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Inhibitory effect of glycoprotein isolated from *Opuntia ficus-indica* var. *saboten* MAKINO on activities of allergy-mediators in compound 48/80-stimulated mast cells

Kye-Taek Lim*

Molecular Biochemistry Laboratory, Biotechnology Research Institute, Chonnam National University, Gwang-ju, 300, Yongbong-Dong 500-757, Republic of Korea

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ABSTRACT

The present study was performed to investigate the anti-allergy potentials of glycoprotein (90 kDa) isolated from *Opuntia ficus-indica* var. *saboten* MAKINO (OFI glycoprotein) in vivo (ICR mice) and in vitro (RBL-2H3 cells). At first, to know whether the OFI glycoprotein has an inhibitory ability for allergy in vivo, we evaluated the activities of allergy-related factors such as histamine and β -hexosaminidase release, lactate dehydrogenase (LDH), and interleukin 4 (IL-4) in compound 48/80 (8 ml/kg BW)-treated ICR mice. After that, we studied to found the effect for anti-allergy in vitro such as nuclear factor kappa B (NF- κ B) and inducible nitric oxide synthase (iNOS), extracellular signal-regulated kinase (ERK) 1/2, arachidonic acid, and cyclooxygenase-2 (COX-2) in compound 48/80 (5 μ g/ml)-treated RBL-2H3 cells. Our results showed that the OFI glycoprotein (5 mg/kg) inhibited histamine and β -hexosaminidase release, lactate dehydrogenase (LDH), and interleukin 4 (IL-4) in mice serum. Also OFI glycoprotein (25 μ g/ml) has suppressive effects on the expression of MAPK (ERK1/2), and on protein expression of anti-allergic proteins (iNOS and COX-2). Thus, we speculate that the OFI glycoprotein is an example of natural compound that blocks anti-allergic signal transduction pathways.

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1. Introduction

Allergy is a disorder of the immune system and it can cause a runny nose, sneezing, itching, rashes, swelling or asthma. From a long time ago, allergic diseases are rapidly increasing chronic health problem in most countries [1]. It is increasing in prevalence and severity and has a rising mortality despite a substantial increase in treatment solutions. Allergic reactions which are caused by the release of histamine, pharmacological mediator, from mast cells occur after exposures to environmental substances known as allergens.

Mast cells and blood basophils are well-known as a critical participant in allergic diseases [2]. Activated mast cells can produce a wide variety of mediators such as histamine, leukotrienes, prostaglandins, proteases and chemotactic cytokines such as IL-4 [3]. Also, it starts the process of degranulation which results in the releasing of mediators, such as products of arachidonic acid metabolism [4–6]. Among these substances, histamine is the best characterized and most potent vasoactive mediator implicated in the acute phase of immediate hypersensitivity [7]. Also it is usually considered as a degranulation marker in experiments on immediate allergic reaction in vivo and in vitro. Mast cell degranulation

can be elicited by a number of positively charged substances, known as the basic secretagogues of mast cells [8,9]. The synthetic compound 48/80 is known to be one of the most potent secretagogues, so an appropriate amount of compound 48/80 has been used as a direct and convenient reagent to study the mechanism of anaphylaxis [10,11]. It strongly activates cellular exocytosis, inducing a rapid release of allergic mediators including such as histamine. At that reason, compound 48/80 has been used as a direct reagent to study the mechanism of allergy and anaphylaxis [12]. And β -hexosaminidase is also stored in secretory granules of mast cells and is released concomitantly with histamine when mast cells are immunologically activated [13,14].

Nuclear factor (NF)- κ B is a transcription factor that represents a potential target for pharmacologic intervention [15]. NF- κ B generally exists as a heterodimer comprised of p50 and p65 in the cytosol that is bound to the protein that makes up the I κ B-a, I κ B-b, and I κ B-c subunits [16]. NF- κ B activation is accomplished by phosphorylation and degradation of I κ B-a though the ubiquitin pathway, followed by translocation of NF- κ B to the nucleus. Mitogen-activated protein kinases (MAPKs) are a widely conserved family of serine/threonine protein kinases involved in many cellular programs such as cell proliferation, differentiation, motility and death. Extracellular signal-regulated kinase (ERK) play a critical role in the signaling of cytokine expression [17–19]. For this reason, inhibition of NF- κ B and MAPKs activation is an important

* Fax: +82 62 530 2129.

E-mail addresses: ktlim@chonnam.ac.kr, kyetaeklim@hotmail.com

target of anti-allergic treatment [20]. Cyclooxygenase (COX) is the key enzyme responsible for the conversion of arachidonic acid via oxygenation to prostaglandins (PGs), which are pleiotropic mediators of immune responses [21]. It exists in two isoforms (COX-1 and COX-2), with different physiological functions. COX-2 is inducible and plays a role in inflammatory cells, such as macrophages, fibroblasts, and endothelial cells. Like COX-2, inducible nitric oxide synthase (iNOS) is also strongly induced by bacterial endotoxin and inflammatory cytokines. It has been reported that the expression of COX-2 and iNOS under inflammatory circumstances is elevated through the activation of transcriptional factors in response to pro-inflammatory cytokines and ROS [22,23].

1.1. *Opuntia ficus-indica* var. *saboten*

MAKINO (OFI) has been used to advance nutritional level and to protect various diseases in South Korea [24]. For instance, it has been widely used traditional herbal medicine for asthma, sores, diarrhea, allergy and inflammation. According to clinical reports, especially, it has been used in Korea for treatment of chronic bronchitis, asthma, chronic hepatitis and coronary heart disease. Previous studies have shown that the roots of OFI have various effects, including anti-oxidation, anti-inflammation, antibacterial activity, apoptosis modulator properties, and anti-tumor activities.

The result of preliminary experiment for screening of biological activities was brought about that ethanol extract of OFI has a potent anti-allergic inflammatory activity in mast cell-mediated reaction. It is very important to study allergic responses using RBL-2H3 cells, investigating inflammation-related cytokines in the molecular signaling pathways [25,26]. Also, the passive cutaneous anaphylaxis (PCA) animal model is one of the most important on the mast cell-mediated allergic reactions in vivo [27]. The degranulation from the mast cells also can be induced by the synthetic compound 48/80, and it has been used to study the mechanism of allergic reaction [28].

We figured out that most glycoproteins originated from plants have a character of modulation for inflammation-, immunity-, and allergy-related factors. Thus, we assume that OFI glycoprotein can also contribute to inhibit allergy-related factors and activity of allergy-mediated inflammation cytokines caused by compound 48/80. Therefore, this study was firstly evaluated allergy-related factors such as mortality, IL-4 and histamine in ICR mice serum, because the compound 48/80 cannot stimulate any cytokines in the mast cells. After that it was evaluated allergy-related factors (COX-2, NF- κ B, and ERK) in RBL-2H3 cells.

2. Materials and methods

2.1. Chemicals

All the plastic materials were purchased from Falcon Labware (Becton–Dickinson, Franklin Lakes, NJ, USA). *N*-methyl-*p*-methoxyphenethylamine, penicillin G, streptomycin, and trypsin were from Sigma (St. Louis, MO, USA). Cell culture reagents, including Dulbecco's modified essential medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). The other chemicals and reagents were of the highest quality available.

2.2. Preparation of OFI glycoprotein

The *Opuntia ficus-indica* var. *saboten* MAKINO (OFI) were collected from Naju traditional market in the Chonnam province of South Korea, and OFI glycoprotein was isolated from it as described previously [29]. The purity of OFI glycoprotein is more than 95%.

The dried sample (4.5 mg, 0.05% from the original sample) was stored at -20°C during the experimental period. We used OFI glycoprotein with a molecular weight of 90 kDa in this study.

