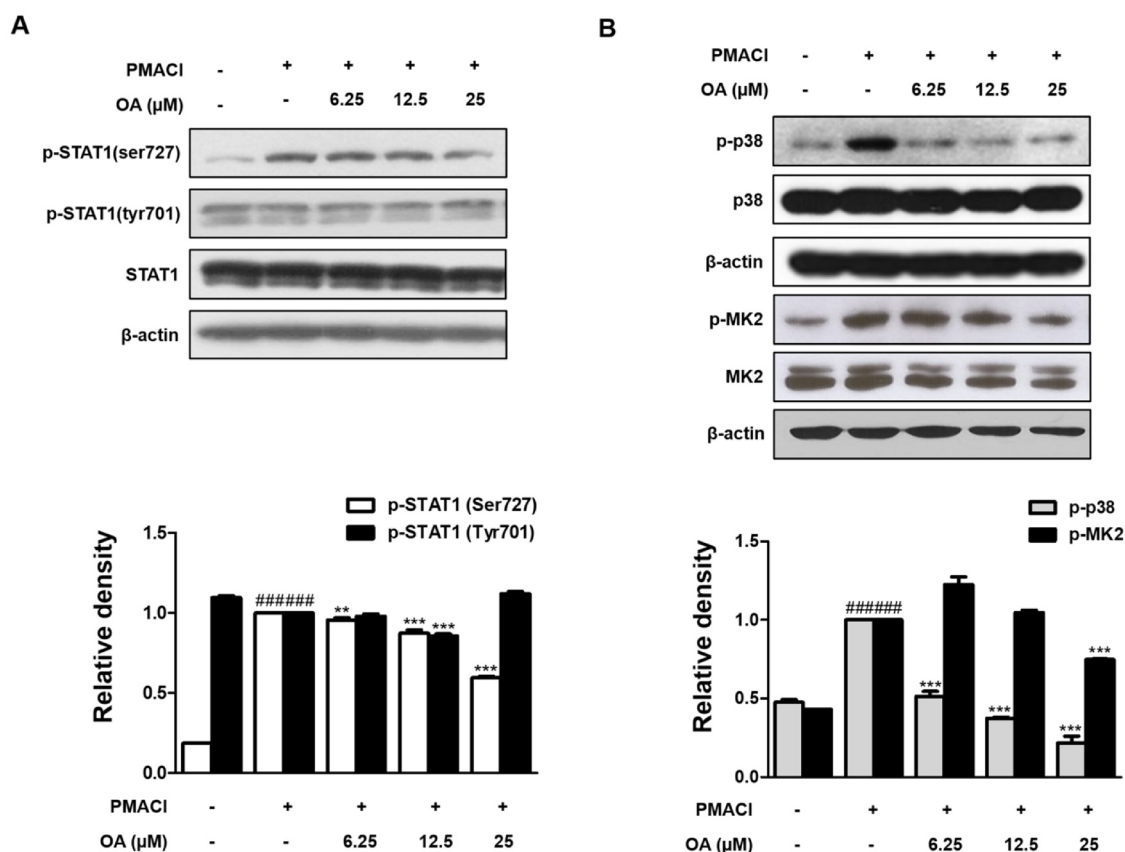


Fig. 4. Effect of OA on PMACI-induced activation of p38 and STAT1 pathway in HMC-1 cells.

HMC-1 cells were pre-treated with OA for 30 min prior to the addition of PMACI. Total proteins were prepared, and the expressions of STAT1 (A), p38, and MK2 (B) were determined by Western blot analysis using specific antibodies. β -actin was used as internal control. Densitometric analysis was performed using Bio-Rad Quantity One® Software. The data shown represent mean $\pm$ S.D. of three independent experiments. ### $p < 0.001$ vs. the control group; ** $p < 0.01$, and *** $p < 0.001$ vs. PMACI-treated group.

HMC-1 cells was responsible for this inhibitory effect was eliminated by the finding that OA did not affect cell viability, as determined by MTT assay, at concentrations of 6.25 to 100 μ M (Figure S1).

