International Immunopharmacology 76 (2019) 105867

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Magnolin inhibits IgE/Ag-induced allergy *in vivo* and *in vitro*

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ARTICLE INFO

Keywords:
Magnolin
Anti-anaphylactic
Mast cells
Calcium activation
Degranulation

ABSTRACT

Mast cells (MCs) play critical roles in allergic reactions and modulating the activation of MCs could be an effective strategy to treat allergic diseases, which cause a rapidly increasing threat to the public health. Herein, we described that Magnolin, a major component from Flos magnoliae could inhibit IgE-dependent MCs activation. We found Magnolin inhibited IgE/Ag-induced calcium mobilization, degranulation, and cytokines release in LAD2 cells. Magnolin was also found to attenuate IgE/Ag-induced mice paw swelling in a dose-dependent manner. Further mechanistic studies suggested a possible anti-allergic and anti-inflammatory effects of Magnolin in IgE/Ag-induced anaphylactic reactions. Thereby, Magnolin could be a potential therapeutic agent for preventing mast cell-related immediate and delayed allergic diseases.

1. Introduction

In recent decades, the population of patients with allergic disease induced by Type I hypersensitive reactions has rapidly increased [1]. Type I hypersensitive reactions were IgE-induced allergic response which could cause severe systemic damage [2]. IgE-induced allergic diseases include eczema, allergic rhinitis, asthma and so forth [3]. Mast cells (MCs) play a key role in IgE/Ag-mediated allergic reaction [4,5]. It is well established now that MCs trigger immediate type I allergic reactions in response to allergens by releasing chemical mediators [6]. Degranulation of MCs can be caused by extracellular ATP [7], a non-immunologic secretagogue like substance P [8], compound 48/80 and many other stimuli which result in a rapid releasing of marked histamine (HA) [9].

The secretory response of MCs can be induced by aggregation of high-affinity receptors for IgE (FcεRI) by the corresponding antigen [10–12]. In MCs, FcεRI is a tetrameric complex of four protein subunits [13–15]. When allergen binds to serum IgE attached to their FceRI receptors that activates MCs, then intracellular allergic signaling is initiated. The Lyn tyrosine kinase which is the main SRC-family kinase and involved in these initial stages can activate PLCγ1 [16,17]. PLCγ1 associated with phosphorylated LAT, and hydrolyzes PIP2 to generate IP3 and triggers the release of Ca^{2+} from the ER, which leads to the opening of the plasma membrane (PM)-bound calcium channels and influx of extracellular Ca^{2+} into the cytoplasm [18,19]. Fyn and some other signaling proteins (p38, ERK), which involved in Ca^{2+} influx, degranulation, actin rearrangement, chemotaxis, and/or gene transcription, are also indicated [20]. Then they release neutral proteases, cytokines, leukotrienes, HA and chemokines [21,22]. Because of these, MC-activation is now widely regarded as a critical step in different IgE/Ag-mediated immune responses, and a potential immunoregulatory roles in various immune disorders [23,24]. Currently, anti-histamines, steroids, small-molecule inhibitors and mast cell stabilizers are used to treat allergic diseases, but some compounds showed different levels of adverse effects and can only relieve symptoms of allergic diseases [25–28].

Natural compounds or traditional medicinal can be used as anti-allergic and anti-inflammatory medicinal with reduced adverse effects [25]. Magnolin (Fig. 1), a major component in Flos magnoliae, has been used to treat inflammation, nasal congestion and allergic rhinitis. However, the efficacy and mechanism of Magnolin in inhibiting IgE/Ag-mediated allergic reaction hasn't been well studied.

This study was to evaluate the inhibitory effect of Magnolin on IgE/Ag-mediated allergy both *in vitro* and *in vivo*. We tried to elucidate the functional targets of Magnolin in various processes. For *in vitro* experiments, we used human mast cells Laboratory of Allergic Disease 2 (LAD2) to elucidate the function of Magnolin on inhibiting IgE/Ag-induced MCs activation by receding calcium mobilization, degranulation, and other steps involved in triggering anaphylactic reactions. Then a murine model of IgE/Ag-induced allergy was used to further evaluate the anti-allergic effects of Magnolin *in vivo* we used. Those findings highlight the therapeutic potential of Magnolin in the treatment of IgE/

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https://doi.org/10.1016/j.intimp.2019.105867
Received 25 June 2019; Received in revised form 27 August 2019; Accepted 27 August 2019
Available online 11 September 2019

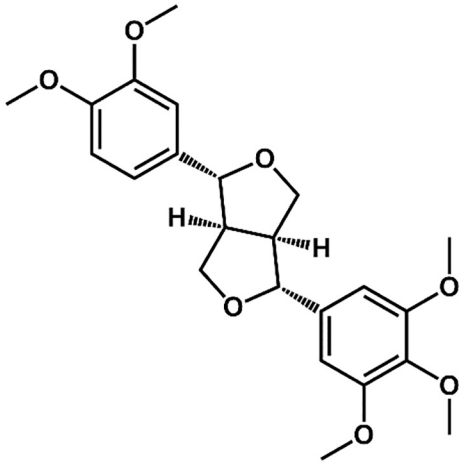

Fig. 1. The molecular structure of Magnolin.

Ag-induced allergy diseases.

2. Materials and methods

2.1. Drugs and reagents

Magnolin (purified to ≥98%) (Lot.16072501) was supplied by Baoji Chenguang biological Co., Ltd. (Baoji, China). Anti-DNP-specific IgE, DNP-HSA, FITC-avidin, *p*-nitrophenyl *N*-acetyl-β-D–glucosamide, Evans blue, and HA were purchased from Sigma-Aldrich (St. Louis, MO, USA). L-glutamine was purchased from BOMEI. (Hefei, China). Pentobarbital sodium was supplied by XIYA (Shandong, China). Triton X-100 was supplied by Qingdao Meigao Chemical Co., Ltd. (Qingdao, China). The Human TNF-α ELISA Kit were supplied by ExCell Biology, Inc. (Shanghai, China). A Mouse TPS (Tryptase) ELISA Kit and Mouse CMA1 (Chymase 1, Mast Cell) ELISA Kit were purchased from Elabscience Biotechnology Co., Ltd. (Wuhan, China). Fluo-3 was supplied by Thermo Fisher Scientific (Waltham, MA, USA). Pluronic F-127 was supplied by Biotium (Fremont, CA, USA).

2.2. Mouse models

BALB/c (wild-type [WT]) mice (18-22 g) were supplied by the Experimental Animal Center of Xi'an Jiao tong University (Xi'an, China). They were provided food and water *ad libitum* in cages individually in a large colony room. All experiments in the animals with identical treatment administration were conducted by technicians blinded to the conditions.