2.3. Animals and treatments

Male ICR mice, aged 5 weeks, were purchased from Daehan Lab. (Animal Research Center Co., Ltd., Dae Jeon, Korea) and housed according to the animal care guidelines approved by the Animal-care Committee of the American Society of Mammalogists at the Experimental Animal Room of Veterinary College of Chonnam National University. All mice were fed a commercial diet and water *ad libitum*, and kept for at least 1 week prior to the experiments. Mice were divided into the following five study groups:

- (1) Control ($n = 6$).
- (2) Compound 48/80 (8 mg/kg) ($n = 6$).
- (3) Compound 48/80 + OFI glycoprotein (5 mg/kg, BW) ($n = 6$).
- (4) Compound 48/80 + OFI glycoprotein (10 mg/kg, BW) ($n = 6$).
- (5) OFI glycoprotein was dissolved in PBS and then administered orally with 5 and 10 mg/kg body weight.

OFI glycoprotein was orally administrated into mice once a day for 3 days. One hour after the final treatment, mice were treated with compound 48/80 at a dose of 8 mg/kg, body weight (BW) intraperitoneally dissolved in saline. At 24 h after compound 48/80 administration, the mice of each group were anesthetized with ether and the blood drawn by cardiac puncture. Blood samples were collected from anesthetized mice by orbital sinus or maxillary vein-puncture. Samples were collected into 1.5 ml centrifuge tubes, allowed to clot, and centrifuged at 10,000 rpm at 4°C for 5 min. The serum was transferred to a new tube and stored at -20°C until testing. The samples were centrifuged and the plasma was isolated and stored at -70°C to use for future experiments of lactate dehydrogenase (LDH) assay, histamine release assay, β -hexosaminidase secretion assay, and IL-4 expression. Mice were weighed before and after the defined treatments for each group.

2.4. Cell culture

RBL-2H3 cells were incubated in DMEM containing 10% FBS, 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin at 37°C and 5% CO_2 atmosphere. The medium was renewed twice a week. The number of cells (1×10^6 cells/ml) was divided into 35-mm culture dishes or 96-well flat bottom plates. Here, it notes that RBL-2H3 cells were co-treated with the compound 48/80 (5 $\mu\text{g}/\text{ml}$) for 15 min to detect activity of ERK1/2, for 1 h to detect revelation of NF- κ B, for 2 h to detect activity of Cox-2, iNOS. Additionally, the OFI glycoprotein was pre-treated at appropriate concentration for 30 min prior to compound 48/80 addition into culture media in all experiments.

2.5. Compound 48/80-induced systemic reaction

ICR mice were given an intraperitoneal injection of 0.008 g/kg body weight (BW) of compound 48/80 for test the mast cell degranulator. OFI glycoprotein was dissolved in saline and administered orally at doses ranging from 5 to 10 mg/kg BW 1 h before the injection of compound 48/80 ($n = 6/\text{group}$). The responses in the compound 48/80 experiments were represented in anaphylaxis and death rate, because it could not detect pain or discomfort which are appropriate indicators for measurement of physiological condition. Such responses (pain or discomfort) were appeared too quickly in short period time after injection of compound 48/80. Therefore, we did not measure and represent the data of pain

and discomfort responses in this study. Mortality was monitored for 1 h after induction of anaphylactic shock.

2.6. Lactate dehydrogenase assay

For measurement of the LDH level of mouse plasma, plasma samples (35 μ l) were mixed with reaction mixture containing 0.6 mM pyruvate in 48 mM potassium phosphate buffer (pH 7.5), and the final volume of the reaction mixture was brought up to 3.15 ml. The reaction was initiated by the addition of 0.18 mM β -NADH and LDH activity was measured as the rate of loss of β -NADH absorption at 340 nm for 2 min. Data were represented as relative percentages, compared with the control value.

2.7. Histamine release and β -hexosaminidase secretion assay

The amount of histamine from mice serum or supernatant of cell culture medium was assayed using a fluorometric assay [30]. To determine the amounts of total cellular histamine, RBL-2H3 cells (1×10^6 cells) were suspended and sonicated in a Tyrode buffer. Then, the cell lysates were centrifuged at 10,000g for 10 min and the supernatants were harvested. Afterwards, 2 ml of the supernatant was mixed with 0.75 g of NaCl and 0.5 ml of 1 N NaOH, 5 ml of the 3:2 (v/v) mixture of *n*-butanol and 1.5 ml of 0.1 N HCl. After centrifugation at 300 g for 5 min, 1 ml of the lower HCl layer was recovered, mixed with 0.15 ml of 1 N NaOH and the subsequent product was added to 0.1 ml of 0.2% *o*-phthalaldehyde dissolved in methanol for 5 min at room temperature to yield a fluorescent product. The reaction was terminated by adding 0.14 ml of 0.5 N H_2SO_4 and fluorescence intensity was measured using a fluorescent microplate reader (Dual Scanning SPECTRAmax, Molecular Devices Corporation, Sunnyvale, CA, USA) with the excitation set at 360 nm and the emission set at 440 nm. Histamine content was quantified by comparison to a standard curve generated by coupling histamine dihydrochloride to *o*-phthalaldehyde. β -hexosaminidase in the supernatant was measured using a previously reported method [31]. Cells were treated with 5 μ g/ml compound 48/80 in the absence or presence of OFI glycoprotein (25, 50 and 100 μ g/ml) for 30 min. After 30 min, degranulation was terminated by placing the cells on ice. To determine the amount of β -hexosaminidase activity released by the cells, 20 μ l of each sample was incubated with 20 μ l of 1 mM *p*-nitrophenyl-*N*-acetyl- β -D-glucosaminide dissolved in a 0.1 M citrate buffer, pH 4.5, in a 96-well microtiter plate at 37 °C for 1 h. The reaction was terminated with 200 μ l/well of 0.1 M carbonated buffer, pH 10.0. Absorbance was measured at 410 nm with a MicroReader (Hyperion, Inc., USA).

2.8. Enzyme-linked immunosorbent assay (ELISA)

From mice serum, the amount of IL-4 was determined by sandwich ELISA using monoclonal antibodies specific to IL-4 (BD Biosciences, San Diego, SA, USA). ELISA plates were coated with rat anti-mouse IL-4. After coating, serially diluted serum was added to the plates and incubated overnight at 4 °C. IL-4 was used as a standard for the quantization of IL-4 in the ICR mice serum. After washing, biotinylated anti-mouse IL-4 were added. The plates were washed and streptavidin–horseradish peroxidase was added. After additional washes, TMB solution was added and developed for 10 min. The OD was determined at 405 nm in a Microplate Reader (Molecular Devices, Sunnyvale, CA, USA).

2.9. Preparations of protein extracts (whole and nuclear)

For immunoblotting of inflammatory-related proteins, protein extracts were prepared as described previously [32]. The cells were treated with 5 μ g/ml compound 48/80 in the presence of the OFI

glycoprotein (25–100 μ g/ml). For the immunoblotting of ERK, the treated cells were rinsed twice with PBS after removing the medium and scraped in 300 μ l of buffer A (20 mM HEPES, pH 7.9, 0.4 M NaCl, 0.2 mM EDTA, 1 mM DTT, 1 mM PMSF, 1.5 mM $MgCl_2$, 0.5% NP40, 25% glycerol) containing a protease inhibitor cocktail. After lysis, the cells were centrifuged at 14,000g for 30 min. The supernatant was collected and used as whole cell lysates. To separate the cytosolic and nucleic protein extracts, the cells were scraped in 500 μ l of buffer B (10 mM HEPES, pH 7.9, 10 mM KCl, 0.1 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 1.5 mM $MgCl_2$, 0.5% NP-40), followed by centrifugation at 3000g for 5 min. The supernatant and pellet were designated as nucleic protein extracts separately. The isolated nucleic protein extracts for the immunoblotting of NF- κ B were then re-suspended in 200 μ l of buffer C (20 mM HEPES, pH 7.9, 0.4 M NaCl, 0.1 mM EDTA, 1 mM EGTA, 1 mM DTT, 1 mM PMSF, 1.5 mM $MgCl_2$, 0.5% NP-40) containing a protease inhibitor cocktail (Boehringer, Mannheim, Germany) and centrifuged at 12,000g for 15 min. The amounts of proteins were measured using the Lowry method [33]. The samples of protein were stored at –70 °C.

