

Full length article

Paeoniflorin inhibits IgE-mediated allergic reactions by suppressing the degranulation of mast cells through binding with FcεRI alpha subunits

Yongjing Zhang, Shiling Hu, Shuai Ge, Jue Wang, Langchong He^{*}

School of Pharmacy, Xi'an Jiaotong University, Xi'an, China

ARTICLE INFO

Keywords:

Paeoniflorin
IgE-mediated allergy
Mast cells
FcεRI antagonist

ABSTRACT

Paeoniflorin (PF), a monoterpene glycoside isolated from the aqueous extract of the Chinese herb *Radix Paeoniae Alba*, has been used for treating various inflammatory diseases. In this study, we aimed to investigate the anti-allergic activities of PF. The anti-anaphylactic activity of PF was investigated using mast cell (MC) degranulation assay as well as Ca^{2+} influx *in vitro* and skin swelling and extravasation assays *in vivo*. The results showed that PF inhibited MC degranulation (histamine release from 74.5 ± 4.95 ng/ml to 58.7 ± 6.06 ng/ml) and Ca^{2+} influx challenged by DNP-BSA *in vitro*. In addition, PF reduced the degree of swelling and Evans blue exudation in mice paws. Furthermore, PF dose-dependently reduced serum inflammatory mediator release in mice sensitized with ovalbumin for 48 h by inhibiting MC degranulation. Molecular docking study revealed that PF bound better with the α subunit of FcεRI than with the β subunit. SPR revealed that PF had a strong affinity interaction with FcεRI α subunit and the K_D value was $(7.08 \pm 0.97) \times 10^{-6}$ M. Our findings revealed that PF inhibited anaphylactic responses *in vivo* and *in vitro*, and it can be considered a novel FcεRI inhibitor for preventing MC-related allergic diseases.

1. Introduction

The incidence of allergic diseases is increasing worldwide (Peden, 2018; Shimanaka et al., 2017). Allergic diseases are characterized by increased isotype switching of B cells and T helper 2 cells, resulting in the production of IgE antibodies against specific allergens (Johansson, 2016; Eckl-Dorna et al., 2018). MCs play a vital role in promoting IgE-mediated allergies (Espinosa and Valitutti, 2018). The crosslinking of high-affinity IgE receptor (FcεRI) and immunoglobulin E (IgE) on MC surface triggers secretory granules, which are filled with inflammatory mediators that induce multiple biological activities (Oettgen and Burton, 2015; Yip et al., 2016). IgE-dependent allergic reaction is usually called "Type I reaction" (Wang et al., 2018). Type I reaction can be severe and pose a threat of death (Simons and Sheikh, 2013). Although the mechanism of IgE-mediated allergy has been studied for long and some anti-IgE therapies are in practice (MacGlashan, 2012), there is still no drug targeting FcεRI.

Paeoniflorin (PF), the main effective component found in *Radix Paeoniae Alba*, has been used as a traditional Chinese medicine for treating inflammation for centuries (Xin et al., 2019). It has been shown to possess extensive pharmacological activities (Hu et al., 2018; Zhao

et al., 2019). PF modulates the functions and activation of immune cells, decreases the production of inflammatory mediators, and restores abnormal signaling pathways (Zhang and Wei, 2020; Li et al., 2018; Liu et al., 2019). There is a study reported that PF could ameliorate RA (rheumatoid arthritis) by inhibiting the mTOR, Rho kinase, and NF-κB signaling pathways and decreasing the expression of LIFR and ASPN proteins (Zhai et al., 2018). PF can balance immune cell subsets by inhibiting abnormally activated cell subsets and restoring regulatory cell subsets. Fei et al. 2019 suggested that PF accumulates in the intestine and primarily modulates Th1 and Th17 responses in the mesenteric lymph nodes and Peyer's patches, rather than in the spleen, to exert anti-arthritis effects. Therefore, PF has been used in the treatment of various diseases (Wang and Cheng, 2018; Circu et al., 2017; Shao et al., 2019; Shou et al., 2019; Zhao et al., 2018), including asthma, cerebral infarction, psoriasis, nephritis, arthritis, and diabetic inflammation.

PF may serve as an FcεRI inhibitor. In the present study, we aimed to investigate the inhibitory effects of PF on allergic reactions both *in vitro* and *in vivo* and the underlying mechanism.

^{*} Corresponding author. School of Pharmacy, Xi'an Jiaotong University, Yanta Westroad, Xi'an, 710061, China.

E-mail address: helc@mail.xjtu.edu.cn (L. He).

<https://doi.org/10.1016/j.ejphar.2020.173415>

Received 15 April 2020; Received in revised form 20 July 2020; Accepted 23 July 2020

Available online 6 August 2020

0014-2999/© 2020 Elsevier B.V. All rights reserved.

2. Materials and methods

2.1. Drugs and reagents

PF (purity $\geq 98\%$) Lot: 1711601, was supplied by Chengdu Pufei De Biotech Co. Ltd. (Chengdu, Sichuan, China). Anti-DNP-specific IgE, DNP-streptomycin, albumin from chicken egg white, FITC-avidin, p-nitrophenyl N-acetyl- β -D-glucosamide and Triton X-100 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluo-3, AM ester, and F-127 were procured from Biotium (California, USA). Tyrode's solution buffer (6.954 g/l NaCl, 0.353 g/l KCl, 0.282 g/l CaCl_2 , 0.143 g/l MgSO_4 , 0.162 g/l KH_2PO_4 , 2.383 g/l HEPES, 0.991 g/l glucose, and 1 g/l BSA, pH 7) was prepared fresh on the day of use and filter with 0.22 μm membrane. All aqueous solutions were prepared using ultrapure water produced by MK-459 Millipore Milli-Q Plus ultra-pure water system.

2.2. Animals

Adult male C57BL/6 mice (18–22 g) were purchased from the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). Animals were free access to water, fed a standard dry chow twice a day, and housed in individual cages in a large colony room, with a room temperature of 20–25 °C and a relative humidity of 40% on a 12 h light/dark cycle. The mice were randomly divided into the following groups (6 mice per group): blank, control, and the experimental group. All experiments involving equal treatments in animals were conducted by experimenters who were blinded to the conditions.

2.3. Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health. The experimental protocols for using mice were approved by the Animal Ethics Committee at Xi'an Jiaotong University, Xi'an, China (Permit Number: XJTU, 2011–0045).

2.4. Cell lines

The Laboratory of Allergic Disease 2 (LAD2) human MCs were kindly provided by A. Kirshenbaum and D. Metcalfe (NIH, USA). Cells were maintained in StemProTM-34 medium supplemented with 10 mL/L StemPro nutrient supplement, 1:100 penicillin-streptomycin, 2 mM L-glutamine, and 100 ng/ml human stem cell factor in 5% CO_2 at 37 °C. Cells maintained at a density of 2×10^6 cells/ml.