2.3. Ethics statement

The study was obeyed in strict accordance with the recommendations stated in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. The protocols for the animal experiment were approved by the Animal Ethics Committee at Xi'an Jiao tong University, Xi'an, China (Permit Number: XJTU 2011-0045).

2.4. Cell lines

LAD2 cells were kindly provided by A. Kirshenbaum and D. Metcalfe from NIH (MD, USA). Cells were maintained in StemPro-34 medium supplemented with StemPro nutrient supplement, 100 U of penicillin streptomycin, 2 mM L-glutamine and 100 ng/mL human stem cell factor in a 37 °C incubator with 5% CO_2. It is necessary to replace Culture medium every 7 days, and maintain the density cells at 2×10^6 cells/mL.

2.5. Cytotoxicity assay

5×10^3 LAD2 cells per well were seeded in 96-well plate and the repetitive wells were set as 6. The experiments were repeated thrice. An abbkine-Cell Counting Kit assay (California, USA) was used to determine the Cytotoxicity assay Cell viability. Annexin-V-FITC/PI Staining Kit from 7Sea Pharmatech Co., Ltd. to was used to evaluate apoptosis. LAD2 cells were seeded into 6-well plates at a density of 5×10^7 cells/mL, and treated with different concentrations of Magnolin (0, 25, 50, 100, 200 and 400 μM) for 24 h. Then apoptosis was evaluated with Accuri™ C6 Plus Flow Cytometer. All the steps were finished followed the instructions strictly.

2.6. Hindpaw swelling assay

8–10 weeks adult BALB/c male mice (18-22 g) were injected intravenously with 0.2 mL DNP-IgE (2 μg/mL) through caudal vein. 24 h later, they were gavaged with Magnolin (0, 5, 10, and 20 mg/kg). After 1 h, 2 μg/mL DNP-HSA in saline was injected into the left paw and saline only into the right paw as a blank control. After 15 min, paw thickness was measured again and recorded. Mice were then killed by CO_2, and a photo of each paw was taken. Paw tissues were collected, dried for 24 h at 50 °C, and weighed. Evans blue was extracted by a 12 h incubation in 500 μL acetone-saline (7:3) at 37 °C. Tissues were cut into pieces, placed for 10 min in an ultrasonic machine, centrifuged for 20 min at 3000 rpm. The supernatant was equally distributed in 200 μL aliquots into 96 well cell culture plates, and the OD was read at 620 nm using a spectrophotometer. For studies using drug substances, mice were gavaged with saline as a blank control.

2.7. β-Hexosaminidase and HA release assay

After LAD2 cells (2.5×10^5 cells/mL) were seeded in 96-well plate and sensitized with 2 μg/mL of anti-DNP-IgE for 24 h, the sensitized cells were washed with Tyrode's buffer (120 mM NaCl, 4.7 mM KCl, 10 mM HEPES, 1.2 mM MgSO4, 2.5 mM $CaCl_2$, 5.5 mM glucose, 1.2 mM KH_2PO4, 5 mM BSA), and were pretreated with Magnolin (100, 200, 400 μM) for 20 min. Then Magnolin was added at indicated concentrations with DNP-HSA (2 μg/mL) to the wells for 1 h at 37 °C. DNP-HSA was used as a control, and. Tyrode's buffer used as a vehicle. The activity of β-hexosaminidase released into the supernatants and in cell lysates was quantified by hydrolysis of *p*-nitrophenyl-β-D-glucosamide.

For histamine analysis, histamine·2HCl was used as an interior label. An LC-MS 8040 mass spectrometer (Shimadzu Corporation, Kyoto, Japan) was used applying the LC-ESI-MS/MS method. Histamine was evaluated with the system by employing an HILIC column (Venusil HILIC, 2.1 mm × 150 mm, 3 μm, Agela Technologies, Tianjin, China) and an isocratic elution with acetonitrile-water containing 0.1% formic acid and 20 mM ammonium formate (77:23, v/v) at a flow rate of 0.3 mL/min.

2.8. Intracellular Ca^{2+} mobilization assay

LAD2 cells were washed once with calcium imaging buffer (CIB; 125 mM NaCl, 3 mM KCl, 2.5 mM $CaCl_2$, 0.6 mM $MgCl_2$, 10 mM HEPES, 20 mM glucose, 1.2 mM $NaHCO_3$, 20 mM sucrose, pH 7.4) after sensitized with 2 μg/mL of DNP-IgE for 24 h. Magnolin was diluted to different concentration. Then cells were incubated for 30 min with incubation buffer (3.5 μM Fluo-3 AM, 0.1% (w/v) F-127, diluted with CIB) at 37 °C. Then the cells were washed twice in CIB, and imaged at excitation wavelengths of 488 nm. CIB was used as vehicle. Responses were monitored at 1-s intervals for 120 s additionally. Inverted fluorescent microscope (Nikon, Ti-U, Japan) was used in this experiment.

2.9. Cytokine, chemokine and protease levels assay

After cells were incubated with 2 μg/mL anti-DNP-IgE for 24 h, 1×10^5 LAD2 cells per well were seeded in 96-well plate and the repetitive wells were set as 6. The experiments were repeated thrice. Cells were further incubated with Magnolin (100, 200, 400 μM) with DNP-HSA (2 μg/mL) for 6 h. Tumor necrosis factor (TNF-α), monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8) in cell supernatant and mice serum were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kit.

2.10. Skin avidin stain assay and H&E stain assay in the mice

The paw skin was prepared using the same method as in the hindpaw swelling and extravasation assay. Then the paw skin was isolated, washed with PBS, and fixed with 4% paraformaldehyde at 4 °C for overnight. The tissues were prepared for skin avidin stain assay and H&E stain assay. The skin for avidin stain assay was same as previously described [29]. Skin tissues were detected by the immune-histochemical SP method. Before immunostaining, skin sections were dewaxed in xylene, rehydrated through decreasing concentrations of ethanol, washed in PBS and then stained with hematoxylin and eosin (H&E). After staining, sections were dehydrated through increasing concentrations of ethanol and xylene. Images were taken with the inverted fluorescent microscope (Nikon, Ti-U, Japan).