2.10. Immunoblot analysis for NF- κ B, ERK, COX-2, and iNOS

Intracellular protein extracts were analyzed on 10% polyacrylamide mini-gel electrophoresis at 100 V for 2 h for at room temperature using a Mini-PROTEIN II electrophoresis cell (Bio-Rad). After transfer onto nitrocellulose membranes (Millipore, Bedford, MA), the membrane was incubated for 1 h at room temperature in TBS-T solution (10 mM Tris–HCl, pH 7.6, 150 mM NaCl, and 0.1% Tween-20) containing 5% non-fat dry milk. The membranes were subsequently incubated for 2 h at room temperature with a 1:3000 dilution of rabbit polyclonal antibodies [NF- κ B (p50 and p65), ERK1/2, COX-2, and iNOS] in TBS-T solution. After three washes with TBS-T, the membranes were incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit IgG (1:10,000; Cell Signaling, Beverly, MA) in TBS-T buffer. The protein bands were visualized by enhanced chemiluminescence (Amersham Pharmacia Biotech, Buckinghamshire, UK). The results obtained from immunoblot assay were calculated using Scion imaging software (Scion Image Beta 4.02, Frederick, MD) as the relative intensity.

2.11. Arachidonic acid release assay

The [^3H] AA release experiments were performed using a modification of the method reported by according to the method of Xing et al. [34]. RBL-2H3 cells were incubated for 24 h in DMEM containing 0.5 μ Ci/ml [^3H] AA. The cells were then washed three times with DMEM and incubated at 37 °C for 0–24 h in incubation medium. At the end of the incubation period, the culture medium was transferred to ice-cold tubes containing 100 μ l of 55 mM EGTA and EDTA (final concentration, 5 mM each). The culture medium was then centrifuged at 12,000g to eliminate the cell debris. The level of radioactivity in the supernatant was determined by placing samples in scintillation vials containing scintillation fluid and measuring the radioactivity using a liquid scintillation counter (LS 6500, Beckman Instruments, Fullerton, CA).

2.12. Statistical analysis

All experiments were carried out in triplicate separately, and data were expressed as means SE. One-way analysis of variance (ANOVA) and the Duncan test were carried out to determine significant differences of multiple comparisons (SPSS program, ver. 11.0).

3. Results

3.1. Mortality and LDH assay

After the intraperitoneal injection of compound 48/80 (0.008 g/kg, BW), the mice were monitored for 1 h, and then the mortality rate was determined. As shown in Table 1 and 100% of the mice injected with compound 48/80 underwent a fatal shock. However, when the OFI glycoprotein was pre-administered orally at concentration (5–10 mg/kg, BW) 1 h prior to compound injection, the mortality rate after treatment with compound 48/80 was reduced in a dose-dependent manner.

In the LDH assay, the amount of LDH was 1.7-fold after treatment with compound 48/80 alone, compared to the control as shown in Fig. 1. However, when the mice were orally pre-administered with the OFI glycoprotein prior to the injection of compound 48/80, amounts of LDH decreased by 0.11- and 0.35-fold at 5 mg/kg and 10 mg/kg (BW) of OFI glycoprotein in the presence of compound 48/80, compared to the compound 48/80 treatment alone.

3.2. Inhibitory effects of the OFI glycoprotein on histamine release in the compound 48/80-induced ICR mice and RBL-2H3 cells and β -hexosaminidase by compound 48/80 in the ICR mice

Histamine release of mice serum and RBL-2H3 cells increased after treatment with compound 48/80 alone compared to the con-

trol. However, in the mice that were pre-administered with the OFI glycoprotein in the presence of compound 48/80, histamine release decreased significantly after treatment with compound 48/80 in the presence of OFI glycoprotein (50 and 100 μ g/ml) in a dose-dependent manner. For instance, the amount of releasing histamine and β -hexosaminidase were increased by 1.52- and 1.95-fold compared with the control. However, histamine release decreased 0.25- and 0.26-fold after the treatment with compound 48/80 in the presence of OFI glycoprotein (5 and 10 mg/kg, BW) (Fig. 2A), while the release of β -hexosaminidase decreased 0.17- and 0.45-fold after the treatment with compound 48/80 in the presence of OFI glycoprotein (5 and 10 mg/kg) (Fig. 2B).

For histamine release from RBL-2H3 cell, the cells were treated with compound 48/80 5 μ g/ml for 30 min. As shown in Fig. 2C, when RBL-2H3 cells were exposed to compound 48/80, the amount of releasing histamine was increased by 1.36-fold compared with the control. However, the cells treated with the OFI glycoprotein inhibited the release of histamine in the presence of compound 48/80, dose-dependently. For instance, when the cells were treated with 50 and 100 μ g/ml OFI glycoprotein, the values of the release of histamine decreased 0.22- and 0.27-fold compared with the compound 48/80 treatment alone.

3.3. Inhibitory effect of OFI glycoprotein on the expression of IL-4 in compound 48/80-stimulated ICR mice

Levels of IL-4 production increased after treatment with compound 48/80 alone compared to the control. However, when the mice were orally pre-administered with the OFI glycoprotein in the presence of compound 48/80, levels of IL-4 production after treatment with compound 48/80 decreased significantly in the presence of OFI glycoprotein in a dose-dependent manner. For instance, the amount of IL-4 was 1.99-fold after treatment with compound 48/80 alone, as shown in Fig. 3. However, when the mice were orally pre-administered with the OFI glycoprotein prior to the injection of compound 48/80, amounts of IL-4 decreased by 0.45-fold at 10 mg/kg (BW) of OFI glycoprotein in the presence of compound 48/80, compared to the compound 48/80 treatment alone.

3.4. Inhibitory activity of the OFI glycoprotein on activity of NF- κ B and iNOS in compound 48/80-stimulated RBL-2H3 cells

We investigated whether or not OFI glycoprotein has inhibitory effect on the activation of NF- κ B in compound 48/80-stimulated RBL-2H3 cells. As shown in Fig. 4, when the RBL-2H3 cells were treated with 5 μ g/ml compound 48/80, NF- κ B activity was markedly increased compared with control. However, its activity (p50 and p65) was decreased at the treatment with the OFI glycoprotein (50–100 μ g/ml) in the presence of 5 μ g/ml compound 48/80. For instance, the relative intensities of NF- κ B was augmented by 1.62 for p50 and 1.86 for p65 at treated with 5 μ g/ml compound 48/80, compared with the control. However, the activity (p50) was diminished by 0.90- and 0.91-fold at the treatment with OFI glycoprotein (50–100 μ g/ml), while the activity (p65) was diminished by 0.47- and 0.80-fold at the treatment with OFI glycoprotein (50–100 μ g/ml) in the presence of compound 48/80, compared with compound 48/80 treatment alone (Fig. 4A). In addition, we further examined whether OFI glycoprotein modulates an expression of pro-inflammatory proteins (iNOS) in compound 48/80-stimulated RBL-2H3 cells (Fig. 4B). As expected, the relative level of iNOS was significantly increased after exposed to 5 μ g/ml compound 48/80 for 2 h, whereas when the cells were pre-treated with OFI glycoprotein in presence of the compound 48/80, the relative levels were attenuated by 0.55 and 0.85 at 50 and 100 μ g/ml of OFI glycoprotein, respectively, compared to the compound 48/80 treatment alone (Fig. 4B).