2.5. Cytotoxicity assay

Cell viability was determined using Cell Counting Kit assays (7 Sea, China). LAD2 cells were seeded into 96-well plates at a density of 5×10^3 cells per well and then treated with 10 μl of PF at concentrations (0, 1, 10, 100, 500, and 1000 μM) for 24 h. Next, 10 μl of Cell Counting Kit solution was added to each well followed by the incubation for 2 h. Further, the relative cell viability was assessed by the detection of the absorbance at 450 nm using a microplate reader (Bio-Rad, Carlsbad, CA, USA). Survival rate of LAD2 cells was calculated as

$$\left[\frac{(\text{OD}_{\text{Treated}} - \text{OD}_{\text{Blank}})}{(\text{OD}_{\text{Control}} - \text{OD}_{\text{Blank}})} \right] \times 100\% \quad (1)$$

2.6. The β -hexosaminidase release assay

LAD2 cells were seeded into 96-well plates and incubated with 2 μg /ml of DNP-IgE for at 37 °C and 5% CO_2 for 24 h. The culture medium was then replaced with 50 μl per well of Tyrode's solution containing different concentrations of PF (10, 100, 500 μM), and incubated for 30 min at 37 °C in 5% CO_2 . Blank groups were treated with Tyrode's solution alone under the same incubation conditions. The plates were

centrifuged at $200 \times g$ for 5 min at 4 °C and buffer removed. 50 μl of Tyrode's solution containing 4 μg /ml DNP-streptomycin was added to the PF-treated cells and control group. Equal volume of Tyrode's solution was added to blank group. Cells were lysed with 0.1% Triton X-100 in Tyrode's solution to analyze the total β -hexosaminidase content. The β -hexosaminidase released into the supernatants and in cell lysates was quantified by hydrolysis of p-nitrophenyl N-acetyl- β -D-glucosamide in 0.1M citric acid/sodium citrate buffer (pH 4.5) for 90 min at 37 °C. The reaction was stop by adding stop buffer (0.1M Na_2CO_3 : 0.1M NaHCO_3 = 9:1, pH 11). The absorbance was measured at 405 nm using a microplate spectrophotometer and the percentage of β -hexosaminidase release was calculated.

IC50 value was calculated by the inhibition ratio of β -hexosaminidase release which caused by different concentration of PF.

2.7. Histamine release assay

The 1×10^6 LAD2 cells per well were incubated with 2 μg /ml of DNP-IgE for at 37 °C and 5% CO_2 overnight. The culture medium was removed, and 100 μl indicated concentrations PF (0, 10, 100, and 500 μM) prepared by Tyrode's solution containing 4 μg /ml DNP-streptomycin was added, only Tyrode's solution was added in blank group. Cells were incubated for 30 min at 37 °C with 5% CO_2 . The 96-well plate was centrifuged at $200 \times g$ for 5 min at 4 °C, and the buffer was removed. Histamine release assay was carried out using the LC-ESI-MS/MS method, with a HILIC column (Venusil HILIC, 2.1 mm $\times$ 150 mm, 3 μm , Agela Technologies, Tianjin, China). Isocratic elution buffer was made up with acetonitrile-water containing 0.1% formic acid and 20 mM ammonium formate (77:23, v/v), using at a flow rate of 0.3 ml/min for eluting the histamine. Histamine2HCl was used as an interior label.

2.8. Intracellular Ca^{2+} mobilization assay

The incubation buffer consisted of 0.5 μl Fluo-3, 2 μl Pluronic F-127 and 997.5 μl CIB. Different concentrations of PF (0, 10, 100, and 500 μM) were prepared in incubation buffer, an incubation buffer without PF was used as a blank group. LAD2 cells were pre-treated with 2 ng/ml DNP-IgE at 37 °C and 5% CO_2 for 24 h. LAD2 cells were washed twice with CIB followed by the addition of the required incubation buffer. After 30 min incubation, the cells were washed twice with CIB and plated into a 96-well plate for the calcium imaging. The cells were magnified 120 times, and one photo per second was obtained under blue light for the calcium imaging. Cells were identified as responding if the $[\text{Ca}^{2+}]$ rose by at least 50% after the DNP-streptomycin were injected.

2.9. Chemokine release assay

The 1×10^6 LAD2 cells per well were incubated with 2 μg /ml of DNP-IgE for at 37 °C and 5% CO_2 overnight. The culture medium was removed, and 100 μl indicated concentrations PF (0, 10, 100, and 500 μM) prepared by Tyrode's solution containing 4 μg /ml DNP-streptomycin was added, only Tyrode's solution was added in blank group. Cells were incubated for 6 h at 37 °C with 5% CO_2 . 5-HT, MCP-1, IL-8 and TNF- α ELISA Kits were purchased from Excell Bio Co., Ltd. (Beijing, China) and finished according to the provided instructions.

2.10. Hind paw swelling and extravasation

Adult male C57BL/6 mice were sensitized with 200 μg /kg OVA 48 h ago, then given PF (0, 0.625, 1.25, and 2.5 mg/kg) by gavage. Mice were anaesthetized with an intraperitoneal(i.p.) injection of 80 mg/kg 1% pentobarbitalum natricum 30 min later. Mice were injected intravenously (i.v.) with 0.2 ml of 0.4% Evans blue in saline. A Vernier caliper was used to measure the thickness of the paw. 5 μl OVA (100 μg /ml) in saline was injected into the left paw 5 min later, and saline only was

injected into the right paw as blank control. After 15 min, paw thickness was measured again and recorded. Paw tissues were collected, dried for 12 h at 50 °C, then weighed. Evans blue was extracted in 500 μ l acetone: saline = 7:3 at 37 °C. Tissues were cut up and then centrifuged for 20 min at 3000 rpm. The supernatant was equally distributed in 150 μ l aliquots into 96-well plate, and the OD was read at 620 nm using a spectrophotometer.

2.11. Avidin staining

Adult male C57BL/6 mice were sensitized with 200 μ g/kg OVA 48 h ago, then given PF (0, 0.625, 1.25, and 2.5 mg/kg) by gavage. Anaesthetized mice by pentobarbitalum natricum were injected with 5 μ l of 100 μ g/ml OVA saline in the left paw 30 min later; saline was used as blank control. The injection site on the skin in paw was excised, washed with PBS, and fixed with 4% formaldehyde for 48 h at room temperature and subjected to avidin staining 15 min later.

After staining, the slides were dried at 37 °C for 30 min and pre-incubated in the blocking solution [10% normal goat serum (v/v), 0.2% Triton X-100 (v/v) in PBS, pH 7.4] for 2 h at 25 °C, followed by incubation with 1/500 FITC-avidin for 45 min. Sections were washed three times with PBS, and a drop of Fluoro-mount G (Southern Biotech, AL, USA) was added. Images were taken immediately using an inverted fluorescence microscope (Nikon, Tokyo, Japan).

2.12. Mouse serum analysis

Adult male C57BL/6 mice were sensitized with 200 μ g/kg OVA 48 h ago, then given PF (0, 0.625, 1.25, and 2.5 mg/kg) by gavage. Mice were injected with 0.2 ml OVA (100 μ g/ml) saline though tail vein 30 min later, only saline was set as a negative control. Blood was collected and centrifuged to get serum 6 h later. Mouse IL-8, TNF- α and MCP-1 were analyzed by ELISA Kits (Excell Bio Co., Ltd. Beijing, China). A liquid chromatography–mass spectrometry (LC–MS) 8040 mass spectrometer (Shimadzu Corporation, Kyoto, Japan) was used in applying the liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS) method.