2.11. Western blot analysis

After incubated with 2 μg/mL DNP-IgE for 24 h, total proteins from the untreated and treated with Magnolin (100, 200 and 400 μM) and DNP-HAS LAD2 cells (24 h) were extracted in ice cold condition. The method was reported previously [28]. Primary antibodies were used as follow: anti-Lyn (1:1000, #2796, Cell Signaling Technology [CST]), anti-phosphorylated-Lyn (1:1000, #2731, CST), anti-Fyn (1:1000, #4023, CST), anti-phosphorylated-Fyn (1:1000, Phospho-Src Family (Tyr416), #6943, CST), anti-PLCγ1 (1:1000, #5690, Cell Signaling Technology CST), anti-phosphorylated-PLCγ1 (P-PLCγ1, Ser1248) (1:1000,#8713, CST), anti-P38 (1:1000, #8690, CST), anti-phosphorylated-P38 (P-P38, Thr180/Tyr182) (1:1000, #4511, CST), anti-Akt (1:1000, #4691, CST), anti-phosphorylated-Akt (P-Akt, Ser473), (1:1000, #4060, CST), anti-ERK1/2 (1:1000, p44/42 MAPK (ERK1/2), #9102, CST), anti-phosphorylated-ERK1/2 (P-ERK1/2, P-p44/42 MAPK (ERK1/2) [Thr202/Tyr204], 1:1000, #9101, CST) or anti-GAPDH (1:2000, #2118, CST) antibodies overnight at 4 °C anti-GAPDH (1:2000, #2118, CST).

2.12. Statistical analysis

The group data are expressed as mean ± S.E.M from at least three independent experiments. To determine the significance of statistical comparisons with SPSS software, independent sample variance analysis was used. Differences were considered significant at $p < 0.05$ ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

3. Results

3.1. Magnolin showed low cytotoxicity on LAD2

The potential cytotoxicity of Magnolin was evaluated with Cell Counting kit assay, and the effect of apoptosis of LAD2 cells was evaluated with Annexin-V-FITC/PI Staining Kit. Magnolin was tested in concentrations range from 25 to 400 μM. Magnolin showed low cytotoxicity in LAD2 cells and didn't induce apoptosis of LAD2 cells even at 400 μM. These results showed that Magnolin didn't influence the viability of LAD2 cells significantly (Fig. 2). Thus, we can use the concentrations of Magnolin under 400 μM in subsequent *in vitro* experiments.

3.2. Magnolin attenuated IgE/Ag-induced calcium flux in LAD2 cells

IgE/Ag-induced Type I hypersensitive reactions activate the MCs by Ca^{2+} mobilization. The effect of Magnolin in Ca^{2+} flux of IgE/Ag-induced LAD2 cells was then evaluated. Cell images were applied to demonstrate whether Magnolin affects Ca^{2+} flux. DNP-HSA can mobilize Ca^{2+} in IgE/Ag-induced LAD2 cells (Fig. 3A) compared to cells treated with vehicle only (Fig. 3E). Magnolin (100, 200 and 400 μM) induced a dose-dependent decrease in the IgE/Ag-induced calcium flux in LAD2 cells (Fig. 3B-D).

3.3. Magnolin decreased IgE/Ag-induced degranulation in LAD2 cells

Rapid secretion of preformed inflammatory mediators such as β-hexosaminidase, HA, synthesized cytokines and chemokine including TNF-α, MCP-1 and IL-8 resulted from the activation of MCs [17,21,22]. Thus, the levels of secreted β-hexosaminidase, HA and TNF-α were measured to evaluate the inhibitory effect of Magnolin on inflammatory mediators releasing from MCs. Compared to the group treated only with 2 μg/mL DNP-HSA, LAD2 cells treated with DNP-HSA and Magnolin (100, 200 and 400 μM) showed a decrease in the secretion of β-hexosaminidase and HA in a dose-dependent manner (Fig. 4A-B). Additionally, the levels of TNF-α, MCP-1 and IL-8 were measured. Compared to the group treated with 2 μg/mL DNP-HSA alone, although 100 μM Magnolin didn't inhibit the release of TNF-α, MCP-1 and IL-8200 μM and 400 μM Magnolin significantly inhibited the secretion of them induced by DNP-HSA (Fig. 4C-E). The results showed that Magnolin could inhibit the degranulation of IgE/Ag-induced LAD2 cells and inhibit the release of chemokine and cytokine.

3.4. Magnolin decreased IgE/Ag-induced cytokine and protease levels in mice

Then we used ELISA to measure the cytokine and protease levels in mice. Magnolin reduced histamine release in mice serum (Fig. 5A). Mice treated with 2 μg/mL DNP-HSA alone used as control. Compared to control, Magnolin showed a significant inhibition effect on the level of histamine, MCP-1, TSP, IL-8, CMA1, and TNF-α in the mice (Fig. 5B-F).

3.5. In vivo assessment of the anti-anaphylactic effect of Magnolin on IgE/Ag-induced cutaneous flare in mice

After MCs was activated, the release of β-hexosaminidase, HA, cytokines and chemokine can cause passive cutaneous anaphylaxis (PCA), such as vasodilation and tissue fluid exudation. So that we use hindpaw swelling assay, H&E stain assay and Avidin stain assay to evaluate the effect of Magnolin in IgE/Ag-induced hypersensitive reaction mice. Compared to the mice treated with DNP-HSA alone, Magnolin can inhibit paw edema and dye diffuse in mice. The difference in paw thickness and the amount of diffused dye between left and right paw was decrease. When treated with 10 and 20 mg/kg of Magnolin, it showed a decrease effect on diffused Evans blue dye compared with the control group (Fig. 6A-C). The H&E stain assay shows that Magnolin can decrease permeability and vasodilation of the vascular caused by IgE/Ag-induced hypersensitive reactions (Fig. 6D). Avidin was used to show the decrease the degranulation of MCs after Magnolin treated (Fig. 6E). The results show that Magnolin can inhibit the IgE/Ag-induced hypersensitive reaction in mice.

3.6. Magnolin inhibition of the two key tyrosine protein kinase (Lyn and Fyn) and downstream PLC, p38/MAPK signaling pathway