OA inhibited activation of NF- κ B, Akt, and STAT1 in PMACI-induced HMC-1 cells

Next, the effect of OA on transcription factors was examined using Western blot analysis. As shown in Fig. 3, our results show that treatment of PMACI markedly increased NF- κ B translocation to the nucleus, whereas in the presence of OA, it was decreased (Fig. 3A). We found that OA prevented PMACI-induced phosphorylation of Akt and IKK α/β , which are upstream regulators of the NF- κ B signal pathway, but did not affect their total amounts in PMACI-induced HMC-1 cells. In addition, PMACI treatment resulted in a remarkable increase in phosphorylation and degradation of I κ B, whereas OA reversed its effects (Fig. 3B). This indicated that its effect involves the inhibition of NF- κ B activation by p65 nuclear translocation. Moreover, PMACI induced the phosphorylation of STAT1 at Ser727, whereas pre-treatment with OA suppressed the PMACI-induced activation of STAT1 at Ser727, but did not affect the phosphorylation of STAT1 at Tyr701 or the total amounts in PMACI-induced HMC-1 cells (Fig. 4A). This result showed that OA has an anti-allergic effect by suppressing pro-inflammatory cytokines that initiate allergic inflammatory responses via inhibition of the transcription factor NF- κ B and STAT1 signal pathways in PMACI-stimulated HMC-1.

OA inhibited activation of p38 MAPK in PMACI-induced HMC-1 cells

The p38 mitogen-activated protein kinase (MAPK), a member of MAPK family, have been implicated in the control of the transcription activity of the NF- κ B as well as phosphorylation of STAT1 at Ser727 (Lee et al., 2017; Ramsauer et al., 2002). To determine whether the p38 MAPK signaling pathway is involved in the effect of OA, Western blot analysis was performed on PMACI-induced HMC-1 cells. The phosphorylations of p38 MAPK and its downstream target MK2 are increased upon PMACI stimulation, whereas pre-treatment with OA suppressed the activation, but did not affect their total amounts in PMACI-induced HMC-1 cells (Fig. 4B). It was found that OA inhibited PMACI-induced phosphorylation of p38 MAPK signaling pathway, resulting in blocking STAT1 Ser727 phosphorylation and translocation of NF- κ B, subsequently its transcriptional activity for allergy-related genes.

OA suppressed pro-inflammatory cytokines in a mouse model of compound 48/80-induced anaphylactic shock

Anaphylaxis is defined as a severe, systemic, life-threatening, immediate hypersensitivity reaction in which mast cells are involved (Tomasiak-Lozowska et al., 2018). Compound 48/80 is a basic amino acid that is one of the most powerful stimulators of mast cells, promoting histamine release, and therefore is often used in studies on anaphylaxis (Lee et al., 2015). To further confirm the effect of OA *in vivo*, we used a mouse model of compound 48/80-induced anaphylactic shock. We examined the production and mRNA levels of cytokines in anaphylactic mice using serum and liver tissue. The compound 48/80

