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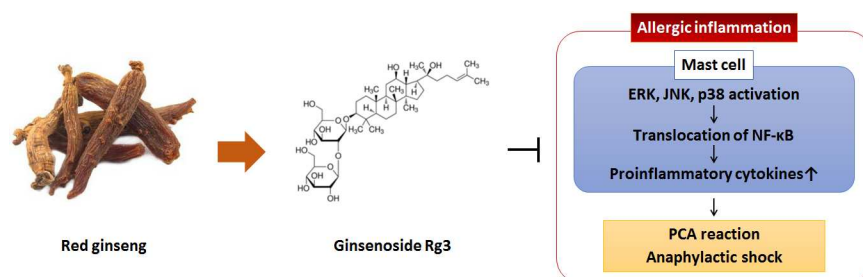
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Ginsenoside Rg3 suppresses mast cell-mediated allergic inflammation via MAPK signaling pathway

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Running title: Rg3 inhibits allergic inflammation via MAPK

ABSTRACT

Background: Ginsenoside Rg3 (G-Rg3) is the major bioactive ingredient of *Panax ginseng* and has many pharmacological effects including anti-adipogenic, anti-viral, and anti-cancer effects. However, the effect of G-Rg3 on mast cell-mediated allergic inflammation has not been investigated.

Method: The anti-allergic effects of G-Rg3 on allergic inflammation were evaluated using the human and rat mast cell lines HMC-1 and RBL-2H3. Anti-allergic effects of G-Rg3 were detected by measuring cAMP, detecting calcium influx, real-time reverse transcription-PCR, enzyme-linked immunosorbent assay, western blotting, and *in vivo* experiments.

Results: G-Rg3 decreased histamine release from activated mast cells by enhancing cAMP levels and calcium influx. Proinflammatory cytokine production was suppressed by G-Rg3 treatment via regulation of the mitogen-activated protein kinases/nuclear factor kappa B and RIP2/caspase-1 signaling pathway in mast cells. Moreover, G-Rg3 protected mice against the IgE-mediated passive cutaneous anaphylaxis reaction as well as compound 48/80-induced anaphylactic shock.

Conclusion: G-Rg3 may serve as an alternative therapeutic agent for improving allergic inflammatory disorders.

Key words: Ginsenoside Rg3, Allergic inflammation, Mast cells, Mitogen-activated protein kinase, Nuclear factor- κ B

1. Introduction

Mast cells are the main immune effector cells involved in allergic responses [1]. They circulate in the blood as progenitors and are involved in allergic diseases including asthma, atopic dermatitis, and eczema as initiators. During allergic responses, they trigger degranulation by various stimuli and release several bioactive substances, such as histamine and proinflammatory cytokines, including tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , and IL-6. These mediators promote inflammatory progression through the migration and activation of immune effector cells [2]. Therefore, inhibition of degranulation and proinflammatory cytokines release may be an efficient therapeutic strategy for allergic inflammatory disorders.

The mitogen-activated protein kinase (MAPK) signaling pathway regulates a wide range of cellular processes. This signaling pathway is involved in the activation, differentiation, and survival of immune cells. Extracellular signal-regulated kinase (ERK), c-jun N-terminal kinase (JNK), and p38 are included in the MAPK pathway [3]. In allergic responses, MAPKs signaling is associated with nuclear factor-kappa B (NF- κ B) activation. NF- κ B controls the transcription of proinflammatory cytokine genes, and the secretion of these cytokines has been observed in the progression of allergic responses [4,5].

The cysteine protease caspase-1 is related to the production of proinflammatory cytokines in allergic inflammation [6]. Caspase-1 activation induces proinflammatory cytokine production via NF- κ B translocation to the nucleus [7]. Particularly, caspase-1 can convert pro-forms of IL-1 β into mature form via the IL-1 β -converting enzyme [8]. RIP2 binds to the caspase-1 prodomain and subsequently regulates the activation of caspase-1. RIP2 depletion decreases the release of pro-inflammatory cytokines such as TNF- α and IL-6 [9]. Thus, blockage of RIP2/caspase-1 activation reduces the severity of inflammatory

diseases.

Ginsenosides are active pharmaceutical constituents isolated from *Panax ginseng*. Among the approximately 40 types of ginsenosides, ginsenoside Rg3 (G-Rg3) is derived from red ginseng. Red ginseng is a type of root prepared by steaming and drying [10]. Red ginseng extract improves skin lesions in atopic dermatitis models by inhibiting chemokine and proinflammatory cytokine expression in mast cells and keratinocytes [11]. G-Rg3 has shown positive therapeutic effects on cancer, diabetes mellitus, viral diseases, and inflammation [12-15]. Particularly, G-Rg3 inhibits oxazolone-induced contact dermatitis by decreasing the expression of lipopolysaccharide (LPS)-induced cyclooxygenase (COX)-2 and cytokines in macrophages [16]. This compound also ameliorates 2,4,6-trinitrochlorobenzene-induced skin lesions by inhibiting the infiltration of mast cells and expression of proinflammatory cytokines [17]. However, the effects and mechanisms of G-Rg3 on allergic inflammation in activated mast cells is unclear.