After the cells were treated with 0, 100, 200 and 400 μM Magnolin

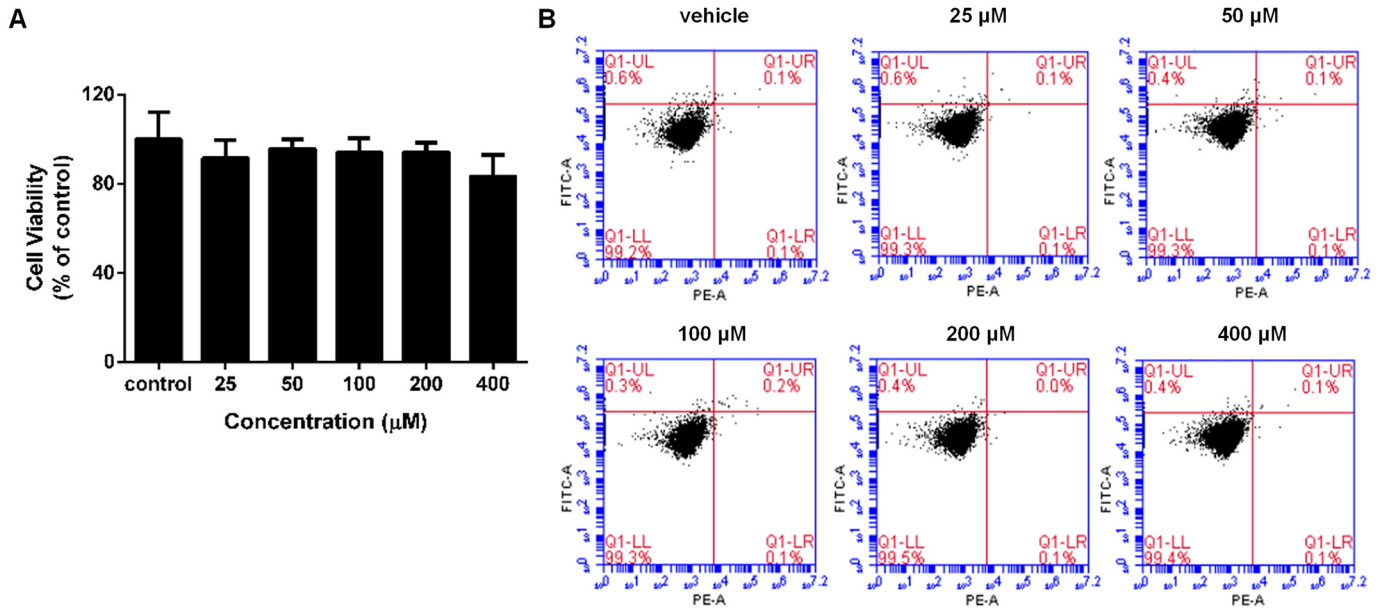

Fig. 2. Magnolin showed no effect on activity and apoptosis in LAD2 cells. LAD2 cells were pretreated with different doses of Magnolin. (A) Viability of LAD2 cells treated with Magnolin for 24 h. (B) Magnolin did not induce LAD2 apoptosis even at 400 μM. The results are expressed as the means ± S.E.M values of three independent experiments.

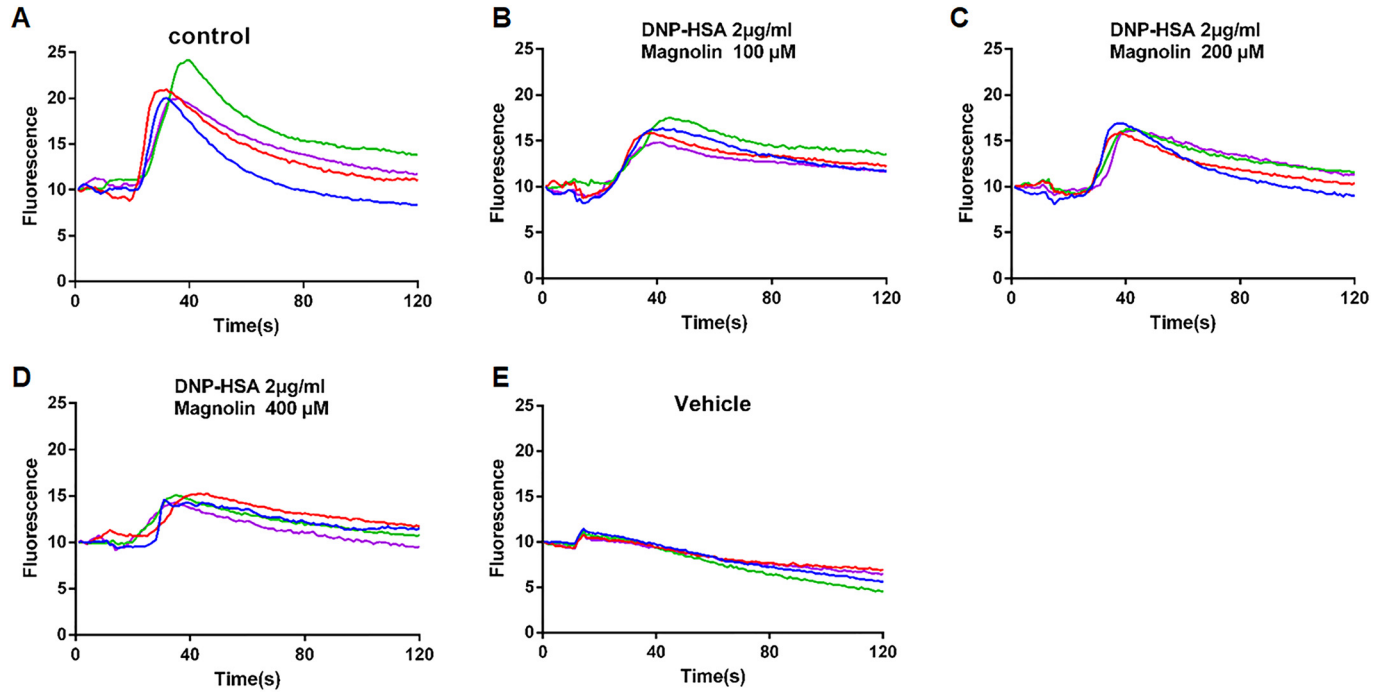

Fig. 3. Magnolin reduced IgE-induced calcium flux in LAD2 cells. Each colour line represents an individual cell. (A) LAD2 cells treated with 2 μg/mL DNP-HSA only. (B–D) LAD2 cells were pretreated with different doses of Magnolin, and treated with 2 μg/mL DNP-HSA, (E) LAD2 cells treated with CIB as vehicle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with 2 μg/mL DNP-HSA. We then studied the two key tyrosine protein kinases Lyn and Fyn activity change after Magnolin treatment. Cells were treated with 0, 100, 200 and 400 μM Magnolin with 2 μg/mL DNP-HSA, after which Lyn and Fyn activity was tested. As seen in Fig. 7, compared with DNP-HSA treated alone group, the levels of phosphorylated Lyn and Fyn in LAD2were decreased in a dose-dependent manner. The decrease of Lyn and Fyn can lead to the deactivation of MCs also inhibition the ERK and P38 MAP kinase pathways. Compared to DNP-HSA only treated LAD2 cells, the levels of phosphorylated PLCγ1, Akt, P38 and ERK decreased in a dose-dependent manner in LAD2 cells (Fig. 7B-C. The results showed that Magnolin may inhibit release of chemokine, cytokine and degranulation through decreasing the levels of phosphorylation of Lyn, Fyn, PLCγ1, Akt, P38 and ERK.