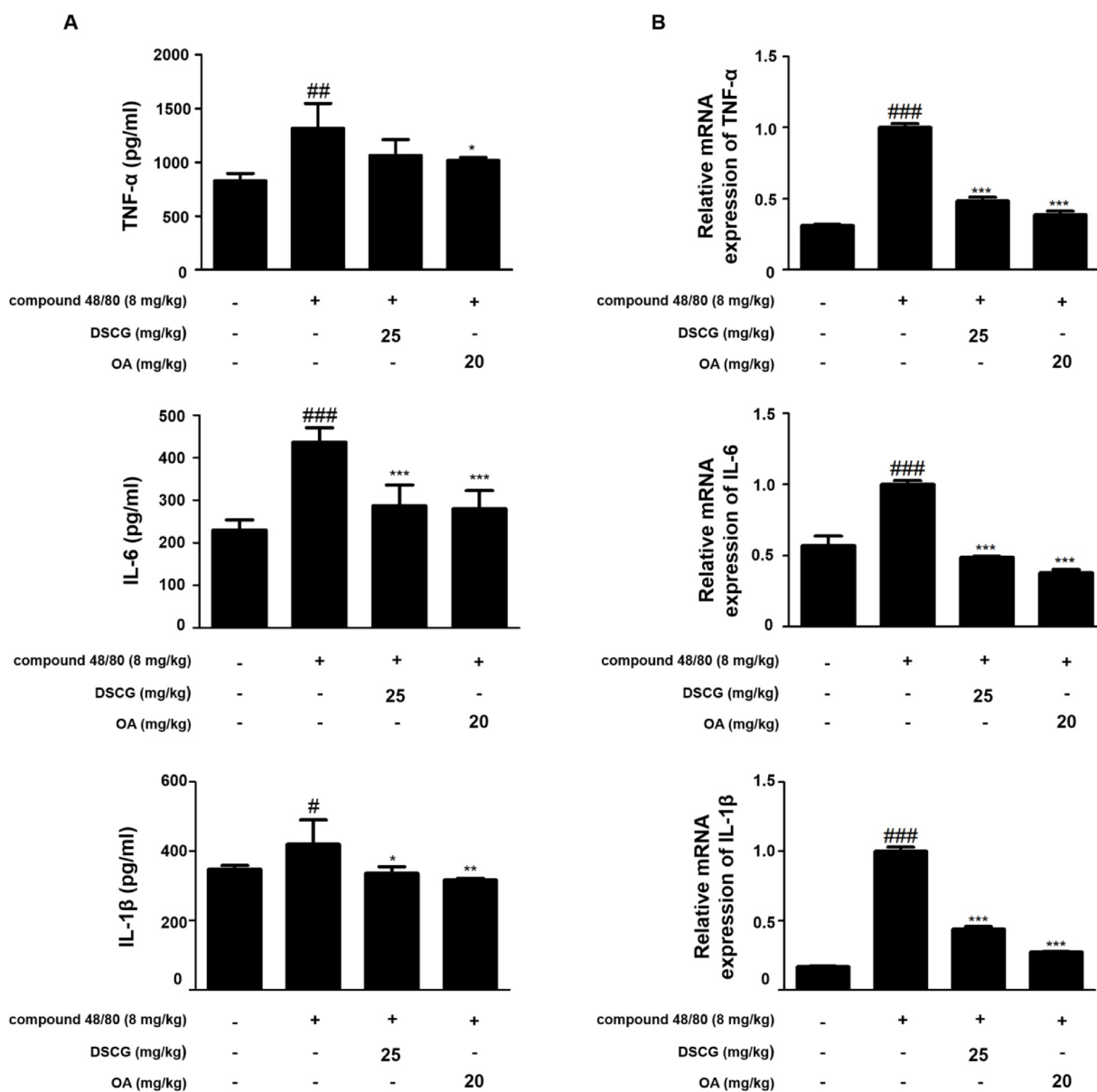


Fig. 5. Effects of OA on compound 48/80-induced pro-inflammatory cytokines in a mouse model of anaphylactic shock.

Mice were injected with OA, DSCG and PBS as a vehicle ($n = 6$ per group or total) for 1 h before compound 48/80 injection (8 mg/kg i.p.). (A) Cytokines production from serum was measured using ELISA kit. (B) Total RNA prepared from the liver tissue, and the levels of TNF- α , IL-6 and IL-1 β were determined by quantitative qRT-PCR. Densitometric analysis was performed using Bio-Rad Quantity One® Software. The data shown represent mean $\pm$ S.D. of three independent experiments. # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs. the control group; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. compound 48/80-treated group.

administration significantly increased the production and mRNA expression of TNF- α , IL-6, and IL-1 β , but pre-treatment with OA (20 mg/kg) for 1 h before compound 48/80 administration markedly decreased these pro-inflammatory cytokines. These results suggest that OA provides an inhibitory effect on transcription and pro-inflammatory cytokine release, similar to the results for HMC-1 cells (Fig. 5A and B).