Table 1
Effect of OFI glycoprotein on compound 48/80-induced systemic reaction.

OFI glycoprotein treatment (mg/kg BW)	Compound 48/80 (8 mg/kg BW)	Mortality (%)
None (Saline)	–	0
None (Saline)	+	100.0
5	+	50.0
10	+	16.6

Groups of mice ($n = 6$ /group) were orally administered with either 100 μ l saline or OFI glycoprotein. OFI glycoprotein was orally administered to mice in various doses (5–10 mg/kg) 1 h prior to the injection of compound 48/80. The compound 48/80 solution was intraperitoneally injected to the mice in each group. Mortality (%) within 1 h after compound 48/80 injection was calculated as following equation: (the number of dead mice/total number of mice) $\times$ 100 obtained from triplicate experiments, separately. BW, body weight.

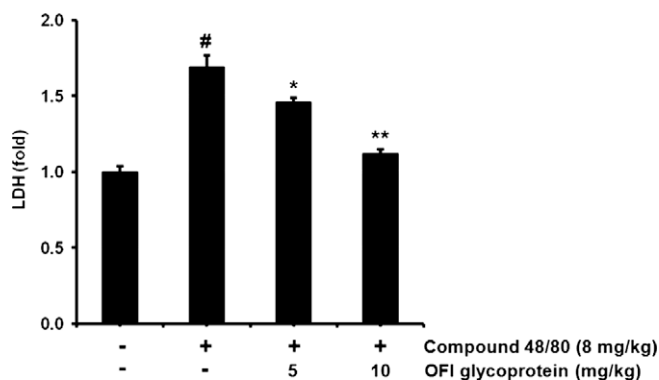


Fig. 1. Inhibitory effect of OFI glycoprotein on LDH activity in compound 48/80-treated ICR mice serum. OFI glycoprotein was orally administered into mice once a day for 3 days. One hour after the final treatment, mice were treated with compound 48/80 at a dose of 8 mg/kg, body weight (BW) intraperitoneally dissolved in saline. From mice serum after pretreatment with OFI glycoprotein (5, 10 mg/kg, BW), LDH activity by compound 48/80 (8 mg/kg, BW) was evaluated and each bar represents the means $\pm$ SE from triplicate separately ($n = 6$). # represents a significant difference between compound 48/80 treatment alone and control, $P < 0.05$. * and ** represent a significant difference between compound 48/80 treatment alone and OFI glycoprotein treatment in the presence of compound 48/80, $P < 0.05$.

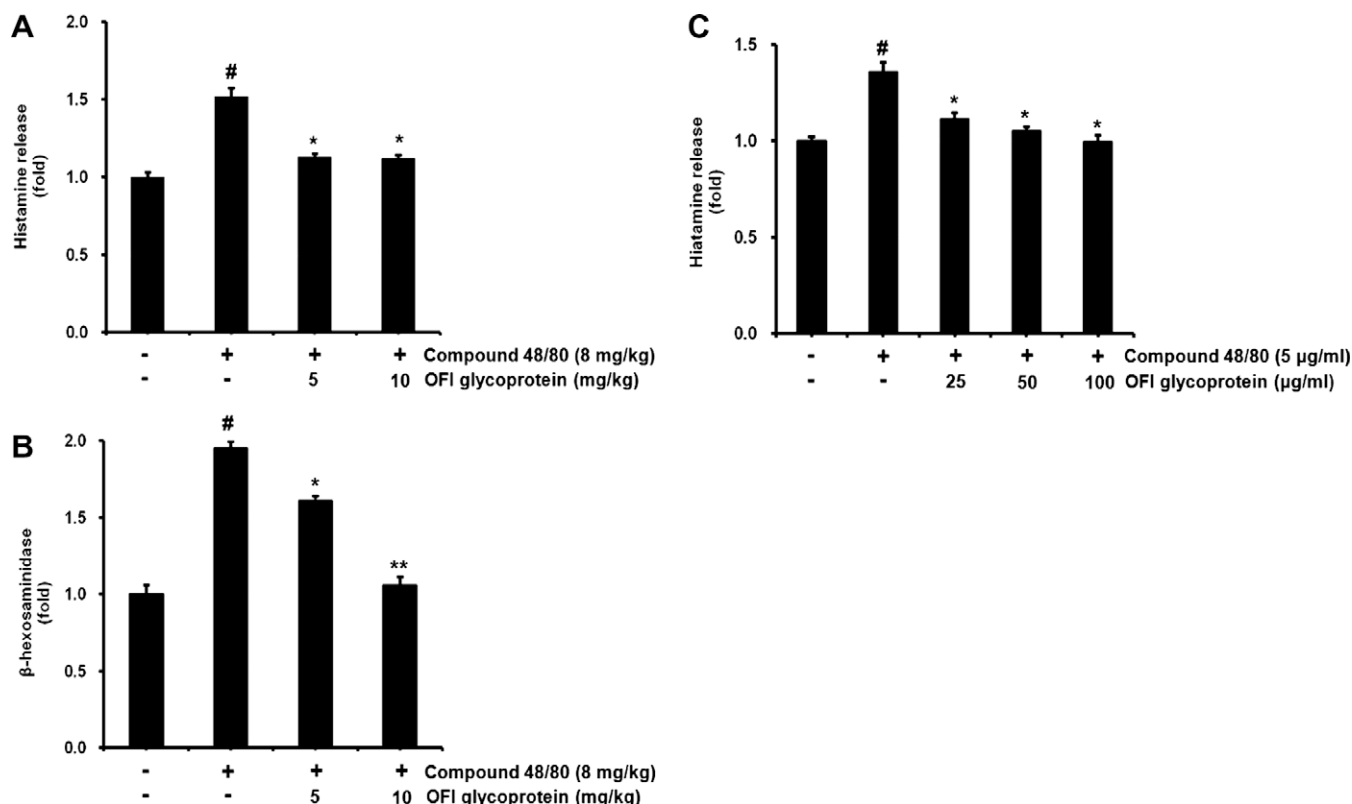


Fig. 2. Inhibitory effects of OFI glycoprotein on histamine release and β -hexosaminidase in the compound 48/80-treated in mice serum. Mice were treated with 8 mg/kg of compound 48/80 in the presence of the various concentrations of OFI glycoprotein (5, 10 mg/kg, BW) for 30 min. Release of histamine in ICR mice serum was measured at with the excitation set at 360 nm and the emission set at 440 nm (A). For β -hexosaminidase, the absorbance was measured with a microplate reader (Molecular Devices, Sunnyvale, CA) at 405 nm (B). RBL-2H3 cells were treated with 5 μ g/ml compound 48/80 in the presence of the various concentrations of OFI glycoprotein (25, 50 and 100 μ g/ml) for 30 min. Release of histamine in RBL-2H3 cells was measured at with the excitation set at 360 nm and the emission set at 440 nm (C). Each bar represents the means $\pm$ SE from triplicate separately. [#] represents a significant difference between compound 48/80 treatment alone and control, $P < 0.05$. * and ** represent a significant difference between compound 48/80 treatment alone and OFI glycoprotein treatment in the presence of compound 48/80, $P < 0.05$.