In this study, we examined the effects of G-Rg3 on allergic inflammation using HMC-1 and RBL-2H3 mast cells. The inhibitory effect of G-Rg3 on degranulation of mast cells and its related mechanisms were confirmed in HMC-1 and RBL-2H3 cells. Systemic anaphylactic shock and the PCA reaction after G-Rg3 administration were conducted to confirm the anti-allergic effect of G-Rg3.

2. Materials and methods

2.1. Antibodies and reagents

Prednisolone (PDN) and antibodies against phospho-ERK, phospho-JNK, phospho-p38, I κ B α , and phospho-NF- κ B, were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-ERK, p38, JNK, α -tubulin, Lamin B, RIP2, and caspase-1 antibodies were

purchased from Santa Cruz Biotechnology (Dallas, TX, USA). G-Rg3 (CAS no. 14197-60-5, purify: 95–99%) was purchased from Chengdu Biopurify Phytochemical Ltd. (Chengdu, Sichuan, China). WST reagent (EZ-cytox) was purchased from DoGen (Seoul, Korea). Fluo-4 AM was purchased from Thermo Fisher Scientific (Waltham, MA, USA). MTT, Compound 48/80, Evans blue, DNP-IgE, DNP-BSA, phorbol 12-myristate 13-acetate (PMA), and A23187 were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Cell culture

The human mast cell line (HMC-1) and rat basophilic leukemia mast cell line RBL-2H3 were maintained in Iscove's Modified Dulbecco's Medium and Dulbecco's Modified Eagle's Medium, respectively. Fetal bovine serum (FBS) and penicillin-streptomycin were added to the medium and the cells were maintained in a cell culture incubator.

2.3. Cell viability

The viability of G-Rg3-treated mast cells was quantified by the MTT and WST assays. After 24 h incubation with G-Rg3, MTT and WST reagents were added to HMC-1 and RBL-2H3 cells, respectively. The absorbance after MTT and WST treatment were measured at 540 and 405 nm.

2.4. Measurement of histamine

Histamine release was measured by the histamine enzyme-linked immunosorbent assay kit according to the manufacturer's protocols, (ALPCO, Salem, NH, USA).

2.5. cAMP and intracellular calcium (Ca^{2+}) levels

To measure cAMP levels, a cAMP direct immunoassay kit (BioVision, Inc., Milpitas, CA, USA) was employed. Cells (5×10^4 cells/well) were added to a 24-well plate and treated with G-Rg3 at the indicated times. After treatment, the cells were harvested, and cAMP levels were measured according to the manufacturer's protocol.

Intracellular Ca^{2+} levels were detected with a fluorescence microscope. Cells were cultured in an 8-well chamber slide and incubated with Fluo-4 AM (5 μM) for 30 min. G-Rg3 was used to treat the cells for 30 min and stimulator was treated to slide. The results were visualized with a fluorescence microscope (Observer A1 microscope, Carl Zeiss, Oberkochen, Germany).

2.6. Measurement of proinflammatory cytokines

An OptEIA ELISA kit (BD Pharmingen, San Jose, CA, USA) was used to detect proinflammatory cytokines. Briefly, the capture antibody was diluted and pre-coated in 96-well plates at 4°C overnight. 5% FBS in phosphate-buffered saline was added to the plate for blocking for 1 h. The plate was washed with 0.05% phosphate-buffered saline containing Tween 20, and then samples and cytokine standards were added. After 3 h of incubation, biotinylated detection antibodies plus streptavidin-horseradish peroxidase solution were added and incubated for 1 h. Absorbance was measured within 30 min after stop solution treatment.

2.7. Real-time RT-PCR

Total RNA extraction and cDNA synthesis were carried out with QIAzol lysis reagent

(Qiagen, Hilden, Germany) and the High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer instructions. SYBR[®] Green Realtime PCR Master Mix (Toyobo, Osaka, Japan) and Step-One plus[™] Real-Time PCR Systems (Applied Biosystems) were used for PCR. Table 1 shows the sequences of the primers used for real-time reverse transcription (RT)-PCR.

2.8. Western blotting

Cells were lysed and cell lysates were used to protein quantification. An NE-PER Nuclear and Cytoplasmic Extraction kit was used to prepare the nuclear extracts. Proteins were separated using electrophoresis and transferred to membranes and detected using primary antibodies. Specific primary antibodies were captured using secondary antibodies and detected using the FluorChem M system (ProteinSimple, San Jose, CA, USA).