OA protected against mortality and inhibits histamine release in a mouse model of compound 48/80-induced anaphylactic shock

We assessed the effect of OA on the survival rate with compound 48/80-induced hypersensitive anaphylaxis. As shown in Fig. 6A, all mice died within 35 min of injection of compound 48/80, whereas DSCG (25 mg/kg) and OA (20 mg/kg) increased the survival rate after compound 48/80 injection. OA is more effective than DSCG at lowering mortality in the anaphylaxis animal model. Histamine plays a pivotal

role in the increased vascular permeability caused by various chronic and/or life-threatening inflammatory responses. Several studies have shown that histamine is a sensitive marker of the anaphylactic shock reaction (Mikelis et al., 2015). IgE is an important mediator of the pathology of allergic disorders, including fatal anaphylaxis, binding to receptors expressed on mast cells. The binding of IgE to Fc ϵ R1 sensitizes mast cells and other effector cells to release various mediators (Galli and Tsai, 2012). We measured the serum levels of histamine and IgE after compound 48/80-induced anaphylactic shock to determine whether OA inhibits mast cell related markers. Our results showed that OA reduced the compound 48/80-induced histamine levels in serum (Fig. 6B and C). This indicates that OA has a protective effect against allergic inflammation through the inhibition of histamine release and level of IgE during anaphylactic shock.