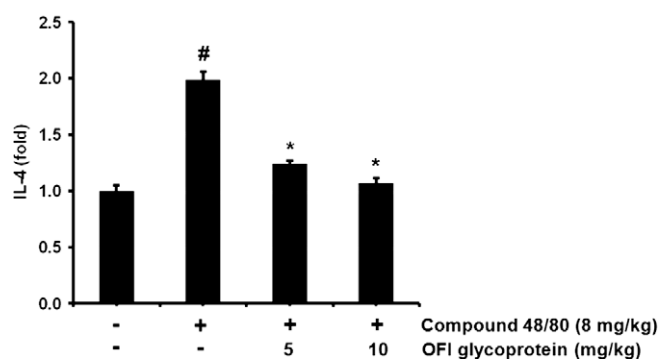


Fig. 3. Inhibitory effect of OFI glycoprotein on amount of IL-4 in the compound 48/80-treated in mice serum. Mice were treated with 8 mg/kg of compound 48/80 in the presence of the various concentrations of OFI glycoprotein (5 and 10 mg/kg, BW). Amount of IL-4 was measured at with the excitation set at 360 nm and the emission set at 440 nm. Each bar represents the means $\pm$ SE from triplicate separately. [#] represents a significant difference between compound 48/80 treatment alone and control, $P < 0.05$. * represents a significant difference between compound 48/80 treatment alone and OFI glycoprotein treatment in the presence of compound 48/80, $P < 0.05$.

3.5. Inhibitory effects of the OFI glycoprotein on phosphorylations of ERK1/2 MAPK in compound 48/80-stimulated RBL-2H3 cells

We further studied whether or not OFI glycoprotein has inhibitory effect on phosphorylation of ERK 1/2 in compound 48/80-stimulated in RBL-2H3 cells (Fig. 5). The results revealed that the phosphorylation of ERK 1/2 significantly increased 2.02-fold at

15 min after treatment with compound 48/80 (5 μ g/ml), compared to the control (Fig. 5, lane 2). To know inhibitory effect of OFI glycoprotein, RBL-2H3 cells were treated with compound 48/80 in the presence of OFI glycoprotein. For instance, when the cells were treated with compound 48/80 in presence of OFI glycoprotein (100 μ g/ml), the expression of phosphor-ERK1/2 decreased by 0.78-fold, compared to the compound 48/80 treatment alone (Fig. 5, lane 4), although total expressions of ERK 1/2 was not changed.

3.6. Inhibitory effect of OFI glycoprotein on activation of arachidonic acid and COX-2 in compound 48/80-stimulated RBL-2H3 cells

To examine the activities of arachidonic acid and COX-2 proteins, radio activity of [^{3} H] AA and Western blot analysis were carried out in whole cell protein extracts. As shown in Fig. 6, OFI glycoprotein inhibited activities of arachidonic acid and COX-2 in compound 48/80-stimulated RBL-2H3 cells. For instance, in cells that were treated with compound 48/80 (5 μ g/ml), the activity of arachidonic acid and COX-2 were markedly increased compared to the control. However, when the cells were treated with compound 48/80 in the presence of OFI glycoprotein (50 and 100 μ g/ml), such activity decreased significantly in a dose-dependent manner. For instance, the relative intensities of arachidonic acid and COX-2 were augmented 2.34- and 3.38-fold after treatment with 5 μ g/ml compound 48/80, compared to the control (Fig. 6). However, such activity was diminished 0.24- and 0.81-fold for arachidonic acid and COX-2 after the treatment with compound 48/80

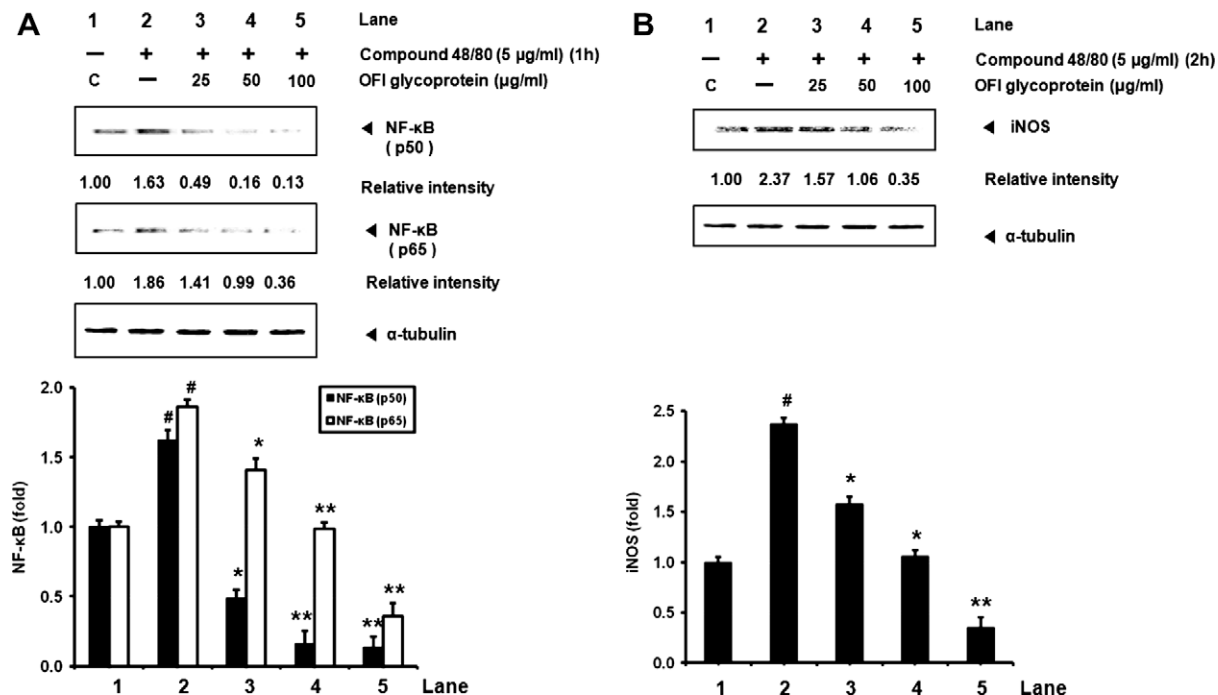


Fig. 4. Inhibitory effect of the OFI glycoprotein on expression of NF-κB and iNOS in the compound 48/80-stimulated RBL-2H3 cells. RBL-2H3 cells were pre-treated with OFI glycoprotein (25, 50, and 100 μg/ml) in the presence of compound 48/80 (5 μg/ml) and then it was evaluated the activities of NF-κB (A) and iNOS (B). Lane 1, cont.; lane 2, compound 48/80; lane 3, OFI glycoprotein (25 μg/ml) in the presence of compound 48/80; lane 4, OFI glycoprotein (50 μg/ml) in the presence of compound 48/80; lane 5, OFI glycoprotein (100 μg/ml) in the presence of compound 48/80. Each bar represents the means ± SE from triplicate separately. # represents a significant difference between compound 48/80 treatment alone and control, $P < 0.05$. * and ** represent a significant difference between compound 48/80 treatment alone and OFI glycoprotein treatment in the presence of compound 48/80, $P < 0.05$. C, control.

in the presence of OFI glycoprotein (100 μg/ml) separately, compared to compound 48/80 treatment alone (Fig. 6A and B).

4. Discussion

Allergy is an adaptive immune response directed against non-infectious environment agents called allergens, including some non-infectious components of certain infectious organisms. Allergic skin disorders, such as urticaria, angioedema, contact dermatitis and atopic dermatitis, are clinically important. Their pathogenesis varies from local to systemic effects depending upon contacts with specific allergens that elicit allergic reactions in the skin [35]. In allergic diseases, immediate-type hypersensitivity reactions are mediated by mast cells either in response to IgE-dependent or IgE-independent stimuli.