2.9. Systemic anaphylactic shock

All *in vivo* experiments were carried out with ICR mice (5 weeks, male) and approved by the Wonkwang University Institutional Animal Care and Use Committee (WKU16-12). Mice were purchased from Samtaco Korea (Osan, Korea) and housed in a ventilated cage system. To establish the systemic anaphylactic model, compound 48/80 (10 mg/kg) was intraperitoneally injected into the mice ($n = 6$). G-Rg3 (10, 25, and 50 mg/kg) was mixed in 0.5% carboxymethylcellulose (CMC) solution, while control mice were administered a 0.5% CMC solution. G-Rg3 and 0.5% CMC solution were used to treat the mice by oral administration 1 h prior to injecting compound 48/80.

2.10. Passive cutaneous anaphylaxis (PCA)

To induce the PCA reaction, back hair was removed using hair clippers before the experiments. DNP-IgE (10 µg/spot) was intradermally injected into mice 24 h prior to antigen injection. G-Rg3 was orally administered 1 h before antigen challenge. DNP-BSA (1 µg/mL) containing 1% Evans blue was injected into the mouse tail vein. Mice were sacrificed, and stained back skin was incubated in formaldehyde overnight. Dissolved blue dye was measured using a microplate reader at 620 nm.

2.11. Statistical analysis

The obtained data were expressed as the mean $\pm$ SD from a minimum of three independent experiments. The results were analyzed by Student's *t*-test using the SPSS statistical program (SPSS, Inc., Chicago, IL, USA). Differences with $p < 0.05$ were regarded as statistically significant.

3. Results

3.1. Effects of ginsenoside Rg3 on histamine release

As shown in Figs. 1A and 1B, the maximum treatment of 50 µg/mL of G-Rg3 did not change the viability of either cell line. During degranulation of mast cells, inflammatory mediators are released and cause allergic reactions. Among inflammatory mediators, histamine is an important protein involved in allergic reactions [18]. Because G-Rg3 reduces β -hexosaminidase secretion from activated RBL-2H3 cells [16], we predicted that G-Rg3 can suppress the release of histamine from mast cells. G-Rg3 (2–50 µg/mL) dose-dependently decreased histamine release from activated HMC-1 and RBL-2H3 cells (Figs. 1C and 1D).

3.2. Effects of G-Rg3 on intracellular cAMP and Ca^{2+} levels in mast cells

During the degranulation of mast cells, the cAMP concentration and Ca^{2+} level affect histamine release [19]. Blocking Ca^{2+} influx and increasing cAMP concentration are effective strategies for treating allergic diseases. Intracellular cAMP and Ca^{2+} levels were measured using a cAMP ELISA kit and Fluo 4-AM, respectively. The intracellular cAMP concentration was increased at 5 min and remained at an increased level until 15 min by G-Rg3 treatment in HMC-1 and RBL-2H3 cells (Figs. 2A and 2B). Ca^{2+} influx into mast cells was elevated by PMA+A23187 or DNP-BSA stimulation, whereas G-Rg3 (10 and 50 $\mu\text{g/mL}$) reduced intracellular Ca^{2+} concentrations (Figs. 2C and 2D). Thus, G-Rg3 may inhibit histamine release by blocking Ca^{2+} influx and enhancing intracellular cAMP content.

3.3. Effects of G-Rg3 on the production of proinflammatory cytokines in mast cells

In response to various stimulation pathogens, mast cells release proinflammatory cytokines, which cause allergic and inflammatory diseases [1]. Thus, inhibition of proinflammatory cytokine secretion may assist in ameliorating allergic diseases. In this study, we investigated whether G-Rg3 modulates the secretion of proinflammatory cytokines in HMC-1 and RBL-2H3 cells. IL-1 β , IL-6, and TNF- α mRNA expression levels were decreased by G-Rg3 treatment (Figs. 3A and 3E). In addition, G-Rg3 significantly suppressed the release of these cytokines in PMA+A23187-stimulated HMC-1 cells (Figs. 3B–D). Similarly, G-Rg3 treatment decreased the production of these cytokines in IgE-sensitized and antigen-stimulated RBL-2H3 cells (Figs. 3F–H).

3.4. Effects of G-Rg3 on MAPK and NF- κB activation in mast cells

The MAPK-NF- κ B signaling pathway is crucial for regulating inflammatory responses, as proinflammatory cytokine production is induced by allergen stimulus [3-5]. Thus, the effect of G-Rg3 on the phosphorylation of MAPKs including ERK, JNK, and p38 was evaluated to determine which molecule is regulated by G-Rg3 in activated mast cells. G-Rg3 dose-dependently blocked ERK, JNK, and p38 activation (Fig. 4). Moreover, degradation of I κ B α and NF- κ B nuclear translocation were suppressed by G-Rg3 in activated-mast cells (Fig. 5).