2.13. Western blot analysis

The 1×10^7 LAD2 cells were incubated with PF (0, 10, 100, and 500 μ M) for 30 min at 37 °C and 5% CO₂ after pre-treated with 2 μ g/ml of DNP-IgE overnight in culture plate. The plate was centrifuged at 200 $\times$ g for 5 min at 4 °C, and the buffer was removed. A 4 μ g/ml DNP-streptomycin was added in the PF groups and control group for 30 min. Total proteins from each group were extracted in ice-cold condition using RIPA lysis buffer containing 10% protease inhibitor and a phosphatase inhibitor cocktail (Roche Diagnostics). After 30 min, insoluble protein lysate was removed by centrifuging the samples at 13,500g for 10 min at 4 °C. The protein concentration was determined using a BCA Protein Quantification kit according to the manufacturer's instructions. The protein in the cell lysates was denatured by boiling the samples for 5 min with a 5 $\times$ loading sample buffer from Thermo Fisher Scientific, Inc. (MA, USA), and equal amounts of protein were separated on a 10% gel using S.D.S-PAGE (Shaanxi Pioneer Biotech Co., Ltd.). The separated proteins were transferred onto polyvinylidene fluoride membranes from Hangzhou Microna Membrane Technology Co., Ltd. (Hangzhou, China) and blocked by constant stirring with 5% nonfat milk in Tris-buffered saline (Baihao) containing Tween-20 (TBST; Shaanxi Pioneer Biotech Co., Ltd.) at room temperature for 2 h. The membranes were then incubated overnight at 4 °C with the following primary antibodies: anti-Lyn (1:1000, #2796, Cell Signaling Technology [CST]), anti-phosphorylated-Lyn (1:1000, #2731, CST), anti-PLC γ 1 (1:1000, #5690, CST), anti-phosphorylated-PLC γ 1 (P-PLC γ 1, Ser1248) (1:1000, #8713, CST), anti-Erk1/2 (1:1000, p44/42 MAPK (Erk1/2), #9102, CST), anti-phosphorylated-Erk1/2 (P-p44/42 MAPK (Erk1/2) [Th202/

Tyr204], 1:1000, #9101, CST), anti-P38 (1:1000, #8690, CST), anti-phosphorylated-P38 (P-P38, Th180/Tyr182) (1:1000, #4511, CST), anti-PKC (1:1000, #GR257247-4, Abcam), anti-phosphorylated PKC (P-PKC, Ser1756) (1:500, #9371, CST), anti-Akt (1:1000, #4691, CST), and anti-phosphorylated-Akt (P-Akt, Ser473) (1:1000, #4060, CST). The membranes were then washed three times with TBST at every 10-min interval and then incubated with secondary antibodies (at a dilution of 1:20,000 in TBST) for 1 h at 37 °C. The membranes were again washed three times with TBST for 10 min and developed using an enhanced chemiluminescence (ECL) kit. A Lane 1DTM transilluminator (Beijing Creation Science Co., Ltd., Beijing, China) was used to capture the images of the developed blots, and Image-Pro Plus 5.1 software (Media Cybernetics, Inc., Rockville, MD, USA) was used to quantify the protein levels.

2.14. Surface plasmon resonance

For the analysis of SPR, Fc ϵ R1 α subunit protein (50 μ g/mL) was fixed on the Carboxyl sensor chip (Nicoya, Canada) by capture-coupling, the interaction of Fc ϵ R1 α subunit with the small molecules fixed was detected by OpenSPR (Nicoya Lifesciences, Waterloo, Canada) at 25 °C. The binding time and disassociation time were both 250 s, the flow rate was 20 μ l/s. A one to one diffusion corrected model was fitted to the wavelength shifts corresponding to the varied PF concentration. The data was retrieved and analyzed with TraceDrawer software. K_D value was also calculated.

2.15. Molecular docking

To investigate the interactions between receptor and ligand, molecular docking studies were conducted using the Sulflex-Dock Mode of Sybyl-X program package (New Tripes International, St. Louis, USA). The docking model of Fc ϵ R1 (PDB: 2Y7Q) used in the study was based on the X-ray Diffraction performed by Holdom, M.D. et al. (Holdom et al., 2011). α and β subunits were extract from the whole receptor structure respectively. The interact ability of PF with both α and β subunits were compared by total score, Crash and Polar. PF binding amino acid residues and the corresponding bond length are marked.

2.16. Statistical analysis

Data are presented as the mean $\pm$ standard error of mean (S.D.) and were statistically analyzed using analysis of variance (ANOVA). Two-tailed tests were used for comparisons between two groups. Differences were considered significant at $P < .05$ (* $P < .05$, ** $P < .01$, *** $P < .001$).

3. Results

3.1. PF inhibited IgE-mediated degranulation in mast cells

First, we determined the cytotoxic effect of PF on LAD2 cells. The effect of PF on MC viability was examined using a Cell Counting kit assay. At a concentration of 1000 μ M, PF showed no significant cytotoxicity on LAD2 cells (Fig. 1A). Thereafter, based on doses used clinically, we selected the doses of 10, 100, and 500 μ M for investigating the protective effect of PF against C48/80-induced MC degranulation.

The release of β -hexosaminidase and histamine, which are pre-stored mediators, was measured to determine whether PF treatment could inhibit IgE-mediated degranulation of MCs. The release rate of β -hexosaminidase was $38.2 \pm 4.86\%$ (Fig. 1B) and that of histamine was 74.5 ± 4.95 ng/ml (Fig. 1C). Pretreatment of LAD2 cells with the indicated concentrations of PF showed a dose-dependent inhibitory effect on DNP-BSA-induced degranulation.

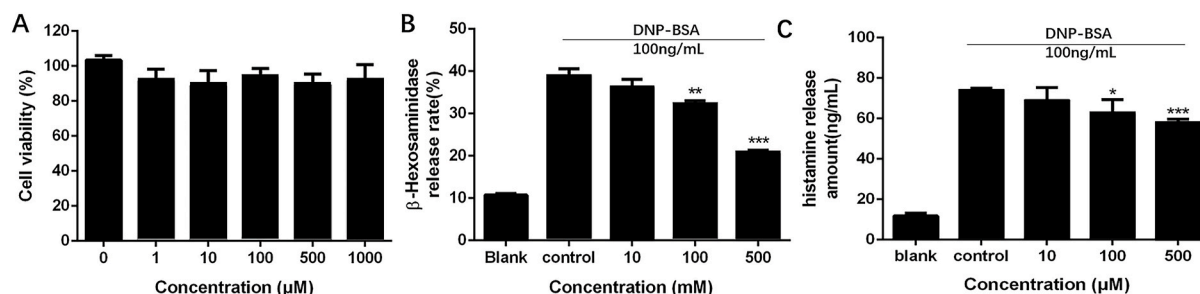


Fig. 1. PF inhibited IgE-mediated degranulation in LAD2 cells. (A) The toxicity of PF at indicated concentrations for 24 h. PF showed no cytotoxic effect on LAD2 cells. (B) β -hexosaminidase release rate was inhibited after pretreated with PF. Tyrode's solution was used as a blank. (C) Histamine release was inhibited after pretreated with PF. Tyrode's solution was used as a blank group. (Experiments were repeated three times, and data are represented as mean $\pm$ S.D. And analyzed using two-tailed unpaired Student's t-test. Differences were considered significant at * $P < .05$, ** $P < .01$, and *** $P < .005$ vs Control), $n = 3$.