Compound 48/80 is a well-known activator of mast cells via phospholipase D and heterotrimeric GTP-binding proteins [36,37]. After activation with allergen and compound 48/80, mast cells and basophils can produce pro-inflammatory mediators, including certain cytokines. Cytokines and chemokines cause allergic diseases and other physiological effects. Also, compound 48/80 is a member of the family of polybasic mast cell secretagogues that are known to activate trimeric G proteins, primarily those of the Gi and Go categories [38,39]. In vitro, compound 48/80 stimulates only certain subtypes of mast cells, such as rat peritoneal mast cells, to induce the rapid release of inflammatory mediators. Rat mucosal mast cells and RBL-2H3 cells are not activated by the compound.

The results obtained from this study showed that OFI glycoprotein protects from mortality caused by compound 48/80 in BALB/c mice in a dose-dependent manner (Table 1). Considering the inhibitory effect of OFI glycoprotein on LDH release, we speculated that OFI glycoprotein is one of non-toxic substances that has chemopre-

ventive potential on immune disorder, because the high level of LDH associates with a number of different diseases and raised from any damage to cells in the mice blood. Degranulation of mast cells results in the release of various mediators, such as histamine and pro-inflammatory cytokines. Mast cells are derived from hematopoietic progenitor cells that enter nearly all vascularized tissues, where they complete their maturation. Therefore, the organ tissues targeted by allergens are particularly enriched in mast cells. As shown in Fig. 1, degranulation in RBL-2H3 cells was induced by compound 48/80, with a maximal response of approximately 17% for 15 min.

To further understand the inhibition mechanism associated with the OFI glycoprotein, the activities of allergy-related signals were evaluated using Western blot analysis. It has been postulated that RBL-2H3 cells participate in allergic diseases and inflammatory disorders, and that histamine release from mice serum or RBL-2H3 cells, and β-hexosaminidase assay have been widely used to assess the degranulation of mast cells. As shown Fig. 2, the results indicated that OFI glycoprotein suppresses histamine release and β-hexosaminidase (degranulation) in RBL-2H3 cells treated by compound 48/80 in a dose-dependent manner.

It has been reported that the mast cell-mediated allergy reaction is associated with an increase in Th2 cytokines and production of IL-4. IL-4 (one of the Th2 cytokines) promotes B cell differentiation to plasma cells that secrete IgE, and also contributes to the proliferation of mast cells. Interestingly, it has been reported that RBL-2H3 cells are useful cells for studying cytokine activation pathways. However, they cannot be expressed pro-inflammatory cytokines by compound 48/80 in the RBL-2H3 cells, specifically. The results from this study provide in vivo evidence that OFI glycoprotein effectively attenuates immediate-type allergic reactions in ICR mice and inhibits expression of IL-4 from mice serum (Fig. 3). Therefore, we suggest that OFI glycoprotein has the potential for anti-allergic effects that is be-

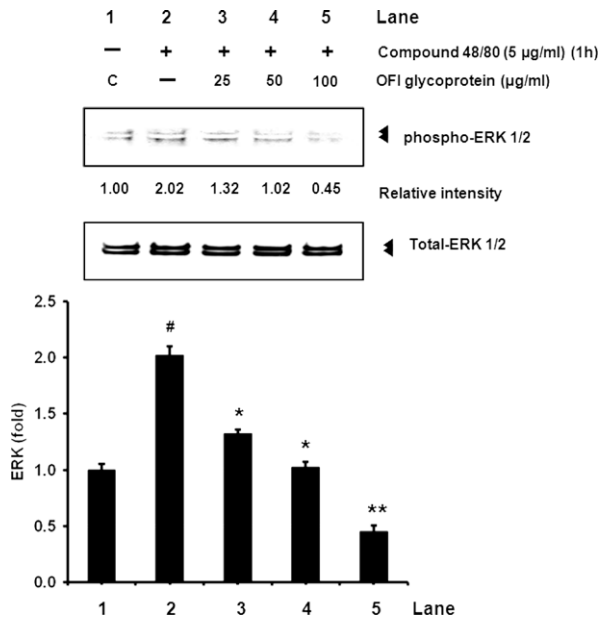


Fig. 5. Inhibitory effect of OFI glycoprotein on the phosphorylation of ERK1/2 in the compound 48/80-stimulated RBL-2H3 cells. RBL-2H3 cells were exposed to the compound 48/80 (5 µg/ml) for 30 min in the absence or presence of OFI glycoprotein (25, 50, and 100 µg/ml). Detection of ERK1/2 was performed by Western blotting using anti-ERK1/2 and -phospho-ERK1/2 polyclonal antibody. Lane 1, cont.; lane 2, compound 48/80; lane 3, OFI glycoprotein (25 µg/ml) in the presence of compound 48/80; lane 4, OFI glycoprotein (50 µg/ml) in the presence of compound 48/80; lane 5, OFI glycoprotein (100 µg/ml) in the presence of compound 48/80. The relative intensities of ERK1/2 bands was quantified using Scion Imaging Software (Scion Image Beta 4.02, Maryland, USA). Data were represented the means $\pm$ S.E from triplicate separately. # represents significant difference between compound 48/80 treatment alone and control, $P < 0.05$. * and ** indicate significant difference between compound 48/80 treatment alone and OFI glycoprotein treatments in the presence compound 48/80, $P < 0.05$. C, control.

lied to have advantages in pharmaceutical use for anti-allergic responses.

In the allergy-related signal proteins (NF- κ B, iNOS, ERK, and COX-2), NF- κ B is one of the transcription factors for the expression of inflammation-related genes including COX-2 and is frequently composed of a p50 and a p65 subunit. Under basal conditions, it is retained in the cytoplasm bound to an inhibitory subunit I κ B. During a response to inflammatory stimulators, the p65 subunit dissociates from the I κ B subunit and translocates from the cytoplasm to the nucleus, where it dimerizes with the p50 subunit and interacts with specific target genes, such as COX-2 leading to increased inflammatory processes. Because of its central role in regulating inflammatory responses, a pharmacological inhibition of NF- κ B activation could be beneficial in the treatment of inflammation. The free radical NO, which is produced by iNOS during conversion of L-arginine to L-citrulline is considered to be associated with occurrences of the pro-inflammatory events. The results in this study indicated that OFI glycoprotein inhibited expression of NF- κ B (P50 and P65) and iNOS (Fig. 4). MAPKs (ERK, JNK, and p38) regulate the expression of many immune- and inflammatory-related genes and are critical factors of the important signaling pathways in immune response. The expression of inflammatory cytokines is regulated by MAPKs which are divided into three types, such as p38, extracellular signal-regulated kinase (ERK), and c-jun N-terminal kinase (JNK). In particular, ERK is known to be necessary to express pro-inflammatory cytokines. Recent studies have suggested that the NF- κ B activation needs to activate p38 MAPK in RBL-2H3 cells [40,41]. The results in this study indicated that OFI glycoprotein also inhibited expression of ERK (Fig. 5).