3.5. Effect of G-Rg3 on RIP2/caspase-1 activation in mast cells

RIP2 interacts with caspase-1 and subsequently induces NF- κ B activation. Activated caspase-1 induces cleavage of pro-IL-1 β , which is a proinflammatory cytokine IL-1 β precursor [20]. Active IL-1 β promotes allergic disorders including asthma, atopic dermatitis, and contact hypersensitivity [21]. To determine the effect of G-Rg3 on RIP2/caspase-1 activation in mast cells, protein expression levels of RIP2 and caspase-1 were determined by western blotting. G-Rg3 dose-dependently suppressed RIP2 activation and cleavage of caspase-1 (Figs. 6A–D). In addition, the effects of G-Rg3 on antigen-induced caspase-1 activation were confirmed. As shown in Figs. 6E and 6F, G-Rg3 treatment also significantly reduced caspase-1 activity. These data demonstrate that blockage of the MAPK/NF- κ B and RIP2/caspase-1 signaling pathways by G-Rg3 treatment suppressed proinflammatory cytokine release.

3.6. Effect of G-Rg3 on compound 48/80-induced anaphylactic shock and IgE-mediated PCA reaction

Injection of DNP-IgE and antigen-induced PCA is a suitable *in vivo* model of local

allergic reaction [22]. Oral and intraperitoneal administration of G-Rg3 (25 mg/kg) to mice inhibits the IgE-antigen complex-induced PCA reaction [16]. However, the dose-response effects of G-Rg3 on the antigen-induced PCA reaction are unclear. Compound 48/80 is used as a reagent to trigger a fatal allergic reaction by activating mast cells [23]. In this study, each mouse was administered 0.5% CMC solution as a control or G-Rg3 by oral administration 2 h prior to compound 48/80 injection. As shown in Table 2, the mortality rate of the 50 mg/kg G-Rg3 group was 22.5%, while those of the 10 and 25 mg/kg G-Rg3 groups were 60% and 30%, respectively.

We investigated the dose-dependent effect of G-Rg3 (10–50 mg/kg) on the PCA reaction. The inhibition rate of the 10 mg/kg G-Rg3 group was 17.7%, 25 mg/kg G-Rg3 group was 22.4%, and 50 mg/kg G-Rg3 group was 40.5% (Fig. 7). These results demonstrate that compound 48/80 and antigen-induced allergic reactions are dose-dependently decreased by G-Rg3 treatment.

4. Discussion

Mast cells are involved in allergic reactions through the release of various inflammatory mediators [24]. HMC-1 cells show similar characteristics to human mast cells which were established from leukemia patients [25]. The rat basophilic leukemia cell line RBL-2H3 was isolated from Wistar rat basophilic cells. This cell line is a proper model for studying cellular events in mast cell activation, as it displays characteristics of mucosal-type mast cells [26]. Thus, these cell lines are commonly used in inflammation, allergy, and immunological research.

Mast cell degranulation is triggered by increasing intracellular calcium concentration. Increased calcium influx subsequently activates calcium-dependent processes for the

degranulation of mast cells. Calcium ionophores can activate mast cells to induce degranulation through high-affinity immunoglobulin E receptor-mediated signaling and transporting extracellular calcium into the cytosol [27]. In contrast, degranulation of mast cells is inhibited by increasing intracellular cAMP levels [28]. Therefore, the regulation of Ca^{2+} influx and intracellular cAMP concentration in mast cells plays an important role in degranulation. G-Rg3 treatment increased intracellular cAMP levels within 15 min and decreased antigen-induced calcium influx in mast cells (Fig. 2).

During inflammatory and allergic responses, mast cells activated by allergens release several inflammatory mediators such as histamine, pro-inflammatory cytokines, and chemokines. Ginsenoside Rb1, Re, and Rh1 treatment significantly decreased histamine release from activated-mast cells [29-31]. Additionally, G-Rg5 and G-Rh3 suppressed degranulation of mast cells by decreasing histamine, $\text{TNF-}\alpha$, and IL-6 in RBL-2H3 cells [32]. Moreover, G-Rg3 inhibits the release of $\text{TNF-}\alpha$, IL-1 β , and IL-6 in LPS-stimulated neutrophils and macrophages in bronchoalveolar lavage fluids [33]. Our results showed that the release of pro-inflammatory cytokines including IL-1 β , IL-6, and $\text{TNF-}\alpha$ in mast cells was decreased by G-Rg3 treatment (Fig. 3).

The activation of MAPKs, including ERK, JNK, and p38, is related to mast cell degranulation and subsequent cytokine production [34]. NF- κ B is a major transcription factor that is a critical mediator of the inflammatory reaction. MAPKs activated by various stimuli degrade I κ B proteins; the separated NF- κ B is translocated into the nucleus, and its target genes are transcribed [4]. RIP2/caspase-1 activation is also related to the NF- κ B signaling pathway and production of pro-inflammatory cytokines. It has been reported that Korean red ginseng extract suppresses proinflammatory cytokine release by inhibiting MAPK/NF- κ B activation [11]. Active compounds in Korean red ginseng including G-Re inhibited activation

of p38 MAPK, ERK, and JNK in the LPS-induced lung inflammation model [35].

Additionally, G-Rg3 inhibits RANKL-induced osteoclast differentiation and epithelial-mesenchymal transition of lung cancer cells via MAPK/NF- κ B signaling pathways [12,36].