4. Discussion

The incidence of IgE-induced allergic disease is rapidly increased After the activation of MCs, Ca^{2+} mobilization can induce degranulation obviously. The medicines which treat allergic diseases are focus on reliving the symptoms, but not the origin causes, and they would

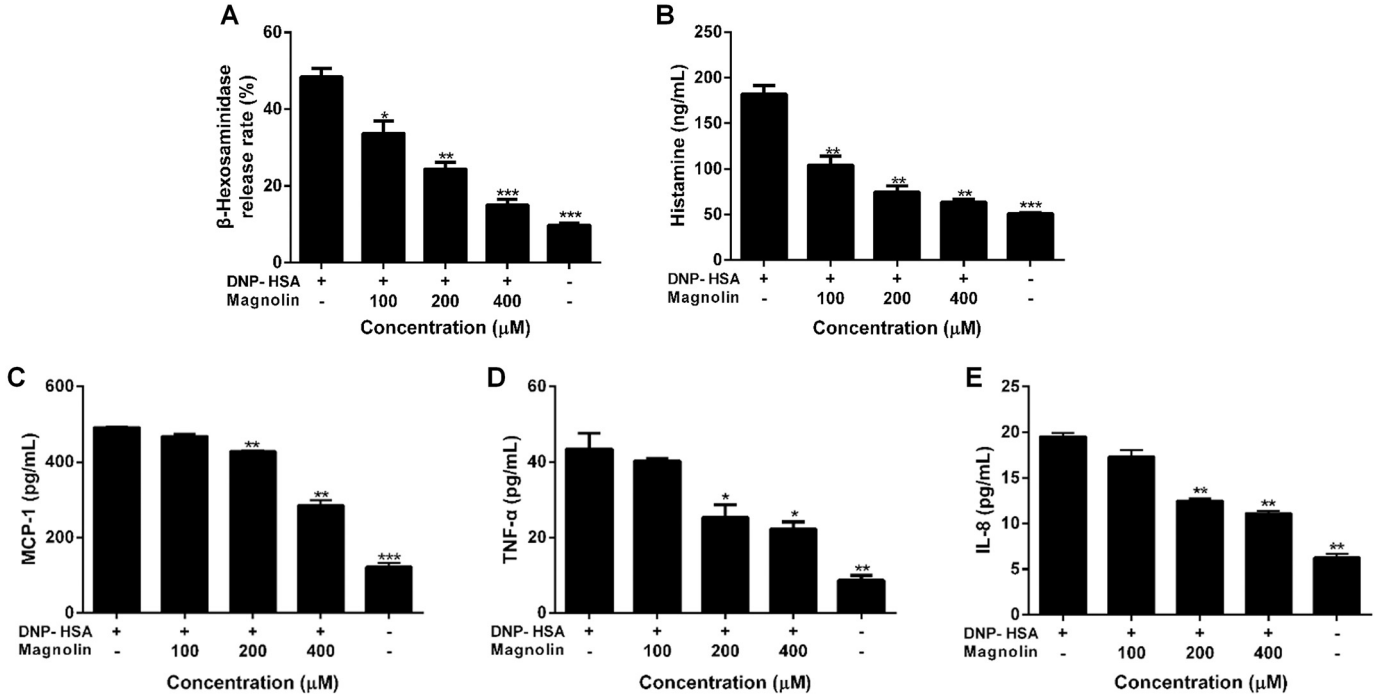

Fig. 4. Magnolin inhibit LAD2 cells degranulation, release of chemokine and cytokine. (A–B) degranulation was evaluated by detecting β-hexosaminidase and HA. (C–E) The release of TNF-α, MCP-1, IL-8 from LAD2 cells (1×10^5 LAD2 cells per well were seeded in 96-well plate and the repetitive wells were set as 6. The experiments were repeated thrice). The results are expressed as the means ± S.E.M values of three independent experiments. Analysis was performed using two-tailed unpaired Student's *t*-test, and statistical significance was defined at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$ and *** $p < 0.001$) *vs.* (DNP-HSA+, Magnolin-).

always come along with adverse effects [30,31]. Magnolin is a main compound from Flos magnoliae which can inhibit passive cutaneous anaphylaxis induced by DNP-IgE in rats. Thus, Magnolin may have anti-allergic effects. However, the role of Magnolin in the anti-allergic effects and its molecular mechanism have not been well studied. Therefore, we investigate whether Magnolin could suppress Ca^{2+}