3.2. PF attenuated IgE-mediated calcium ion influx

Increased concentrations of intracellular Ca^{2+} ions can trigger the degranulation of MCs. MC activation induced Ca^{2+} influx. Pretreatment with PF inhibited Ca^{2+} influx in a dose-dependent manner (Fig. 2).

3.3. Inhibitory effects of PF on FcεRI-mediated signaling pathway

The phosphorylation of Lyn, Akt, and PKC signaling pathways is important for MC Ca^{2+} influx and degranulation. The results of western blotting performed after 30 min treatment with PF showed that PF dose-dependently downregulated the levels of phosphorylated Lyn, Akt, and PKC in IgE-mediated allergy (Fig. 3 A–F).

3.4. PF inhibited IgE-mediated inflammatory mediators release by downregulating the phosphorylation of key kinases

Cytokines and 5-HT released from MCs can induce serious inflammation, which triggers severe allergic reactions. We analyzed chemokine release by using ELISA and found that treatment with PF led to a

significant reduction in the release of IgE-mediated MC inflammatory mediators, including 5-HT, MCP-1, IL-8, and TNF- α (Fig. 4A). In addition, the phosphorylation levels of chemokine-related key kinases were investigated using western blotting. PF was shown to downregulate the phosphorylation levels of key kinases such as Erk1/2, P38, and PLC (Fig. 4B–G), which regulate chemokine release by MCs.

3.5. Molecular docking and SPR revealed that PF might be an FcεRI ligand

To investigate the interactions between PF and FcεRI, molecular docking studies were performed using the Surflex-Dock Mode of the SYBYL-X 2.0 software package. PF has poor lipid solubility and lacks the ability for direct transmembrane delivery. As the α and β subunits of FcεRI are extracellular fragments and mainly responsible for cross-linking with IgE, we investigated these two subunits as potential PF binding sites. The results showed that PF matched the active site of FcεRI α subunit in sphere space field and ribbon models (Fig. 5A–C) and that of β subunit (Fig. 5E–G). The docking results showed that PF binds to FcεRI α subunit though 5 hydrogen bonds with 5 residues (LYS117, VAL118,

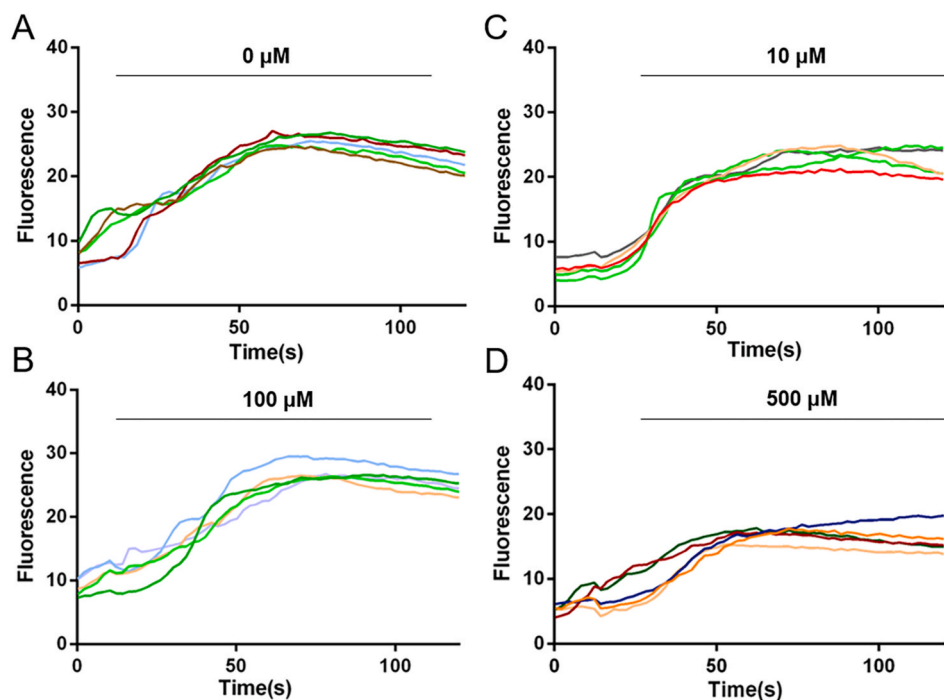


Fig. 2. PF inhibited calcium influx in LAD2 cells mediated by IgE. (A) LAD2 cells were pretreated with CIB and Fluo-3, then stimulated with 100 ng/ml DNP-BSA, was used as Control group. (B–D) LAD2 cells were pretreated with different concentrations of PF (10, 100, and 500 μM) to inhibit DNP-BSA induced calcium influx. From fluorescence intensity curve, PF inhibited calcium influx induced by DNP-BSA in a dose-dependent manner. Experiments were repeated three times.

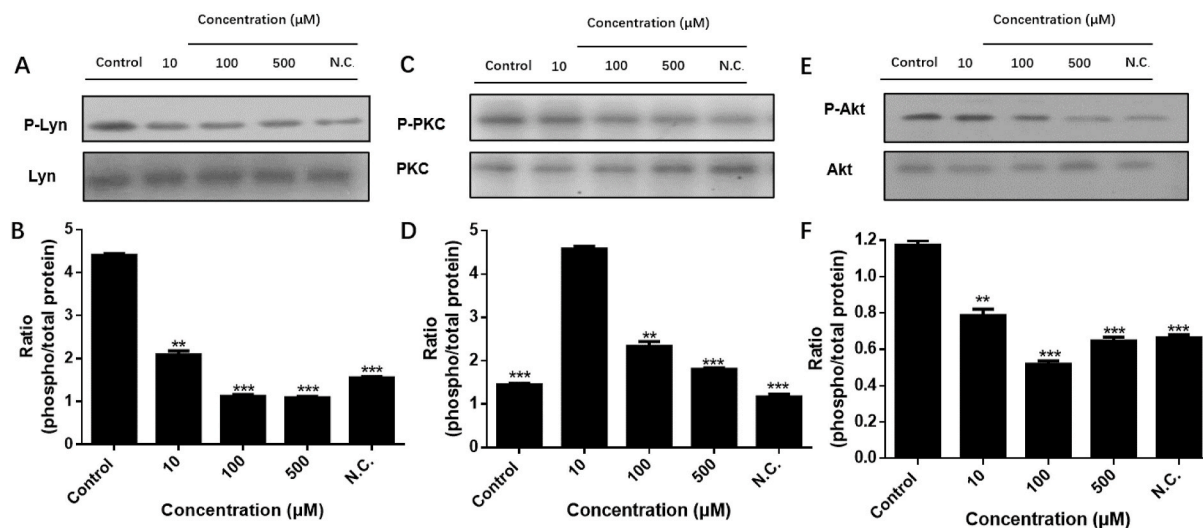


Fig. 3. PF showed an inhibitory effect on FcεRI mediated signaling pathway. PF decreased DNP-BSA induced phosphorylation level rise of Lyn, PKC and Akt in a dose-dependent manner. When concentration came to 100 μM, the phosphorylation level of all three protein were decreased significantly, compared to control groups. (Experiments were repeated three times, and data are represented as mean ± S.D. And analyzed using two-tailed unpaired Student's t-test. Differences were considered significant at $*P < .05$, $**P < .01$, and $***P < .005$ vs Control), $n = 3$.