In this study, we investigated the anti-allergic effects of G-Rg3 on mast cells and in a mouse model using maximum doses of 50 μ g/mL and 50 mg/kg G-Rg3, respectively. It has been reported that a G-Rg3 concentration of 50 μ g/mL is reached in 1–2 h after oral administration of 50 mg/kg G-Rg3 in normal rats [37]. Additionally, oral administration of 50 mg/kg of G-Rg3 showed hepatoprotective effects in *tert*-butyl hydroperoxide-induced liver injury mice [38]. Thus, we investigated the anti-allergic effects using 2–50 μ g/mL in an *in vitro* experiment and 10–50 mg/kg of Rg3 in the *in vivo* model.

Prednisolone, a corticosteroid drug used to treat inflammatory diseases, was used as a positive control in this study. Prednisolone suppresses LPS-induced TNF- α , IL-6, IL-8, and MCP-1 release in the serum and IL-1 β -induced COX-2 expression via the ERK, JNK, and AP-1 signaling pathway in HEI-OC1 murine auditory cells [39,40]. Particularly, prednisolone significantly decreased 2,4-dinitrofluorobenzene-induced scratching behavior and the edematous reaction in IgE-sensitized mice [41]. Although prednisolone showed the greatest effects in the experimental groups, G-Rg3 also significantly suppressed anaphylactic shock and the PCA reaction (Table 2 and Fig. 7).

In conclusion, this study demonstrated that G-Rg3 inhibits mast cell-mediated allergic responses by blocking degranulation. G-Rg3 decreases histamine and proinflammatory cytokine release via the MAPK/NF- κ B and RIP2/caspase-1 pathway. Compound 48/80-induced anaphylactic shock and the IgE-mediated PCA reaction were suppressed by G-Rg3 administration in the mouse model. Thus, G-Rg3 is a potential agent for treating allergic diseases.

298

299 **Conflicts of Interest**

300 The authors have no conflicts of interest to declare.

301

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Table 1

Sequences of the real-time RT-PCR primers

Genes	Forward (5'-3')	Reverse (5'-3')
IL-1 β (h)	TACCTGTCCTGCGTGTGAA	TDTTTGGGTAATTTTGGGATCT
IL-6 (h)	GATGAGTACAAAAGTCCTGATCCA	CTGCAGCCACTGGTTCTGT
TNF- α (h)	CGCTCCCCAAGAAGACAG	AGAGGCTGAGGAACAAGCAC
β -actin (h)	CCAACCGCGAGAAGATGA	CCAGAGGCGTACAGGGATAG
IL-1 β (m)	CACAGCAGCACATCAACAAG	GTGCTCATGTCCTCATCCTG
IL-6 (m)	CTCTGGGAAATCGTGGAAAT	CCAGTTTGGTAGCATCCATC
TNF- α (m)	ATGAGAAGTTCCCAAATGGC	CTCCACTTGGTGGTTTGCTA
GAPGH (m)	GACATGCCGCCTGGAGAAAC	AGCCCAGGATGCCCTTTAGT

Table 2

Effect of ginsenoside Rg3 (G-Rg3) on compound 48/80-induced anaphylactic shock

G-Rg3 (mg/kg) ^a	Compound 48/80 (10 mg/kg)	Mortality (%) ^b
0	-	0
0	+	100
10	+	66.7*
25	+	33.3*
50	+	27.7*
Prednisolone (3 mg/kg)	+	22.1*

^a The groups of mice ($n = 6$) were orally administrated with 0.05% carboxymethylcellulose solution or G-Rg3 1 h prior to injection of compound 48/80.

^b Mortality (%) is presented as the 'number of dead mice $\times$ 100/total number of experimental mice'. This result was represented from three independent experiments.

* $p < 0.05$; significantly different from control group.

Figure Legends

Fig. 1. Ginsenoside Rg3 (G-Rg3) inhibited histamine production from activated mast cells. (A and B) HMC-1 and RBL-2H3 cells (1×10^4 cells/well) were treated with G-Rg3 (2-50 $\mu\text{g/mL}$) for 24 h and cell viability was determined by MTT and WST assays. (C and D) The production level of histamine was measured using the ELISA kit. (C) HMC-1 cells (2×10^5 cells/well) were pretreated with G-Rg3 and PDN (10 μM) for 30 min and stimulated with PMA (50 nM) + A23187 (1 μM) for 24 h. (D) DNP-IgE (100 ng/mL) sensitized RBL-2H3 cells (2×10^5 cells/well) were pretreated with G-Rg3 and PDN (10 μM) for 30 min and stimulated with DNP-BSA (10 $\mu\text{g/mL}$) for 24 h. Results are the mean $\pm$ SD. # $p < 0.05$ vs the blank; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ vs stimulator-treated group.