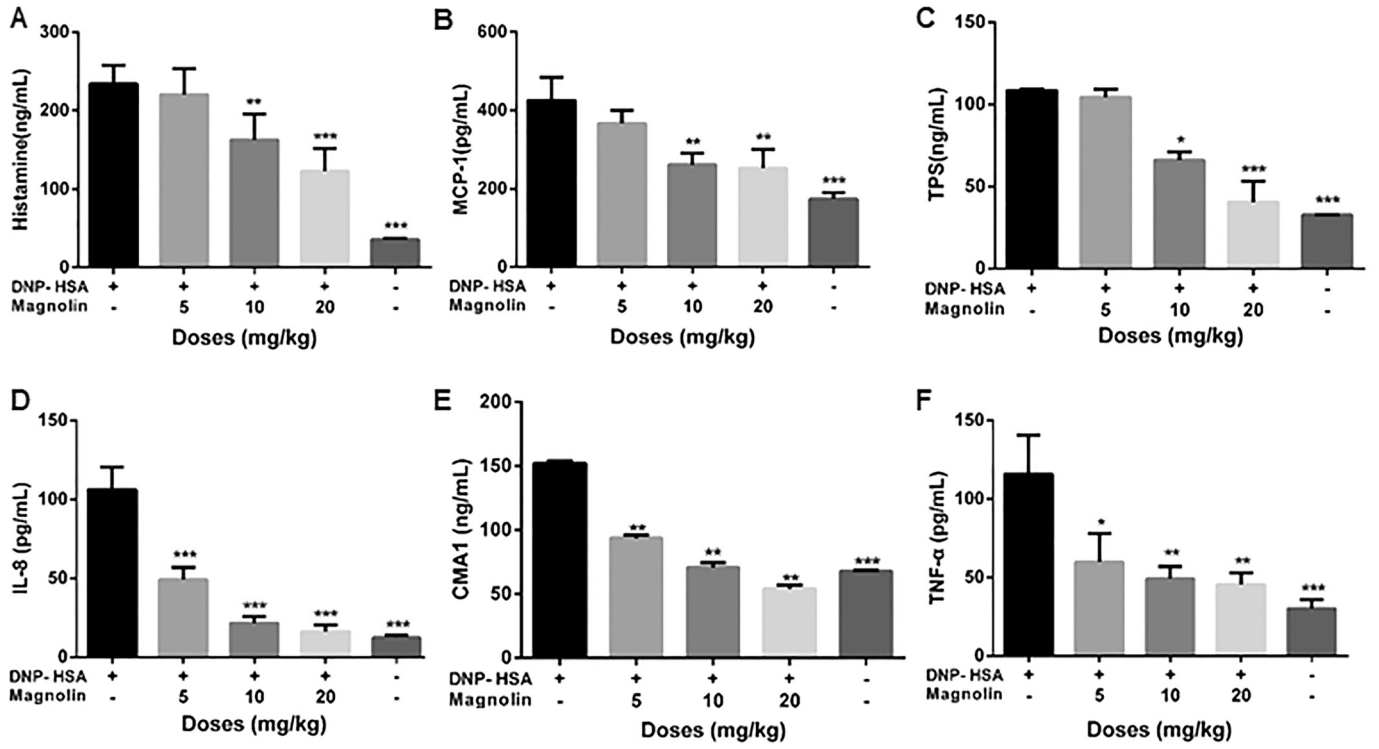

Fig. 5. The levels of chemotactic factors and inflammatory mediator in mice (n = 5) serum were detected. LAD2 cells were treated with 5, 10 and 20 mg/kg of Magnolin, inhibited the secretion of (A) histamine, (B) MCP-1, (C) Tryptase (TPS), (D) IL-8, (E) CMA1, (F) TNF-α. Data are presented as the means ± S.E.M (n = 3). The results are expressed as the means ± S.E.M values of three independent experiments. Two-tailed unpaired Student's *t*-tests were used to determine significance in statistical comparisons, and statistical significance was defined at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$) *vs.* (DNP-HSA+, Magnolin-).

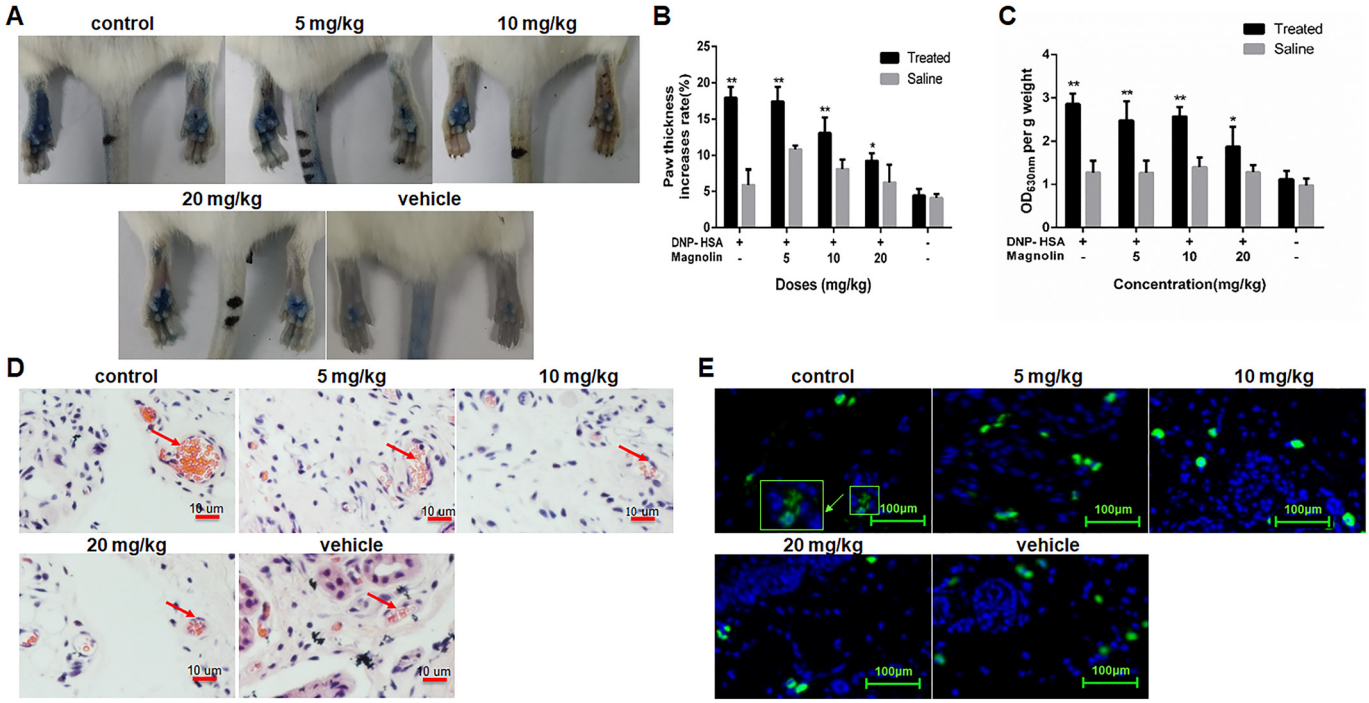

Fig. 6. Magnolin inhibited IgE-induced PCA and MCs degranulation. After orally administered with Magnolin (0, 5, 10, and 20 mg/kg) (A) images of mice paws, Left paw was injected with DNP-HSA, and right paw was injected with the same volume of vehicle, (B–C) PAW thickness and swelling analysis (n = 5), (D) H&E staining of skin tissue sections demonstrate that Magnolin inhibited hemangiectasis (n = 5), (E) avidin staining of the paw skin (n = 5). The results are expressed as the means ± S.E.M values of three independent experiments. Analysis was performed using two-tailed unpaired Student's t-test, and statistical significance was defined at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$) *vs.* (DNP-HSA+, Magnolin-), the ruler is 100 μm.