The availability of arachidonic acid (AA) liberated from membrane phospholipid (PL) is rate-limiting for the synthesis of the 2-series of prostaglandin (PGs). *n*-3 PUFAs such as eicosapentaenoic acid (20:5*n*-3, EPA) and docosahexaenoic acid (22:6*n*-3, DHA) may inhibit AA-dependent PG-production by directing *n*-3

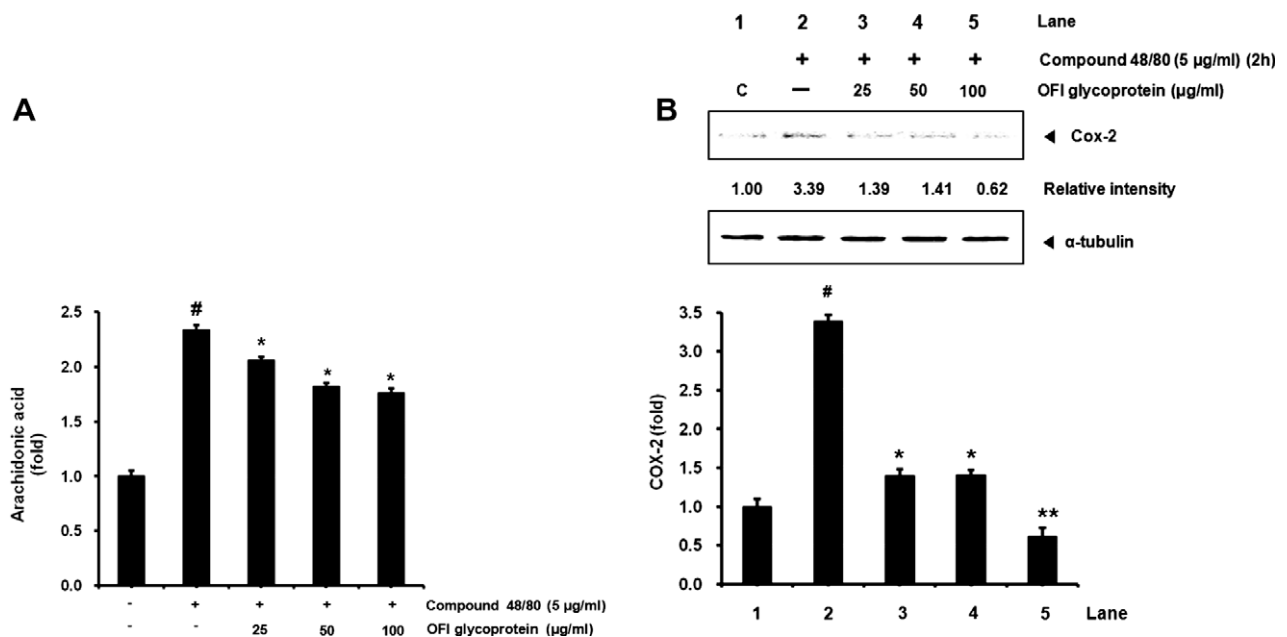


Fig. 6. Inhibitory effects of OFI glycoprotein on expression of arachidonic acid and COX-2 in the compound 48/80-stimulated RBL-2H3 cells. RBL-2H3 cells were pre-treated with OFI glycoprotein (25, 50, and 100 µg/ml) in the presence of compound 48/80 (5 µg/ml) and then it was evaluated expression of arachidonic acid (A) and COX-2 (B). Lane 1, cont.; lane 2, compound 48/80; lane 3, OFI glycoprotein (25 µg/ml) in the presence of compound 48/80; lane 4, OFI glycoprotein (50 µg/ml) in the presence of compound 48/80; lane 5, OFI glycoprotein (100 µg/ml) in the presence of compound 48/80. # represents a significant difference between compound 48/80 treatment alone and control, $P < 0.05$. * and ** represent a significant difference between compound 48/80 treatment alone and OFI glycoprotein treatment in the presence of compound 48/80, $P < 0.05$. C, control.

PUFA into cell membrane phospholipids with a concomitant inhibition of phospholipase A₂ (PLA₂) rendering less AA available as a substrate for COXs. With respect to the COX-2 expression, our finding indicated that the OFI glycoprotein has an inhibitory effect on the expression of COX-2. Generally, COX exists as two isoforms (constitutive COX-1 and inducible COX-2) and COX-2 relates to inflammation and pain. Specifically, COX-2 is barely detectable under normal physiological conditions, but is rapidly and transiently induced by pro-inflammatory mediators. Also, COX-2 is an important factor for anaphylactic reactions induced by compound 48/80. Activation of arachidonic acid and COX-2 was occurred after treatment with compound 48/80 in the RBL-2H3 cells. However, their activities were diminished after treatment with OFI glycoprotein in the presence of compound 48/80 (Fig. 6).

In conclusion, our results showed that the OFI glycoprotein (5 mg/kg) inhibited histamine and β -hexosaminidase release, lactate dehydrogenase (LDH), and interleukin 4 (IL-4) in the compound 48/80-treated ICR mice serum. Also, OFI glycoprotein (25 μ g/ml) has suppressive effects on the expression of MAPK (ERK1/2), and on protein expression of anti-allergic proteins (iNOS and COX-2) in the compound 48/80-treated RBL-2H3 cells. However, it still remains to elucidate its mechanism of action and active components of OFI glycoprotein on the cell membrane. The results obtained from this study suggest that it will give an insight into the prevention or treatment of mast cell-dependent allergic diseases.