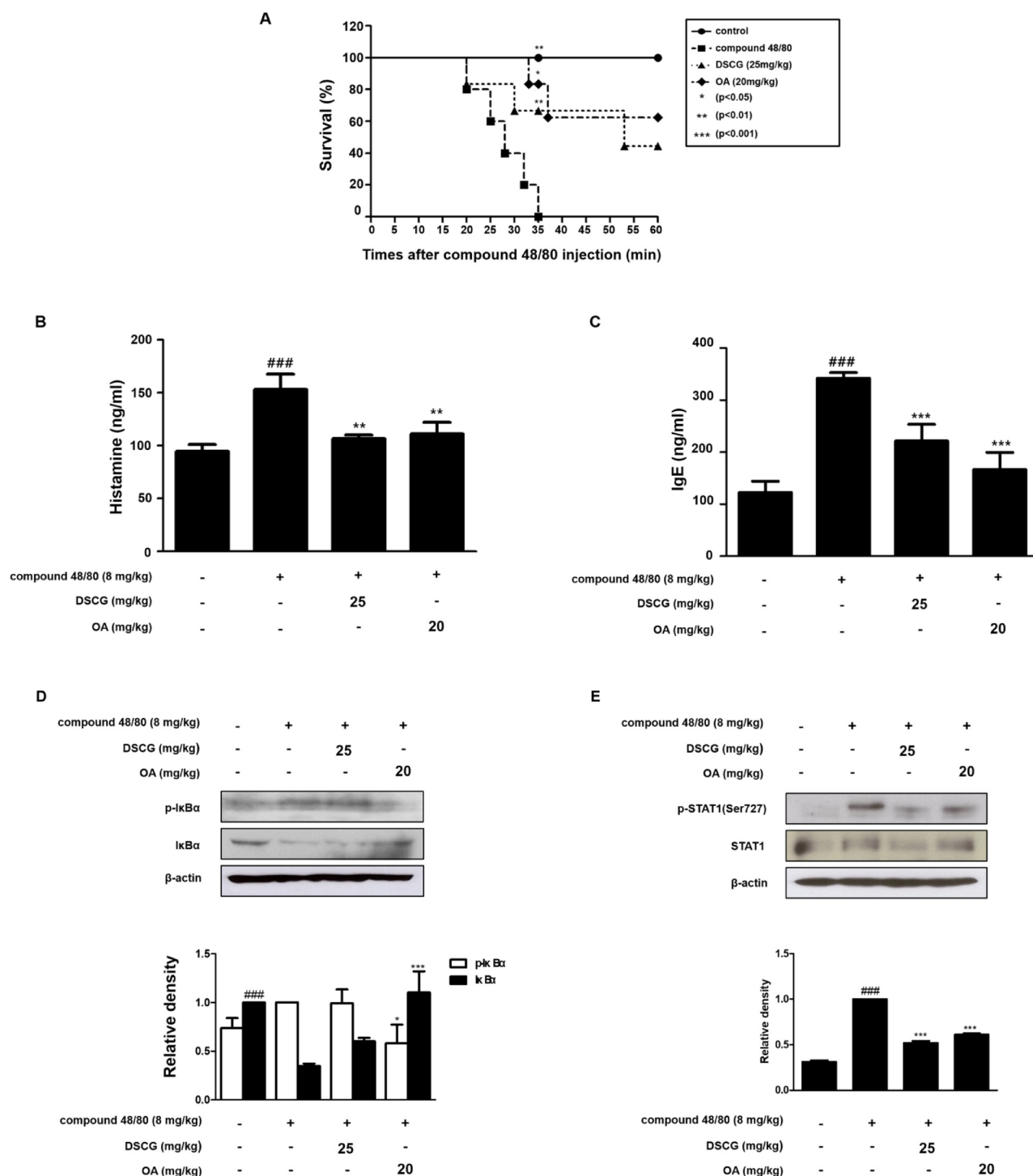


Fig. 6. Effects of OA on compound 48/80-induced mortality and mediators in a mouse model of anaphylactic shock.

Mice were injected with OA, DSCG and PBS as a vehicle ($n = 6$ per group or total) for 1 h before compound 48/80 injection (8 mg/kg i.p.). (A) Survival rates of these mice were monitored for 1 h. (B) Histamine release and (C) IgE serum levels were measured using ELISA kit. Expressions of IκB (D) and STAT1 (E) were determined by Western blot analysis using specific antibodies. Densitometric analysis was performed using Bio-Rad Quantity One® Software. The data shown represent mean $\pm$ S.D. of three independent experiments. ### $p < 0.01$, ### $p < 0.001$ vs. the control group; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs. compound 48/80-treated group.

OA inhibited activation of IκB and STAT1 in a mouse model of compound 48/80-induced anaphylactic shock

To investigate whether OA regulates allergic inflammation through the NF-κB and STAT1 pathways, we examined the protein expressions of IκB and STAT1 in liver tissue of compound 48/80-induced

anaphylactic shock mice. The results of the Western blot analysis revealed that compound 48/80 markedly induced phosphorylation and degradation of IκB, whereas treatment with OA significantly repressed its effect in the anaphylaxis animal model. In addition, the level of phosphorylated STAT1 (at Ser727) was significantly increased in the compound 48/80 treatment group as compared to the control group,

and OA treatment decreased its expression in compound 48/80-induced anaphylactic shock mice (Fig. 6D and E). These results suggest that the anti-inflammatory effect of OA via regulation of NF- κ B and STAT1 activation in an anaphylaxis animal model.

Discussion

Allergy is one of the most common diseases in the world, and its prevalence is increasing rapidly every year. The World Allergy Organization (WAO) estimate of its prevalence by country ranges from 10 to 40% (Pawankar, 2014). Allergen exposure causes allergic reactions in individuals; these can range from mild skin reactions to severe and life-threatening reactions such as anaphylaxis. Mast cells are associated with many pathologies, including type I hypersensitivity reactions, mastocytosis, and urticaria. The classic and most well-known function of mast cells involves the inflammatory cascade and the role of the body's innate immune system (Fong and Crane, 2018). The late reaction may take place about 3 or 4 h after allergen exposure, according to the features of chronic allergic inflammation. At this point, it is characterized by an IgE-dependent local influx of inflammatory cells (Benson et al., 2006) and stimulates mast cells to synthesize and release pro-inflammatory cytokines such as TNF- α , IL-6, and IL-1 β . These pro-inflammatory cytokines are thought to be initiators of allergic inflammatory responses, and the cytokines released also have direct effects on local tissue, such as increased mucus production and increased gut peristalsis to reduce pathogen invasion (Lee et al., 2017). In this study, we observed that OA decreased mRNA and production levels of TNF- α , IL-6, and IL-1 β in PMACI-stimulated HMC-1 (Fig. 2). We also found that OA suppressed not only p65 NF- κ B translocation but also phosphorylation of I κ B, IKK, Akt, as well as that of STAT1 at Ser727 (Figs. 3 and 4A) PMACI-stimulated HMC-1 cell. The p38 MAPK/MK2 signaling pathway was involved in this mechanisms (Fig. 4B). OA showed an anti-allergic effect by suppressing pro-inflammatory cytokines via inhibition of the NF- κ B and STAT1 signal pathways.