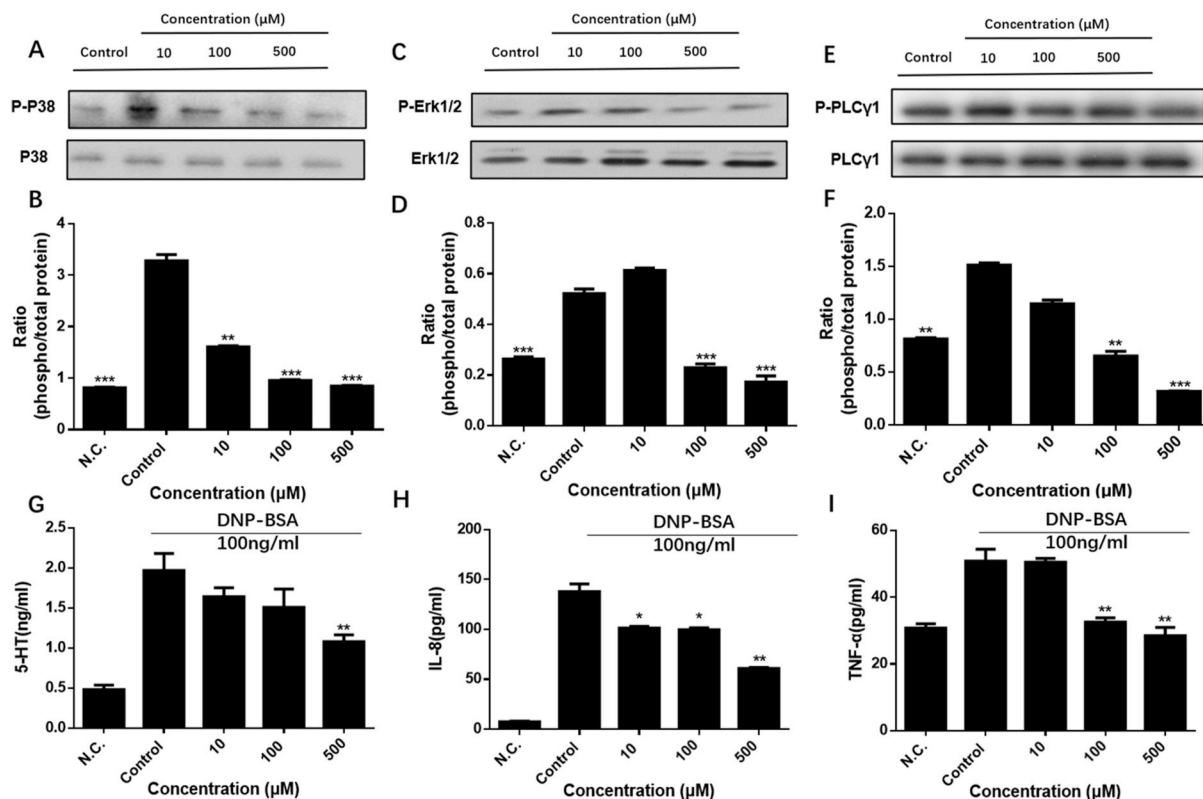


Fig. 4. PF decreased IgE-mediated key protein phosphorylation and cytokine release in LAD2 cells. (A–F) PF inhibited DNP-BSA induced phosphorylation level rise of P38, Erk1/2, and PLCγ1 dose-dependently. (G–I) PF inhibited key cytokines release closely related to allergic reaction, such as 5-HT, IL-8, TNF-α in a dose-dependent manner. (Experiments were repeated three times, and data are represented as mean ± S.D. And analyzed using two-tailed unpaired Student's t-test. Differences were considered significant at $*P < .05$, $**P < .01$, and $***P < .005$ vs Control), $n = 3$.

TYR131, GLU132, HIS134) and the bond length are 1.91, 1.84, 2.22, 2.01, 1.86 Å, respectively. PF bind with β subunit though 3 hydrogen bonds with 2 residues (ALA329, 1.97, 2.20 Å; ALA428, 1.94 Å). The total score, Crash, and Polar were respectively 5.3541, −1.0904, and 4.7132 for the α subunit and 4.4930, −1.7884, and 2.9134 for the β subunit. SPR revealed that PF had a strong affinity interaction with FcεRI α subunit

and the K_D value was $(7.08 \pm 0.97) \times 10^{-6}$ M. Based on the information above, it was considered that PF binds to the extracellular α subunit to inhibit FcεRI activation.