Fig. 2. Ginsenoside Rg3 (G-Rg3) increased the concentration of cAMP levels and blocked calcium influx into mast cells. (A and B) cAMP levels were measured using a colorimetric cAMP ELISA kit. HMC-1 (A) and RBL-2H3 (B) cells were treated with G-Rg3 (50 $\mu\text{g/mL}$) at the indicated time. (C and D) Intracellular calcium levels were detected by fluorescence microscopy (magnification x200). HMC-1 (C) and IgE-sensitized RBL-2H3 (D) cells were treated with G-Rg3 and PDN (10 μM) 30 min prior to stimulation using PMA+A23187 or DNP-BSA for 5 min. Results are the mean $\pm$ SD.

Fig. 3. Ginsenoside Rg3 (G-Rg3) decreased the production of proinflammatory cytokines IL- 1β , IL-6, and TNF- α in activated mast cells. (A–D) HMC-1 cells (5×10^5 cells/well) were treated with G-Rg3 (2–50 $\mu\text{g/mL}$) and PDN (10 μM) for 30 min and stimulated with PMA+A23187 for 24 h. (A) mRNA expression of proinflammatory cytokines were analyzed

by real-time RT-PCR. (B-D) Release of proinflammatory cytokines IL-1 β (B), IL-6 (C), and TNF- α (D) was measured by ELISA. (E-H) DNP-IgE (100 ng/mL) sensitized RBL-2H3 cells (5×10^5 cells/well) were treated with G-Rg3 (2–50 μ g/mL) and PDN (10 μ M) for 30 min and stimulated with DNP-BSA (10 μ g/mL) for 24 h. (E) mRNA expression of proinflammatory cytokines was analyzed by real-time RT-PCR. (F-H) Production of proinflammatory cytokines IL-1 β (F), IL-6 (G), and TNF- α (H). Results are the mean $\pm$ SD. # p < 0.05 vs the control group; * p < 0.05, ** p < 0.01 and *** p < 0.001 vs stimulator-treated group.

Fig. 4. Ginsenoside Rg3 (G-Rg3) inhibited phosphorylation of MAPKs in activated mast cells. Phosphorylation of ERK, JNK, and p38 was detected by western blotting. (A and B) HMC-1 cells (1×10^6 cells/well) were incubated with G-Rg3 and PDN (10 μ M) for 30 min and stimulated with PMA (50 nM) + A23187 (1 μ M) for 30 min. (C and D) DNP-IgE (100 ng/mL) sensitized RBL-2H3 cells (1×10^6 cells/well) were incubated with G-Rg3 and PDN (10 μ M) for 30 min and stimulated with DNP-BSA (10 μ g/mL) for 30 min. Relative levels of MAPKs (B and D) were calculated using the Image J program (NIH, Bethesda, MD, USA). Results are the mean $\pm$ SD. # p < 0.05 vs the control group; * p < 0.05 vs stimulator-treated group.

Fig. 5. Ginsenoside Rg3 (G-Rg3) inhibited the degradation of I κ B α and NF- κ B translocation in activated mast cells. Cytosol I κ B α and nuclear NF- κ B levels were detected by western blotting. (A and B) HMC-1 cells (1×10^6 cells/well) were pretreated with G-Rg3 and PDN (10 μ M) for 30 min and PMA (50 nM) + A23187 (1 μ M) was added to the cells for 2 h. (C and D) DNP-IgE (100 ng/mL) sensitized RBL-2H3 cells (1×10^6 cells/well) were incubated with G-Rg3 and PDN (10 μ M) for 30 min and stimulated with DNP-BSA (10 μ g/mL) for 2 h.

α -Tubulin and Lamin B were used as loading controls. Relative levels of I κ B α and NF- κ B (B and D) were calculated using an Image J program. Results are the mean $\pm$ SD. C, cytosol extracts; N, nuclear extracts. # p < 0.05 vs the control group; * p < 0.05 vs stimulator-treated group.

Fig. 6. Ginsenoside Rg3 (G-Rg3) suppressed the activation of RIP-2 and caspase-1 in activated mast cells. HMC-1 cells (1×10^6 cells/well) were incubated with G-Rg3 and PDN (10 μ M) for 30 min and stimulated with PMA (50 nM) + A23187 (1 μ M) for 1 h. DNP-IgE (100 ng/mL) sensitized RBL-2H3 cells (1×10^6 cells/well) were incubated with G-Rg3 and PDN (10 μ M) for 30 min and stimulated with DNP-BSA (10 μ g/mL) for 1 h. (A and C) The protein levels of RIP2 and caspase-1 in HMC-1 (A) and RBL-2H3 (C) cells. α -Tubulin was used as a loading control. (B and D) Quantification of band intensities were determined using Image J program. (E and F) The activity of caspase-1 in HMC-1 (E) and RBL-2H3 (F) cells was measured with a caspase-1 assay kit. Results are the mean $\pm$ SD. # p < 0.05 vs the control group; * p < 0.05 vs stimulator-treated group.