Anaphylaxis is also an IgE-mediated systemic hypersensitivity reaction involving inflammatory mediators from mast cells and basophils (Sampson et al., 2006). In accordance with previous studies (Chai et al., 2013), injection of compound 48/80 induced anaphylactic shock with mortality and increased the expression of allergic and inflammatory mediators. The result showed that OA decreased mRNA expression and production levels of TNF- α , IL-6, and IL-1 β in an animal model of anaphylactic shock (Fig. 5). Moreover, we could confirm the protective effect of OA against the compound 48/80-induced anaphylactic shock. OA also decreased the levels of histamine and IgE, which trigger immediate hypersensitivity, and inhibits the degradation of I κ B and phosphorylation of STAT1 at Ser727 (Fig. 6). These results were correlated with the prior results from HMC-1 in this study.

Since transcription factor is important determinant in allergic response, contributing to gene production and cellular function, thus, targeting transcription factors is promising strategy for controlling of allergic disease. Therefore, further studies are required to clear the cross-talk between different transcription factors, including NF- κ B and STAT1, and the precise interactions with cytokines resulted from allergic reactions for better understanding of the molecular mechanisms of OA and identify novel targets for pharmacological approach in allergic responses. This will be the subject of our future study. OA is classified as a typical Biopharmaceutics Classification System IV category drug with low water-solubility and poor permeability. Due to its instability and low oral bioavailability, OA's wide application has been restricted (Xia et al., 2017). Recently, OA-phospholipid complex (Jiang et al., 2016) and nanoparticulate drug delivery system (Chen et al., 2011) have been developed to enhance the dissolution rate and bioavailability of OA. Thus, the pharmacological effects of OA should be discussed with how to improve the bioavailability enhancing solubility and permeability of OA.

In conclusion, our result showed that OA inhibited the allergic

inflammatory reaction that is mediated by pro-inflammatory cytokines derived from the mast cells, which play a major role in immediate responses such as anaphylactic shock. Moreover, OA exerts the anti-allergic inflammatory effect via blockade of the NF- κ B and STAT1 signaling pathways (Figure 7). Considering the reported effects of OA on allergic models (Choi et al., 2013; Cordova et al., 2014; Kim et al., 2014), though we need the mouse model that fully recapitulates all the features of allergic disorders, these findings provide an insight of promising potential of OA for prevention and treatment of allergic disorders. Therefore, the potential benefits of OA in the alleviation of allergic inflammatory reactions suggest that OA could be a useful therapeutic agent against allergic inflammatory diseases.

Author contributions

YMK and HJA conceived and designed the experiments. YMK performed the experiments and analyzed the data with ML and HJA. YMK wrote the manuscript. ML contributed to funding acquisition. All authors read and approved the final manuscript. All data were generated in-house, and no paper mill was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP; Ministry of Science, ICT & Future Planning) (No. NRF-2017R1C1B2008617); and the Research fund from Sangji University Graduate School. The authors would like to thank Professor Jong-Sik Jin (Chonbuk University, Republic of Korea) for providing the cells.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.phymed.2020.153340](https://doi.org/10.1016/j.phymed.2020.153340).