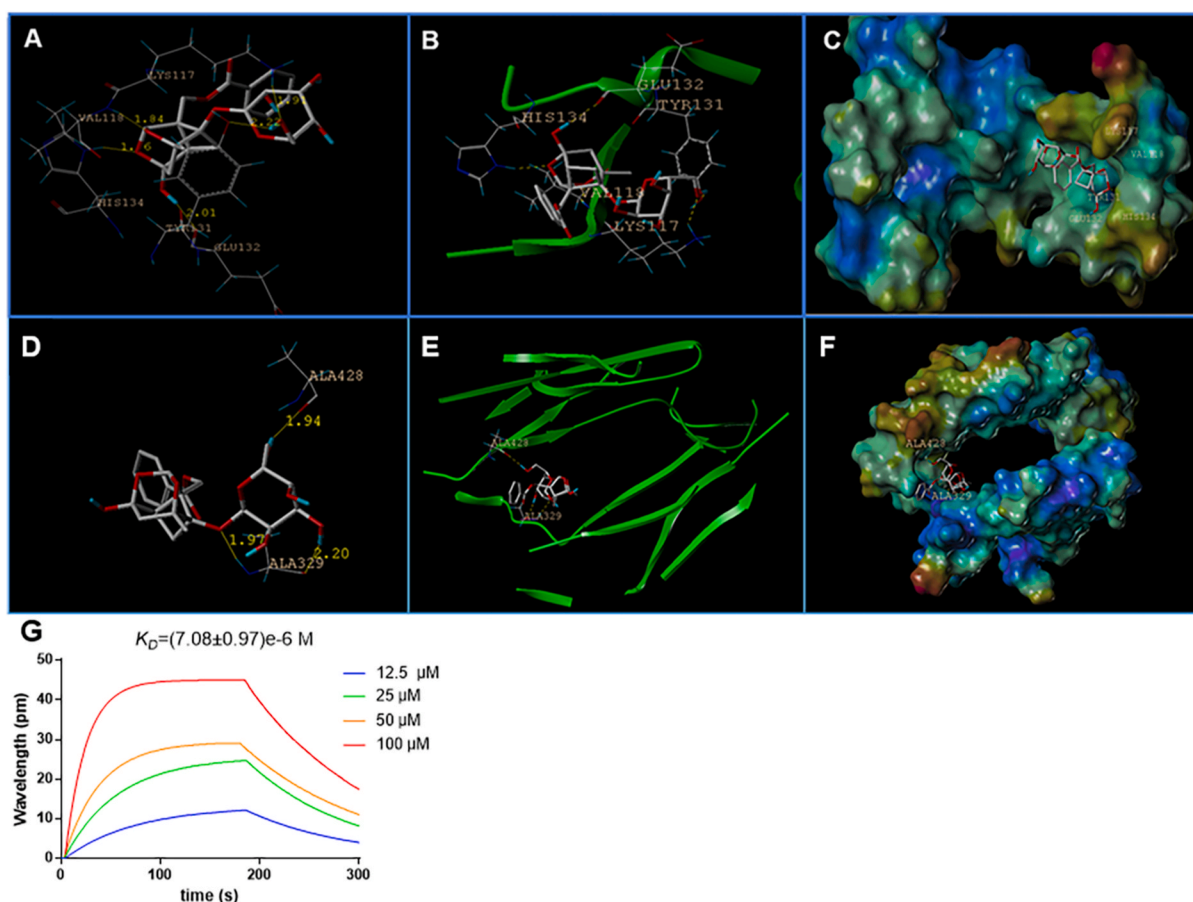


Fig. 5. PF bind to FcεRI protein receptor as seen from molecular docking assay. The docking results showed that PF bind to the FcεRI α subunit through 5 hydrogen bonds with 5 residues (LYS117, VAL118, TYR131, GLU132, HIS134) and the bond length are 1.91, 1.84, 2.22, 2.01, 1.86 Å, respectively (A–C). The β subunit bind with PF through 3 hydrogen bonds with 2 residues (ALA329, 1.97, 2.20 Å; ALA428, 1.94 Å) as shown in (D–F). The total score, Crash, and Polar were respectively 5.3541, −1.0904, and 4.7132 for the α subunit and 4.4930, −1.7884, and 2.9134 for the β subunit. SPR revealed that PF had a strong affinity interaction with FcεRI α subunit and the K_D value was $(7.08 \pm 0.97) \times 10^{-6}$ M in (G). Based on the information above, it was considered that PF binds to the extracellular α subunit to inhibit FcεRI activation.

3.6. PF inhibited IgE-mediated local inflammatory reactions

A mouse model of ovalbumin (OVA)-induced skin inflammation, which was previously shown to be IgE-dependent, was used to investigate the anti-anaphylactic effect of DA. The mice were sensitized with OVA (200 μg/kg) for 48 h. The treatment group was intragastrically administered different concentrations (0.625, 1.25, and 2.5 mg/kg) of PF. Five microliters of OVA (100 μg/ml) in saline were injected into the left hind paws of mice to induce local inflammatory reactions; an equal volume of saline was injected into the right hind paws as control. The increase in hind paw thickness and extravasation of Evans blue were shown to be attenuated in a dose-dependent manner (Fig. 6A–C).

In addition, we analyzed the paw skin sections for degranulated MCs by avidin staining and found that PF antagonized MCs *in vivo* to cause their degranulation (Fig. 6D). PF inhibited MC degranulation in a dose-dependent manner (Fig. 6E). The inhibitory effect was observed as a decrease in hemangiectasis, which was caused by the reduction in histamine release. Subsequently, PF treatment dose-dependently decreased the release of histamine (Fig. 6F) and cytokines, such as IL-8 (Fig. 6G) and MCP-1 (Fig. 6H), in the serum.

4. Discussion

MCs, which are located at the interface between the environment and host, play a vital role in the immune system (Huber and Gibbs, 2015). They respond to immunogenic stimuli to initiate host defense and

hypersensitive reactions by releasing proinflammatory mediators such as histamine and β-hexosaminidase as well as cytokines. Thus, inhibition of MC activation is of great significance in alleviating allergic symptoms such as atopic dermatitis, allergic asthma, and urticaria. As FcεRI and/or IgE are required for the activation of MCs by antigens, blocking either of them can interrupt the effect of antigens (MacGlashan, 2012; Monino-Romero et al., 2019). A previous study reported that anti-IgE methods reduced not only IgE concentrations but also FcεRI expression. Anti-IgE monoclonal antibodies, such as omalizumab, have been widely used in clinical practice (Metz et al., 2017). Omalizumab significantly reduced IgE levels in serum by binding specifically with IgE. However, its application is limited because of the high cost and inconvenience of injection (Metz et al., 2017; Kupczyk and Kuna, 2014). FcεRI, which is present on the surface of MC membrane, is responsible for the downstream signaling pathway related to MC activation.

PF, a main component of *Radix Paeoniae Alba*, has been used in traditional Chinese medicine for its outstanding anti-inflammatory effects (Xin et al., 2019; Zhang and Wei, 2020). The therapeutic effects of PF on many diseases, such as arthritis, liver injury, allergic contact dermatitis, psoriasis, asthma, and autoimmune encephalomyelitis, have already been reported (Zhao et al., 2019; Li et al., 2018; Zhai et al., 2018; Wang and Cheng, 2018; Shao et al., 2019; Shou et al., 2019; Havard et al., 2011). Various signaling pathways are regulated by PF (Xin et al., 2019), including GPCR, MAPK/NF-κB, PI3K/Akt/mTOR, JAK2/STAT3, and TGFβ/Smad pathways. In addition, PF inhibits the activation, proliferation, and differentiation of B lymphocytes, T lymphocytes,