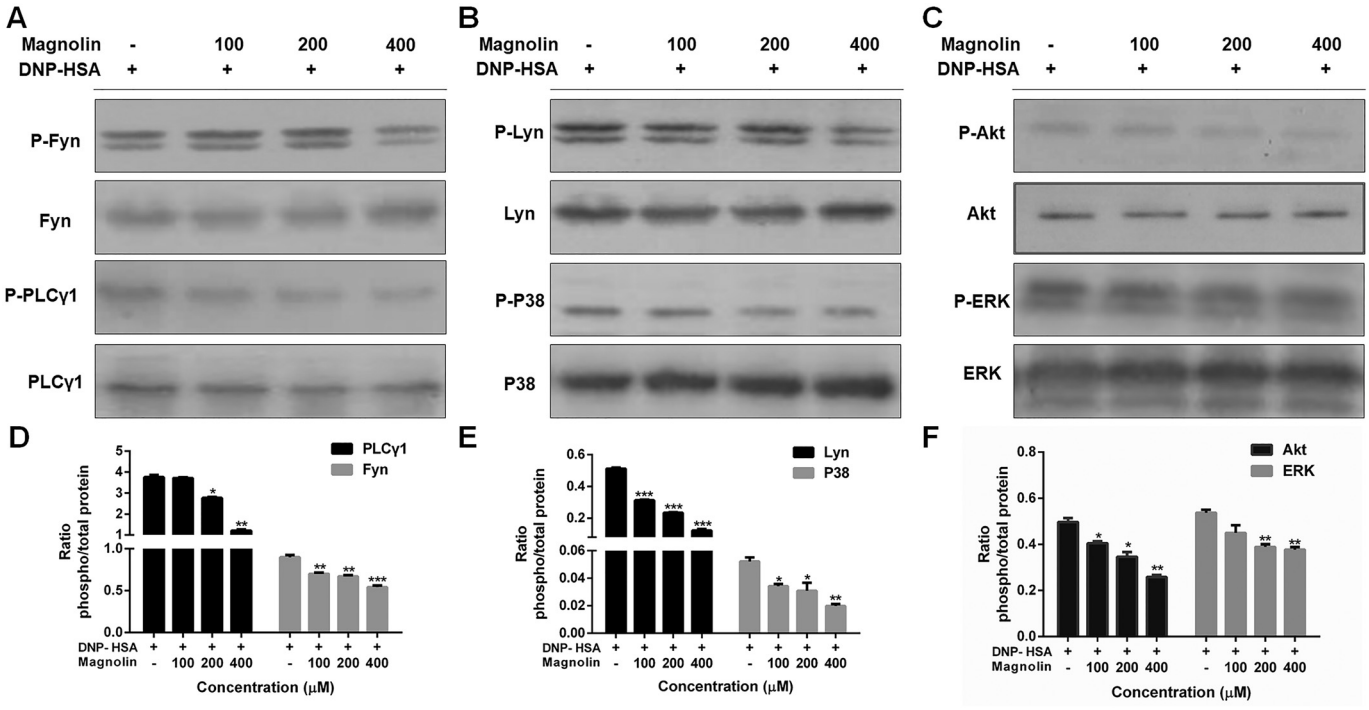

Fig. 7. Effect of Magnolin on two key tyrosine protein kinases and protein. (A) Western blot analysis of the expression levels of Fyn and PLCγ1 in LAD2 cells treated with Magnolin and quantification of Fyn and PLCγ1 protein expression by densitometric analysis. (B) Western blot analysis of the expression levels of Lyn and P38 in LAD2 cells treated with Magnolin and quantification of Lyn and P38 protein expression by densitometric analysis. (C) Western blot analysis of the expression levels of Akt and ERK in LAD2 cells treated with Magnolin and quantification of Akt and ERK protein expression by densitometric analysis. The data are presented as the means ± SEM. (n = 3). Two-tailed unpaired Student's t-test was used to determine significance in statistical comparisons, and statistical significance was accepted at $p < 0.05$ (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$) *vs.* DNP-HSA.

mobilization and the secretion of MCs degranulation, cytokine and protease through the IgE/Ag-induced pathway. In this study, we showed that the anti-allergic effects of Magnolin were induced by the inhibition of IgE activation both *in vitro* and *in vivo*. These results demonstrate the potential of Magnolin as an anti-allergic compound. The results revealed that Magnolin can inhibit IgE/Ag-induced anaphylactoid reactions by suppressing Ca^{2+} mobilization, degranulation, release of chemokine and cytokine in MCs in a dose-dependent manner *in vitro* and *in vivo*. However, MRGPRX2 expresses on MCs, which mediates pseudo-allergic reaction caused by C48/80, substance P and other small molecule drugs. Pseudo-allergic reaction is also caused by the activation of MCs and the release of histamine. Actually, we proved the inhibition effect of Magnolin on pseudo-allergic reaction and found that Magnolin could not reduce the histamine release of MCs stimulated by C48/80 and substance P. That might be due to that MRGPRX2 and FceRI pathway are independent.

FcεRI plays a main role in IgE-induced allergic reactions and can link IgE in the MCs and antigen, so that MCs can be activated. Tyrosine kinases, such as Lyn and Fyn are key to induce degranulation reaction and cytokine release. In our study, we found that Magnolin could inhibit the phosphorylation of Fyn, Lyn that decreased the phosphorylation of PLCγ1 leading to the inhibition of more downstream kinases, such as Akt, P38 and ERK, and finally decreased the degranulation of MCs and the generation of pro-inflammatory cytokines and chemokines. Those results indicate that Magnolin inhibited IgE/Ag-induced Lyn-Fyn-PLC signaling pathway activation. So, we speculate that Magnolin may be an inhibitor of tyrosine kinase such as Fyn and Lyn.

Tyrosine kinases show many function in other physiological function. So Magnolin showed other effect on the growth and migration of tumor cells. Magnolin has shown the effect on inhibition the proliferation and colony growth in ovarian cancer cells [32]. In addition, it was also proved that Magnolin could influence on the ERKs/RSK2 signaling pathway to inhibit cell migration and invasion [33]. As a potential inhibitor of tyrosine kinase, it might decrease the phosphorylation of ERK, this may be another reason for inhibiting cell migration and invasion.

Magnolin is a compound with potential for development in different field. And our study showed that the effect on decrease of the phosphorylation Lyn and Fyn reduced the phosphorylation of PLCγ1, ERK, Akt, and P38 to inhibit the degranulation, release of chemokine, cytokine and protease, permeability and vasodilation of the vascular. So that Magnolin may be a potential candidate for effective anti-allergic medicine that may regulate the activation of MCs.

Funding

This work was supported by the National Natural Science Foundation of China (Grant Number: 81227802), China Postdoctoral Science Foundation (Grant Number: 2016M592808, 2018M643682) and Natural Science Foundation of Shaanxi Province (Grant Number: 2016JQ8025).

Declaration of competing interest

The authors declare no financial or commercial conflict of interest.

References

[1] M. Yuan, J. Li, J. Lv, X. Mo, C. Yang, X. Chen, Z. Liu, J. Liu, Polydatin (PD) inhibits IgE-mediated passive cutaneous anaphylaxis in mice by stabilizing mast cells through modulating Ca^{2+} mobilization, Toxicol. Appl. Pharmacol. 264 (3) (2012) 462–469.

[2] F.E. Simons, A. Sheikh, Anaphylaxis: the acute episode and beyond, BMJ 346 (2013) 31–35.

[3] J.Y. Oh, W.S. Choi, C.H. Lee, H.J. Park, The ethyl acetate extract of Cordyceps militaris inhibits IgE-mediated allergic responses in mast cells and passive cutaneous anaphylaxis reaction in mice, J. Ethnopharmacol. 135 (2) (2011) 422–429.