References

- Ayeleso, T.B., Matumba, M.G., Mukwevho, E., 2017. Oleanolic acid and its derivatives: biological activities and therapeutic potential in chronic diseases. *Molecules* 22.
- Benson, M., Langston, M.A., Adner, M., Andersson, B., Torinsson-Nalwai, A., Cardell, L.O., 2006. A network-based analysis of the late-phase reaction of the skin. *J. Allergy Clin. Immunol.* 118, 220–225.
- Chai, O.H., Shon, D.H., Han, E.H., Kim, H.T., Song, C.H., 2013. Effects of Anemarrhena asphodeloides on IgE-mediated passive cutaneous anaphylaxis, compound 48/80-induced systemic anaphylaxis and mast cell activation. *Exp. Toxicol. Pathol.* 65, 419–426.
- Chen, M., Zhong, Z., Tan, W., Wang, S., Wang, Y., 2011. Recent advances in nanoparticle formulation of oleanolic acid. *Chin. Med.* 6, 20.
- Choi, J.K., Oh, H.M., Lee, S., Park, J.W., Khang, D., Lee, S.W., Lee, W.S., Rho, M.C., Kim, S.H., 2013. Oleanolic acid acetate inhibits atopic dermatitis and allergic contact dermatitis in a murine model. *Toxicol. Appl. Pharmacol.* 269, 72–80.
- Choi, Y.H., Yan, G.H., Chai, O.H., Lim, J.M., Sung, S.Y., Zhang, X., Kim, J.H., Choi, S.H., Lee, M.S., Han, E.H., Kim, H.T., Song, C.H., 2006. Inhibition of anaphylaxis-like reaction and mast cell activation by water extract from the fruiting body of *Phellinus linteus*. *Biol. Pharm. Bull.* 29, 1360–1365.
- Cordova, C., Gutierrez, B., Martinez-Garcia, C., Martin, R., Gallego-Munoz, P., Hernandez, M., Nieto, M.L., 2014. Oleanolic acid controls allergic and inflammatory responses in experimental allergic conjunctivitis. *PLoS ONE* 9, e91282.
- Descotes, J., Choquet-Kastylevsky, G., 2001. Gell and Coombs's classification: is it still valid? *Toxicology* 158, 43–49.
- Fong, M., Crane, J.S., 2018. *Histology, Mast Cells*, StatPearls, Treasure Island (FL).
- Galli, S.J., Tsai, M., 2012. IgE and mast cells in allergic disease. *Nat. Med.* 18, 693–704.
- Giner-Larza, E.M., Manez, S., Recio, M.C., Giner, R.M., Prieto, J.M., Cerda-Nicolas, M., Rios, J.L., 2001. Oleanonic acid, a 3-oxotriterpene from *Pistacia*, inhibits leukotriene synthesis and has anti-inflammatory activity. *Eur. J. Pharmacol.* 428, 137–143.
- Hong, M.H., Kim, J.H., Bae, H., Lee, N.Y., Shin, Y.C., Kim, S.H., Ko, S.G., 2010. *Attractylodes japonica* Koidzumi inhibits the production of proinflammatory