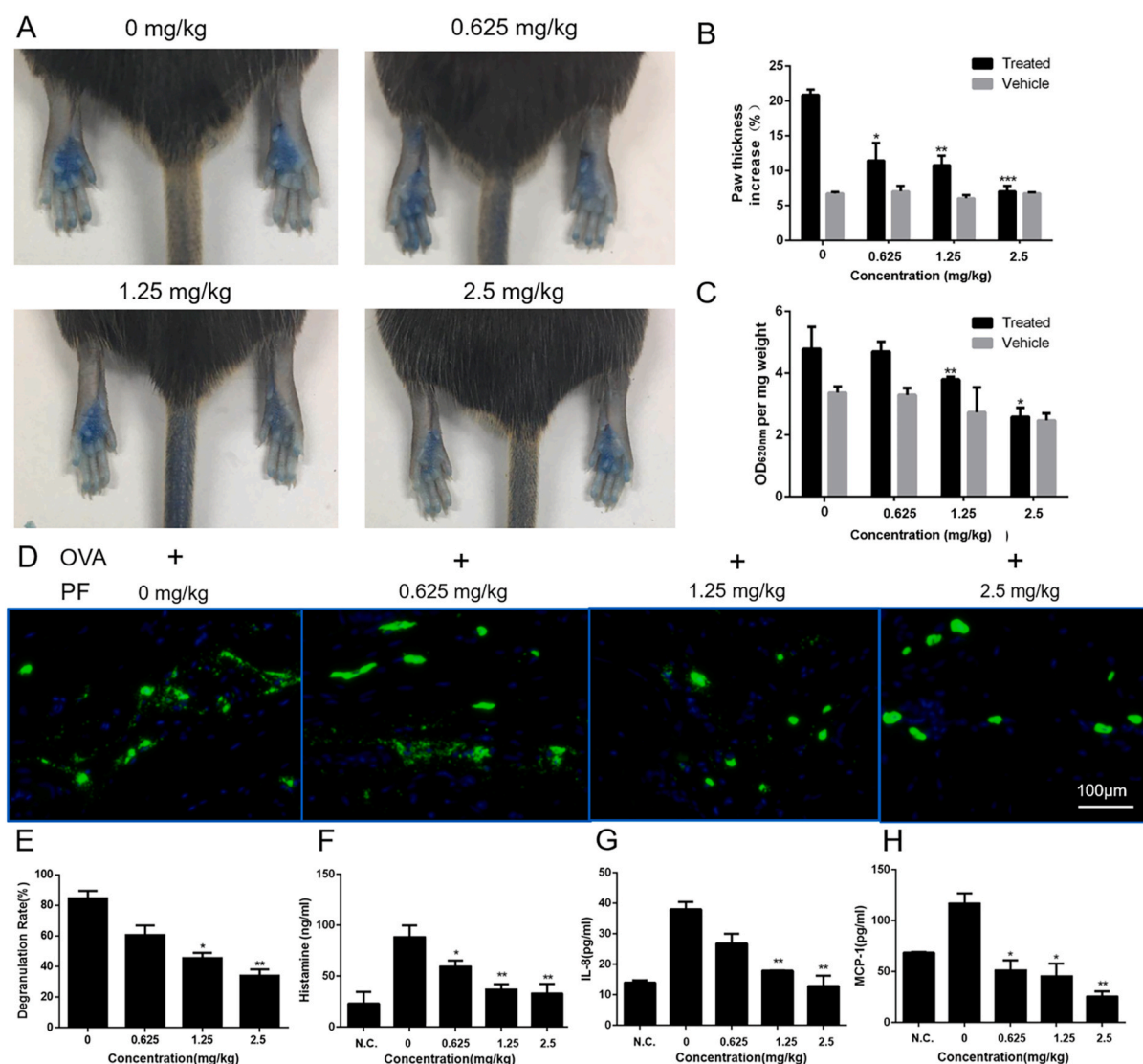


Fig. 6. PF inhibited IgE-mediated local allergic inflammation by inhibiting mast cells (MCs) degranulation. Mice were sensitized with OVA 48 h ago. After oral administration of PF at different concentrations (0.625, 1.25 and 2.5 mg/kg), 100 μ g/ml OVA was injected through the tail vein. (A–C) PF reduced the degree of swelling and Evans blue exudation of mice hind paws induced by OVA in a dose dependent manner. (D) Avidin staining of paw skin. (E) Degranulation rate of MCs in paw skin. (F–G) Inflammatory mediators including histamine, IL-8, TNF- α in serum were inhibited by PF in a dose dependent manner. There were five mice in each group. Experiments were repeated three times, and data are represented as mean $\pm$ S.D. And analyzed using two-tailed unpaired Student's t-test. Differences were considered significant at * $P < .05$, ** $P < .01$, and *** $P < .005$ vs Control).

macrophages, and DCs.

In the present study, we found that PF significantly decreased IgE-dependent MC degranulation in a dose-dependent manner both *in vitro* and *in vivo*. The release of cytokines during allergic reactions was also inhibited by PF. Moreover, PF bound more strongly to the Fc ϵ RI α subunit than to the β subunit, resulting in reduced IgE binding and MC activation. SPR revealed that PF had a strong affinity interaction with Fc ϵ RI α subunit and the K_D value was $(7.08 \pm 0.97) \times 10^{-6}$ M. Interestingly, as we found by molecular docking, PF shared the same residuals (TRY131 and GLU132) with IgE when interacts with α subunit of Fc ϵ RI. It suggested that PF can occupy certain residuals in α subunit of Fc ϵ RI and have the ability to inhibit IgE bind with Fc ϵ RI. The interaction of Fc ϵ RI and other factors (Lyn and Fyn) can regulate Syk and downstream signals. Syk regulates the production of inflammatory cytokines by activating the downstream molecules. To confirm the effects of PF on MCs, the expression of Lyn, Akt, and PKC proteins was measured. PF showed an inhibitory effect by downregulating the phosphorylation of key kinases involved in the regulation of Ca^{2+} ion influx and cytokine

synthesis, such as Lyn and PKC and downstream key proteins. In summary, we have confirmed theoretically and experimentally that PF can prevent binding of IgE to Fc ϵ RI α subunit, thereby inhibiting mast cell activation and allergic reaction. However, how does the receptor change its conformation and lead to key protein decrease is still unclear.

In our previous work, it was found that PF also antagonized MRGPRX2, thereby inhibiting pseudo-allergic reaction induced by C48/80. It is found that pseudo-allergy affected by PF was more obvious though the experimental results of β -hexosaminidase release and calcium influx. Interestingly, molecular docking simulations of PF interactions with both proteins found that PF and Fc ϵ RI interactions were stronger and scored higher. The seemingly contradictory results need further study.

In conclusion, PF inhibited IgE-mediated allergic action both *in vivo* and *in vitro*. PF binds to the extracellular α subunit to inhibit Fc ϵ RI activation and suppress the Lyn/PKC pathway. Thus, PF can be used as a potential anti-IgE-mediated allergy drug.

CRediT authorship contribution statement

Yongjing Zhang: Conceptualization, Methodology, Writing - original draft. **Shiling Hu:** Data curation, Investigation. **Shuai Ge:** Investigation, Software. **Jue Wang:** Formal analysis, Visualization. **Langchong He:** Writing - review & editing, Supervision, Resources.

Declaration of competing interest

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the National Natural Science Foundation of China [Grant number: 81930096], Natural Science Foundation of Shaanxi Province [Grant number: 2016JQ8025].

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ejphar.2020.173415>.