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References

- [1] B. Wutrich, Epidemiology of the allergic diseases: are they really on the increase?, *Int Arch. Allergy Appl. Immunol.* 90 (1989) 3–10.
- [2] R.L. Stevens, K.F. Austen, Recent advances in the cellular, molecular biology of mast cells, *Immunol. Today* 10 (1989) 381–386.
- [3] M.K. Church, F. Levi-Schaffer, The human mast cell, *J. Allergy Clin. Immunol.* 99 (1997) 155–160.
- [4] S.J. Galli, J. Kalesnikoff, M.A. Grimaldeston, A.M. Piliponsky, C.M. Williams, M. Tsai, Mast cells as “tunable” effector and immunoregulatory cells: recent advances, *Ann. Rev. Immunol.* 23 (2005) 749–786.
- [5] I. Miyajima, D. Dombrowicz, T.R. Martin, J.V. Ravetch, J.P. Kinet, S.J. Galli, Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and Fc gammaRIII. Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis, *J. Clin. Invest.* 99 (1997) 901–914.
- [6] S.J. Galli, J.R. Gordon, B.K. Wershil, Cytokine production by mast cells and basophils, *Curr. Opin. Immunol.* 3 (1991) 865–872.
- [7] L.J. Petersen, H. Mosbech, P.S. Skov, Petersen, H. Mosbech, Allergen-induced histamine release in intact human skin in vivo assessed by skin microdialysis technique: characterization of factors influencing histamine releasability, *J. Allergy Clin. Immunol.* 97 (1996) 672–679.
- [8] D. Lagunoff, T.W. Martin, G. Read, Agents that release histamine from mast cells, *Annu. Rev. Pharmacol. Toxicol.* 23 (1983) 331–351.
- [9] T.Y. Shin, S.H. Kim, K. Suk, J.H. Ha, I. Kim, M.G. Lee, C.D. Jun, S.Y. Kim, J.P. Lim, J.S. Eun, H.Y. Shin, H.M. Kim, Anti-allergic effects of *Lycopus lucidus* on mast cell-mediated allergy model, *Toxicol. Appl. Pharmacol.* 209 (2005) 255–262.
- [10] S.H. Kim, S. Lee, I.K. Kim, T.K. Kwon, J.Y. Moon, W.H. Park, T.Y. Shin, Suppression of mast cell-mediated allergic reaction by *Amonum xanthioides*, *Food Chem. Toxicol.* 45 (2007) 2138–2144.
- [11] S.H. Kim, C.H. Choi, S.Y. Kim, J.S. Eun, T.Y. Shin, Anti-allergic effects of *Artemisia iwayomogi* on mast cell-mediated allergy model, *Exp. Biol. Med.* 230 (2005) 82–88.
- [12] M.R. Allansmith, R.S. Baird, R.N. Ross, N.P. Barney, K.J. Bloch, Ocular anaphylaxis induced in the rat by topical application of compound 48/80. Dose response and time course study, *Acta Ophthalmol. Suppl.* 192 (1989) 145–153.
- [13] L.B. Schwartz, R.A. Lewis, D. Seldin, K.F. Austen, Acid hydrolases and tryptase from secretory granules of dispersed human lung mast cells, *J. Immunol.* 126 (1981) 1290–1294.
- [14] D.L. Marquardt, S.I. Wasserman, Modulation of rat serosal mast cell biochemistry by in vivo dexamethasone administration, *J. Immunol.* 131 (1983) 934–939.
- [15] J.R. Sheller, V.V. Polosukhin, D. Mitchell, D.S. Cheng, R.S. Peebles, T.S. Blackwell, Nuclear factor kappa B induction in airway epithelium increases lung inflammation in allergen-challenged mice, *Exp. Lung Res.* 35 (2009) 883–895.
- [16] M.J. May, S. Ghosh, Signal transduction through NF-kappa B, *Immunol. Today* 19 (1998) 80–88.
- [17] S. Arbabi, R.V. Maier, Mitogen-activated protein kinases, *Crit. Care. Med.* 30 (2002) S74–S79.
- [18] B. Kaminska, MAPK signalling pathways as molecular targets for anti-inflammatory therapy—from molecular mechanisms to therapeutic benefits, *Biochim. Biophys. Acta.* 1754 (2005) 253–262.
- [19] J.M. Kyriakis, J. Avruch, Sounding the alarm: protein kinase cascades activated by stress and inflammation, *J. Biol. Chem.* 271 (1996) 24313–24316.
- [20] H. Yamamoto, N. Atsuchi, H. Tanaka, W. Ogawa, M. Abe, A. Takeshita, H. Ueno, Separate roles for H-Ras and Rac in signaling by transforming growth factor (TGF)-beta. H-Ras is essential for activation of MAP kinase, partially required for transcriptional activation by TGF-beta, but not required for signaling of growth suppression by TGF-beta, *Eur. J. Biochem.* 264 (1) (1999) 110–119.
- [21] B. Hinz, K. Brune, Cyclooxygenase-2—10 years later, *J. Pharmacol. Exp. Ther.* 300 (2002) 367–375.
- [22] Y.W. Hsu, K.H. Chi, W.C. Huang, W.W. Lin, Ceramide inhibits lipopolysaccharide-mediated nitric oxide synthase and cyclooxygenase-2 induction in macrophages: effects on protein kinases and transcription factors, *J. Immunol.* 166 (2001) 5388–5397.
- [23] T.E. Hansen, J.B. Jorgensen, Cloning and characterisation of p38 MAP kinase from Atlantic salmon A kinase important for regulating salmon TNF-2 and IL-1beta expression, *Mol. Immunol.* 44 (2007) 3137–3146.
- [24] Y.N. Han, Y. Choo, Y.C. Lee, Y.I. Moon, S.D. Kim, J.W. Choi, Monoamine oxidase B inhibitors from the fruits of *Opuntia ficus-indica* var. *saboten*, *Arch. Pharm. Res.* 24 (2001) 51–54.
- [25] S.H. Kim, S.Y. Baik, K.H. Jung, M.R. Choi, B.H. Yang, J.S. Lee, D.Y. Oh, I.G. Choi, H. Chung, Y.G. Chai, Fluoxetine-induced up-regulation of 14-3-3zeta and tryptophan hydroxylase levels in RBL-2H3 cells, *Neurosci. Lett.* 374 (2005) 53–57.
- [26] C.M. Tsang, C.K. Wong, W.K. Ip, C.W. Lam, Synergistic effect of SCF and TNF-alpha on the up-regulation of cell-surface expression of ICAM-1 on human leukemic mast cell line (HMC)-1 cells, *J. Leukoc. Biol.* 78 (2005) 239–247.
- [27] B.K. Wershil, Y.A. Mekori, T. Murakami, S.J. Galli, ¹²⁵I-fibrin deposition in IgE-dependent immediate hypersensitivity reactions in mouse skin. Demonstration of the role of mast cells using genetically mast cell-deficient mice locally reconstituted with cultured mast cells, *J. Immunol.* 139 (1987) 2605–2614.
- [28] M. Ennis, G. Atkinson, F.L. Pearce, Inhibition of histamine release induced by compound 48/80 and peptide 401 in the presence and absence of calcium. Implications for the mode of action of anti-allergic compounds, *Agents Actions* 10 (1980) 222–228.
- [29] P.S. Oh, S.J. Lee, K.T. Lim, 90 kDa glycoprotein isolated from *Opuntia ficus-indica* var. *Saboten* Makino has an antioxidative activity, *Food Sci. Biotech.* 13 (2004) 781–789.
- [30] P.A. Shore, A. Brukhalter, V.H. Cohn Jr., A method for the fluorometric assay of histamine in tissues, *J. Pharmacol. Exp. Ther.* 127 (1959) 182–186.
- [31] S. Lim, J.W. Choi, H.S. Kim, Y.H. Kim, K. Yea, K. Heo, J.H. Kim, S.H. Kim, M. Song, J.I. Kim, S.H. Ryu, P.G. Suh, A myristoylated pseudosubstrate peptide of PKC-zeta induces degranulation in HMC-1 cells independently of PKC-zeta activity, *Life Sci.* 82 (2008) 733–740.
- [32] P.S. Oh, S.J. Lee, K.T. Lim, Glycoprotein isolated from *Rhus verniciflua* Stokes inhibits inflammation-related protein and nitric oxide production in LPS-stimulated RAW 264.7 cells, *Biol. Pharm. Bull.* 30 (2007) 111–116.
- [33] O.H. Lowry, N.J. Rosebrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, *J. Biol. Chem.* 193 (1951) 265–275.
- [34] M. Xing, B.L. Firestein, G.H. Shen, P.A. Insel, Dual role of protein kinase C in the regulation of cPLA2-mediated arachidonic acid release by P2U receptors in MDCK-D1 cells: involvement of MAP kinase-dependent and -independent pathways, *J. Clin. Invest.* 99 (1997) 805–814.
- [35] S.C. Incorvaia, C. Pravettoni, M. Mauro, M.R. Yacoub, F. Tarantini, G.G. Riarior-Sforza, Effectiveness of omalizumab in a patient with severe asthma and atopic dermatitis, *Monaldi Arch. Chest Dis.* 69 (2008) 78–80.
- [36] J. Senyshyn, R.A. Baumgartner, M.A. Beaven, Quercetin sensitizes RBL-2H3 cells to polyanionic mast cell secretagogues through increased expression of Gi GTP-binding proteins linked to a phospholipase C signaling pathway, *J. Immunol.* 160 (1998) 5136–5144.
- [37] A. Chahdi, P.F. Fraundorfer, M.A. Beaven, Compound 48/80 activates mast cell phospholipase D via heterotrimeric GTP-binding proteins, *J. Pharmacol. Exp. Ther.* 292 (2000) 122–130.
- [38] M. Mousli, J.L. Bueb, C. Bronner, B. Rouot, Y. Landry, G protein activation: a receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides, *Trends Pharmacol. Sci.* 11 (1990) 358–362.
- [39] A. Chahdi, L. Daeflfer, J.P. Gies, Y. Landry, Drugs interacting with G protein alpha subunits: selectivity and perspectives, *Fundam. Clin. Pharmacol.* 12 (1998) 121–132.
- [40] C.K. Wong, C.M. Tsang, W.K. Ip, C.W. Lam, Molecular mechanisms for the release of chemokines from human leukemic mast cell line (HMC)-1 cells activated by SCF and TNF-alpha: Roles of ERK, p38 MAPK, And NF-kappaB, *Allergy* 61 (2006) 289–297.
- [41] Y.D. Min, C.H. Choi, H. Bark, H.Y. Son, H.H. Park, S. Lee, J.W. Park, E.K. Park, H.I. Shin, S.H. Kim, Quercetin inhibits expression of inflammatory cytokines through attenuation of NF-kappaB and p38 MAPK in HMC-1 human mast cell line, *Inflamm. Res.* 56 (2007) 210–215.