- cytokines through inhibition of the NF-kappaB/IkappaB signal pathway in HMC-1 human mast cells. *Arch. Pharm. Res.* 33, 843–851.
- Jiang, Q., Yang, X., Du, P., Zhang, H., Zhang, T., 2016. Dual strategies to improve oral bioavailability of oleanolic acid: enhancing water-solubility, permeability and inhibiting cytochrome P450 isozymes. *Eur. J. Pharm. Biopharm.* 99, 65–72.
- Kamei, R., Fujimura, T., Matsuda, M., Kakiyama, K., Hirakawa, N., Baba, K., Ono, K., Arakawa, K., Kawamoto, S., 2017. A flavanone derivative from the Asian medicinal herb (*Perilla frutescens*) potently suppresses IgE-mediated immediate hypersensitivity reactions. *Biochem. Biophys. Res. Commun.* 483, 674–679.
- Kim, S.H., Hong, J.H., Lee, Y.C., 2014. Oleanolic acid suppresses ovalbumin-induced airway inflammation and Th2-mediated allergic asthma by modulating the transcription factors T-bet, GATA-3, RORgammat and Foxp3 in asthmatic mice. *Int. Immunopharmacol.* 18, 311–324.
- Lee, J.Y., Moon, H., Kim, C.J., 2010. Effects of hydroxy pentacyclic triterpene acids from *Forsythia viridissima* on asthmatic responses to ovalbumin challenge in conscious guinea pigs. *Biol. Pharm. Bull.* 33, 230–237.
- Lee, M., Lee, N.Y., Chung, K.S., Cheon, S.Y., Lee, K.T., An, H.J., 2017. Roxatidine attenuates mast cell-mediated allergic inflammation via inhibition of NF-kappaB and p38 MAPK activation. *Sci. Rep.* 7, 41721.
- Lee, N.Y., Chung, K.S., Jin, J.S., Bang, K.S., Eom, Y.J., Hong, C.H., Nugroho, A., Park, H.J., An, H.J., 2015. Effect of chicoric acid on mast cell-mediated allergic inflammation in vitro and in vivo. *J. Nat. Prod.* 78, 2956–2962.
- Li, M., Han, Z., Bei, W., Rong, X., Guo, J., Hu, X., 2015. Oleanolic acid attenuates insulin resistance via NF-kappaB to regulate the IRS1-GLUT4 pathway in HepG2 Cells. *Evid. Based Complement. Alternat. Med.* 2015, 643102.
- Martin, R., Hernandez, M., Cordova, C., Nieto, M.L., 2012. Natural triterpenes modulate immune-inflammatory markers of experimental autoimmune encephalomyelitis: therapeutic implications for multiple sclerosis. *Br. J. Pharmacol.* 166, 1708–1723.
- Mikelis, C.M., Simaan, M., Ando, K., Fukuhara, S., Sakurai, A., Amornphimoltham, P., Masedunskas, A., Weigert, R., Chavakis, T., Adams, R.H., Offermanns, S., Mochizuki, N., Zheng, Y., Gutkind, J.S., 2015. RhoA and ROCK mediate histamine-induced vascular leakage and anaphylactic shock. *Nat. Commun.* 6, 6725.
- Pawankar, R., 2014. Allergic diseases and asthma: a global public health concern and a call to action. *World Allergy Organ. J.* 7, 12.
- Petronelli, A., Pannitteri, G., Testa, U., 2009. Triterpenoids as new promising anticancer drugs. *Anticancer Drugs* 20, 880–892.
- Rajan, T.V., 2003. The Gell-Coombs classification of hypersensitivity reactions: a re-interpretation. *Trends Immunol.* 24, 376–379.
- Ramsauer, K., Sadzak, I., Porras, A., Pilz, A., Nebreda, A.R., Decker, T., Kovarik, P., 2002. p38 MAPK enhances STAT1-dependent transcription independently of Ser-727 phosphorylation. *Proc. Natl. Acad. Sci. USA* 99, 12859–12864.
- Sampson, H.A., Munoz-Furlong, A., Campbell, R.L., Adkinson Jr., N.F., Bock, S.A., Branum, A., Brown, S.G., Camargo Jr., C.A., Cydulka, R., Galli, S.J., Gidudu, J., Gruchalla, R.S., Harlor Jr., A.D., Hepner, D.L., Lewis, L.M., Lieberman, P.L., Metcalfe, D.D., O'Connor, R., Muraro, A., Rudman, A., Schmitt, C., Scherrer, D., Simons, F.E., Thomas, S., Wood, J.P., Decker, W.W., 2006. Second symposium on the definition and management of anaphylaxis: summary report—Second National Institute of Allergy and Infectious Disease/Food Allergy and Anaphylaxis Network symposium. *J. Allergy Clin. Immunol.* 117, 391–397.
- Sifaoui, I., Lopez-Arencibia, A., Martin-Navarro, C.M., Reyes-Battle, M., Mejri, M., Valladares, B., Lorenzo-Morales, J., Abderabba, M., Pinero, J.E., 2017. Selective activity of oleanolic and maslinic acids on the amastigote form of *Leishmania* Spp. *Iran J. Pharm. Res.* 16, 1190–1193.
- Somova, L.I., Shode, F.O., Ramnandan, P., Nadar, A., 2003. Antihypertensive, anti-atherosclerotic and antioxidant activity of triterpenoids isolated from *Olea europaea*, subspecies *africana* leaves. *J. Ethnopharmacol.* 84, 299–305.
- Sultana, N., Ata, A., 2008. Oleanolic acid and related derivatives as medicinally important compounds. *J. Enzyme Inhib. Med. Chem.* 23, 739–756.
- Tomasiaik-Lozowska, M.M., Klimek, M., Lis, A., Moniuszko, M., Bodzenta-Lukaszyk, A., 2018. Markers of anaphylaxis - a systematic review. *Adv. Med. Sci.* 63, 265–277.
- Wang, X., Ye, X.L., Liu, R., Chen, H.L., Bai, H., Liang, X., Zhang, X.D., Wang, Z., Li, W.L., Hai, C.X., 2010. Antioxidant activities of oleanolic acid in vitro: possible role of Nrf2 and MAP kinases. *Chem. Biol. Interact.* 184, 328–337.
- Xia, X., Liu, H., Lv, H., Zhang, J., Zhou, J., Zhao, Z., 2017. Preparation, characterization, and in vitro/vivo studies of oleanolic acid-loaded lactoferrin nanoparticles. *Drug Des. Devel. Ther.* 11, 1417–1427.