[4] J. Wedemeyer, M. Tsai, S.J. Galli, Roles of mast cells and basophils in innate and acquired immunity, Curr. Opin. Immunol. 12 (6) (2000) 624–631.

[5] H.M. Kim, J.M. Yi, K.S. Lim, Magnoliae flos inhibits mast cell-dependent immediate-type allergic reactions, Pharmacol. Res. 39 (2) (1999) 107–111.

[6] H. Saito, Mast cell research, Chem. Immunol. Allergy 100 (100) (2014) 165.

[7] N. Sudo, K. Tanaka, Y. Koga, Y. Okumura, C. Kubo, K. Nomoto, Extracellular ATP activates mast cells via a mechanism that is different from the activation induced by the cross-linking of Fc receptors, J. Immunol. 156 (10) (1996) 3970–3979.

[8] X.Y. Hua, S.M. Back, E.K. Tam, Substance P enhances electrical field stimulation-induced mast cell degranulation in rat trachea, Am. J. Phys. 270 (1) (1996) 985–991.

[9] F.L. Pearce, Mast cells: function, differentiation and activation, Curr. Opin. Immunol. 1 (4) (1989) 630–636.

[10] D.M. Segal, J.D. Taurog, H. Metzger, Dimeric immunoglobulin E serves as a unit signal for mast cell degranulation, Proc. Natl. Acad. Sci. U. S. A. 74 (7) (1977) 2993–2997.

[11] H. Metzger, G. Alcaraz, R. Hohman, J.P. Kinet, A.V. Pribluda, R. Quarto, The receptor with high affinity for immunoglobulin E, Annu. Rev. Immunol. 4 (1) (1986) 419–470.

[12] G. Alber, L. Miller, C.L. Jelsema, N. Varin-Blank, H. Metzger, Structure-function relationships in the mast cell high affinity receptor for IgE. Role of the cytoplasmic domains and of the beta subunit, J. Biol. Chem. 266 (33) (1992) 22613–22620.

[13] M.S. Helena, F. Sheli, M. David, L.S. Francesca, Novel identified receptors on mast cells, Front. Immunol. 3 (3) (2012) 238.

[14] G. Gri, B. Frossi, F. D'Inca, L. Danelli, E. Betto, F. Mion, R. Sibilano, C. Pucillo, Mast cell: an emerging partner in immune interaction, Front. Immunol. 3 (2012) 120.

[15] L.C. Wu, Immunoglobulin E receptor signaling and asthma, J. Biol. Chem. 286 (38) (2011) 32891–32897.

[16] P. Draber, I. Halova, I. Polakovicova, T. Kawakami, Signal transduction and chemotaxis in mast cells, Eur. J. Pharmacol. 778 (2015) 11–23.

[17] J. Kalesnikoff, S.J. Galli, New developments in mast cell biology, Nat. Immunol. 9 (11) (2008) 1215–1223.

[18] M. Hoth, R. Penner, Depletion of intracellular calcium stores activates a calcium current in mast cells, Nature 355 (6358) (1992) 353–356.

[19] P.J. Jr, R.R. Mckay, Capacitative calcium entry channels, Bioessays 21 (1) (1999) 38–46.

[20] R. Sibilano, B. Frossi, C.E. Pucillo, Mast cell activation: a complex interplay of positive and negative signaling pathways, Eur. J. Immunol. 44 (9) (2014) 2558–2566.

[21] J. Rivera, A.M. Gilfillan, Molecular regulation of mast cell activation, J. Allergy Clin. Immunol. 117 (6) (2006) 1214–1225.

[22] M.A. Beaven, Our perception of the mast cell from Paul Ehrlich to now, Eur. J. Immunol. 39 (1) (2009) 11–25.

[23] S.J. Galli, M. Tsai, Mast cells in allergy and infection: versatile effector and regulatory cells in innate and adaptive immunity, Eur. J. Immunol. 40 (7) (2010) 1843–1851.

[24] K. Amin, The role of mast cells in allergic inflammation, Respir. Med. 106 (1) (2012) 9–14.

[25] J.C. Mi, J.K. Sohng, D.J. Choi, I.P. Yong, Inhibitory effect of phloretin and biochanin A on IgE-mediated allergic responses in rat basophilic leukemia RBL-2H3 cells, Life Sci. 93 (9–11) (2013) 401–408.

[26] P. Ehrlich, Beiträge zur Theorie und Praxis der histologischen Färbung *, Collected Papers of Paul Ehrlich, 2013, pp. 29–64.

[27] T. Zhang, D. Che, R. Liu, S. Han, N. Wang, Y. Zhan, P. Pundir, J. Cao, Y. Lv, L. Yang, Typical antimicrobials induce mast cell degranulation and anaphylactoid reactions via MRGPRX2 and its murine homologue MRGPRB2, Eur. J. Immunol. 47 (11) (2017) 1949–1958.

[28] R. Liu, D. Che, T. Zhao, P. Pundir, J. Cao, Y. Lv, J. Wang, P. Ma, J. Fu, N. Wang, MRGPRX2 is essential for sinomenine hydrochloride induced anaphylactoid reactions, Biochem. Pharmacol. 146 (2017) 214–223.

[29] N. Wang, D. Che, T. Zhang, R. Liu, J. Cao, J. Wang, T. Zhao, P. Ma, X. Dong, L. He, Saikosaponin A inhibits compound 48/80-induced pseudo-allergy via the Mrgprx2 pathway in vitro and in vivo, Biochem. Pharmacol. 148 (2018) 147–154.

[30] D.F. Finn, J.J. Walsh, Twenty-first century mast cell stabilizers, Br. J. Pharmacol. 170 (1) (2013) 23–37.

[31] T.B. Casale, J.J. Oppenheimer, Next generation antihistamines: therapeutic rationale, accomplishments and advances, Expert Opin. Investig. Drugs 11 (6) (2002) 807–817.

[32] J.H. Song, C.J. Lee, H.J. An, S.M. Yoo, H.C. Kang, J.Y. Lee, K.D. Kim, D.J. Kim, H.S. Lee, Y.Y. Cho, Magnolin targeting of ERK1/2 inhibits cell proliferation and colony growth by induction of cellular senescence in ovarian cancer cells, Mol. Carcinog. 58 (1) (2019) 88–101.

[33] C.J. Lee, M.H. Lee, S.M. Yoo, K.I. Choi, J.H. Song, J.H. Jang, S.R. Oh, H.W. Ryu, H.S. Lee, Y.J. Surh, Magnolin inhibits cell migration and invasion by targeting the ERKs/RSK2 signaling pathway, BMC Cancer 15 (1) (2015) 576.