Fig. 7. Ginsenoside Rg3 (G-Rg3) inhibited the IgE-mediated PCA reaction. Anti-DNP IgE (0.5 μ g) was injected into the mouse dorsal skin 24 h before antigen challenge. DNP-BSA (1 μ g) containing 1% Evans blue was injected by intravascular injection into the mouse tail vein (n = 6). G-Rg3, PDN (3 mg/kg) or saline were administered 1 h prior to the challenge with DNP-BSA. Dorsal skins were excised and blue dye was dissolved in formaldehyde. Colorimetric changes were measured by microplate reader at 620 nm. Results are the mean $\pm$ SD. * p < 0.05.

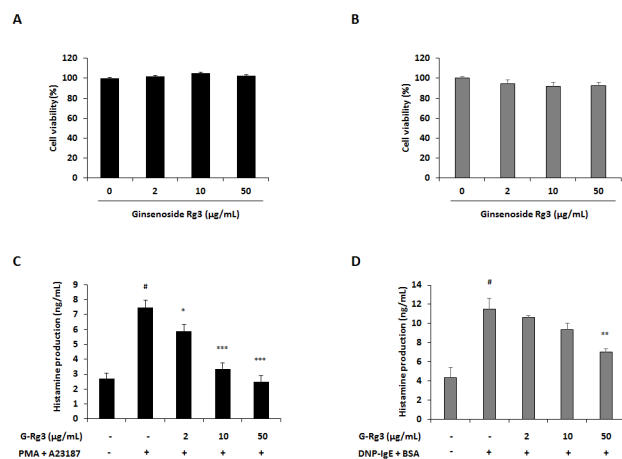


Fig. 1

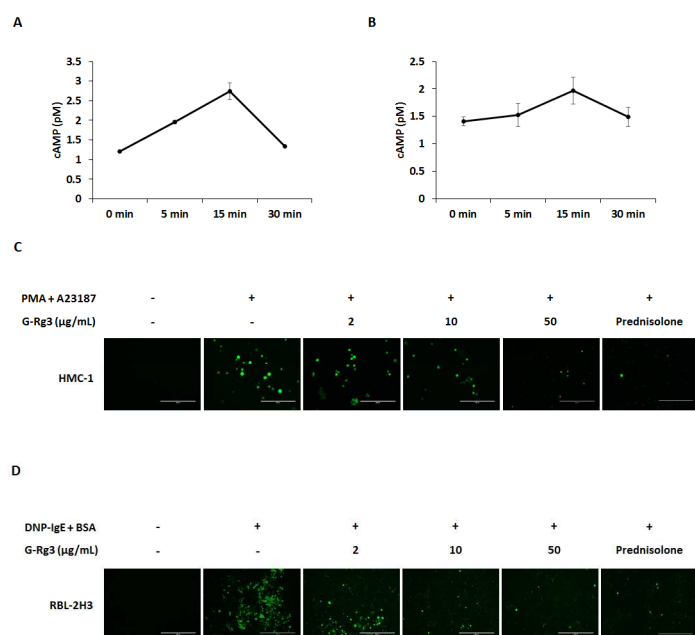


Fig. 2

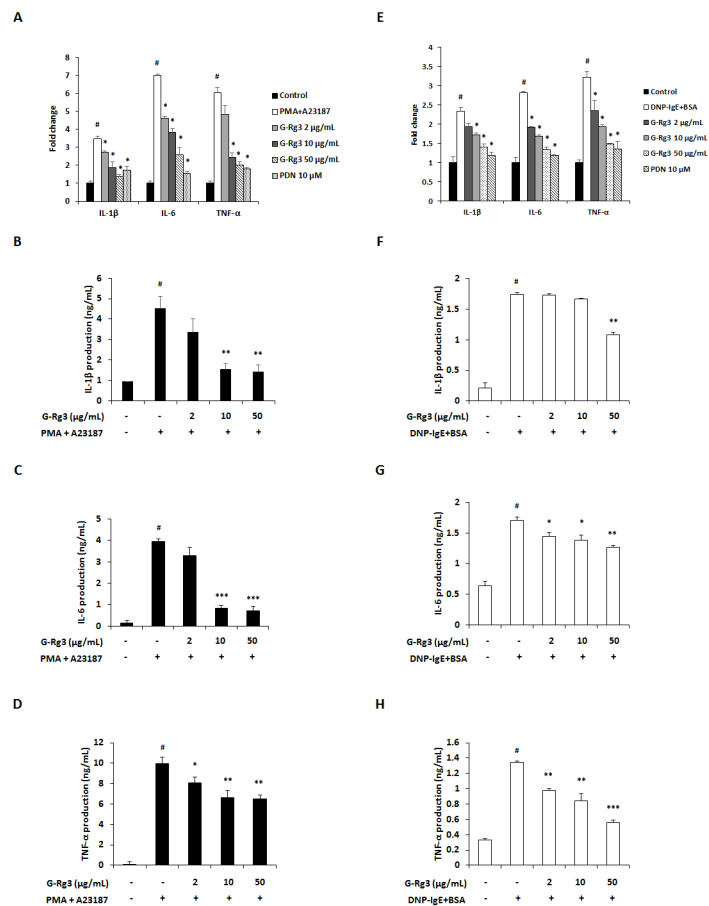


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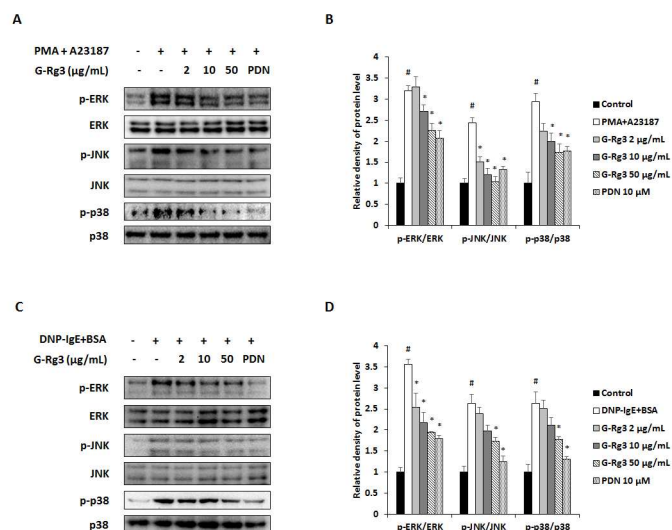


Fig. 4

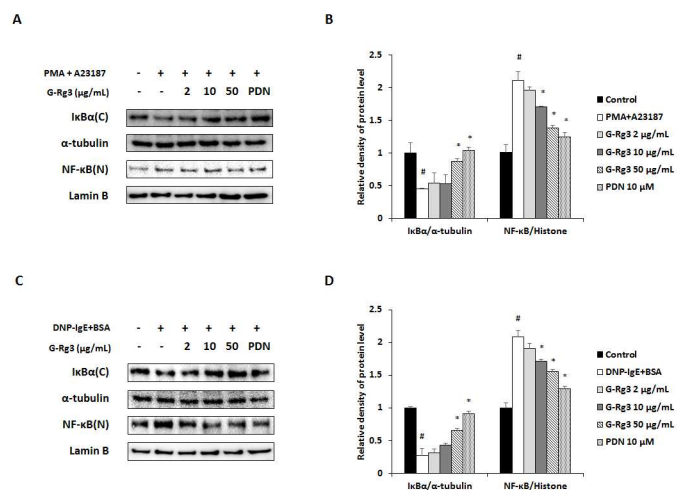


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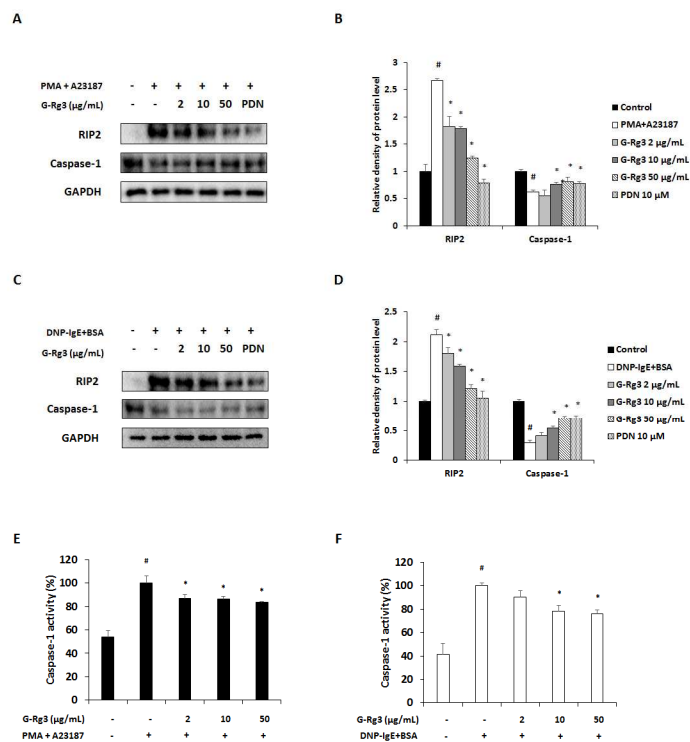


Fig. 6

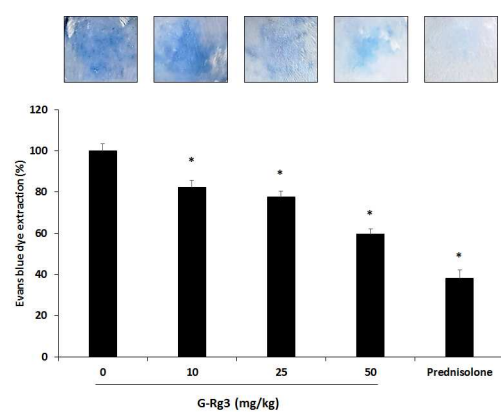


Fig. 7