References

- Circu, M., Cardelli, J., Barr, M.P., O'Byrne, K., Mills, G., El-Osta, H., 2017. Modulating lysosomal function through lysosome membrane permeabilization or autophagy suppression restores sensitivity to cisplatin in refractory non-small-cell lung cancer cells. *PloS One* 12, e0184922.
- Eckl-Dorna, J., Froschl, R., Lupinek, C., Kiss, R., Gattinger, P., Marth, K., Campana, R., Mittermann, I., Blatt, K., Valent, P., Selb, R., Mayer, A., Gangl, K., Steiner, I., Gamper, J., Perkmann, T., Ziegelmayer, P., Gevaert, P., Valenta, R., Niederberger, V., 2018. Intranasal administration of allergen increases specific IgE whereas intranasal omalizumab does not increase serum IgE levels A pilot study. *Allergy* 73, 1003–1012.
- Espinosa, E., Valitutti, S., 2018. New roles and controls of mast cells. *Curr. Opin. Immunol.* 50, 39–47.
- Fei, F., Aa, L.X., Qi, Q., Sun, R.B., Yan, C.X., Aa, J.Y., Wang, G.J., 2019. Paeoniflorin inhibits Th1 and Th17 cells in gut-associated lymphoid tissues to produce anti-arthritis activities. *Inflammopharmacology* 27, 1193–1203.
- Havard, S., Scola, A.M., Kay, L.J., Ishmael, S.S., MacGlashan, D.W., Peachell, P.T., 2011. Characterization of syk expression in human lung mast cells: relationship with function. *Clin. Exp. Allergy* 41, 378–388.
- Holdom, M.D., Davies, A.M., Nettleship, J.E., Bagby, S.C., Dhaliwal, B., Girardi, E., Hunt, J., Gould, H.J., Beavil, A.J., McDonnell, J.M., Owens, R.J., Sutton, B.J., 2011. Conformational changes in IgE contribute to its uniquely slow dissociation rate from receptor Fc epsilon RI. *Nat. Struct. Mol. Biol.* 18, 571. U187.
- Hu, B., Xu, G.T., Zhang, X.M., Xu, L., Zhou, H., Ma, Z.Y., Shen, X.H., Zhu, J., Shen, R.L., 2018. Paeoniflorin attenuates inflammatory pain by inhibiting microglial activation and akt-NF-kappa B signaling in the central nervous system. *Cell. Physiol. Biochem.* 47, 842–850.
- Huber, M., Gibbs, B.F., 2015. SHIP1 and the negative control of mast cell/basophil activation by supra-optimal antigen concentrations. *Mol. Immunol.* 63, 32–37.
- Johansson, S.G.O., 2016. The discovery of IgE. *J. Allergy Clin. Immunol.* 137, 1671–1673.
- Kupczyk, M., Kuna, P., 2014. Omalizumab in an allergology clinic: real life experience and future developments. *Postępy Dermatologii i Alergologii* 31, 32–35.
- Li, X.Y., Wang, Y., Wang, K., Wu, Y.G., 2018. Renal protective effect of Paeoniflorin by inhibition of JAK2/STAT3 signaling pathway in diabetic mice. *Bioscience Trends* 12, 168–176.
- Liu, B.H., Lin, J., Bai, L.X., Zhou, Y., Lu, R.R., Zhang, P.C., Chen, D.D., Li, H.L., Song, J.P., Liu, X.S., Wu, Y.D., Wu, J.B., Liang, C.L., Zhou, J.Y., 2019. Paeoniflorin inhibits mesangial cell proliferation and inflammatory response in rats with mesangial proliferative glomerulonephritis through PI3K/AKT/GSK-3 beta pathway. *Front. Pharmacol.* 10.
- MacGlashan, D.W., 2012. IgE-dependent signaling as a therapeutic target for allergies. *Trends Pharmacol. Sci.* 33, 502–509.
- Metz, M., Staubach, P., Bauer, A., Brehler, R., Gericke, J., Kangas, M., Ashton-Chess, J., Jarvis, P., Georgiou, P., Canvin, J., Hillenbrand, R., Erpenbeck, V.J., Maurer, M., 2017. Clinical efficacy of omalizumab in chronic spontaneous urticaria is associated with a reduction of Fc epsilon RI-positive cells in the skin. *Theranostics* 7, 1266–1276.
- Monino-Romero, S., Lexmond, W.S., Singer, J., Bannert, C., Amoah, A.S., Yazdanbakhsh, M., Boakye, D.A., Jensen-Jarolim, E., Fiebig, E., Szepefalusi, Z., 2019. Soluble FcεRI: a biomarker for IgE-mediated diseases. *Allergy* 74, 1381–1384.
- Oettgen, H.C., Burton, O.T., 2015. IgE and mast cells: the endogenous adjuvant. *Adv. Immunol.* 127, 203–256, 127.
- Peden, D.B., 2018. Effect of pollution on allergy/immunology. *J. Allergy Clin. Immunol.* 141, 878–879.
- Shao, Y.X., Gong, Q., Qi, X.M., Wang, K., Wu, Y.G., 2019. Paeoniflorin ameliorates macrophage infiltration and activation by inhibiting the TLR4 signaling pathway in diabetic nephropathy. *Front. Pharmacol.* 10.
- Shimamaka, Y., Kono, N., Taketomi, Y., Arita, M., Okayama, Y., Tanaka, Y., Nishito, Y., Mochizuki, T., Kusuhara, H., Adibekian, A., Cravatt, B.F., Murakami, M., Arai, H., 2017. Omega-3 fatty acid epoxides are autocrine mediators that control the magnitude of IgE-mediated mast cell activation. *Nat. Med.* 23, 1287.
- Shou, Q.Y., Jin, L., Lang, J.L., Shan, Q.Y., Ni, Z.N., Cheng, C.P., Li, Q.L., Fu, H.Y., Cao, G., 2019. Integration of metabolomics and transcriptomics reveals the therapeutic mechanism underlying paeoniflorin for the treatment of allergic asthma. *Front. Pharmacol.* 9.
- Simons, F.E.R., Sheikh, A., 2013. Anaphylaxis: the acute episode and beyond. *BMJ Br. Med. J. (Clin. Res. Ed.)* 346.
- Wang, G.H., Cheng, N., 2018. Paeoniflorin inhibits mast cell-mediated allergic inflammation in allergic rhinitis. *J. Cell. Biochem.* 119, 8636–8642.
- Wang, N., Che, D.L., Zhang, T., Liu, R., Cao, J., Wang, J., Zhao, T.T., Ma, P.Y., Dong, X.Z., He, L.C., 2018. Saikosaponin A inhibits compound 48/80-induced pseudo-allergy via the Mrgrpx2 pathway in vitro and in vivo. *Biochem. Pharmacol.* 148, 147–154.
- Xin, Q.Q., Yuan, R., Shi, W.L., Zhu, Z.C., Wang, Y., Cong, W.H., 2019. A review for the anti-inflammatory effects of paeoniflorin in inflammatory disorders. *Life Sci.* 237.
- Yip, K.H., Kolesnikoff, N., Hauschild, N., Biggs, L., Lopez, A.F., Galli, S.J., Kumar, S., Grimbaldston, M.A., 2016. The Nedd4-2/Ndfip1 axis is a negative regulator of IgE-mediated mast cell activation. *Nat. Commun.* 7.
- Zhai, W.J., Ma, Z.H., Wang, W.J., Song, L., Yi, J.F., 2018. Paeoniflorin inhibits Rho kinase activation in joint synovial tissues of rats with collagen-induced rheumatoid arthritis. *Biomed. Pharmacother.* 106, 255–259.
- Zhang, L.L., Wei, W., 2020. Anti-inflammatory and immunoregulatory effects of paeoniflorin and total glucosides of paeony. *Pharmacol. Therapeut.* 207.
- Zhao, L., Chang, Q., Huang, T., Huang, C.L., 2018. Paeoniflorin inhibits IL-1 beta-induced expression of inflammatory mediators in human osteoarthritic chondrocyte. *Mol. Med. Rep.* 17, 3306–3311.
- Zhao, M.C., Liu, L., Zheng, Y.T., Liu, G.R., Che, B., Li, P.H., Chen, H.X., Dong, C.Z., Lin, L., Du, Z.Y., 2019. Anti-inflammatory effects of paeoniflorin from *Paeonia lactiflora* Pall. on human corneal epithelial cells and a mouse model of dry eye disease. *RSC Adv.* 9, 12998–13006.

PF hind paw 药足趾

	Before challenge		After challenge	
	L	R	L	R
0	0.234	0.232	0.286	0.246
	0.226	0.236	0.272	0.251
	0.228	0.236	0.277	0.250
	0.229	0.230	0.283	0.244
	0.233	0.231	0.282	0.245
0.625	0.229	0.228	0.255	0.244
	0.232	0.232	0.271	0.247
	0.228	0.23	0.245	0.251
	0.23	0.232	0.267	0.251
	0.226	0.229	0.249	0.243
			0.252	0.244
1.25	0.231	0.228	0.253	0.246
	0.230	0.232	0.254	0.244
	0.229	0.230	0.262	0.247
	0.232	0.232	0.252	0.245
	0.228	0.229	0.251	0.242
		0.231	0.249	0.245
2.5	0.234	0.234	0.245	0.236
	0.232	0.225	0.246	0.242
	0.227	0.23	0.252	0.241
	0.228	0.228		
	0.231			

年 11 月 25 日

温度: °C

相对湿度: % 第 页

Weight 重量

	L	R	OD	
0	0.0559	0.0555	0.244	0.177
	0.0523	0.0507	0.251	0.153
	0.0517	0.0491	0.298	0.163
	0.0516	0.0520	0.248	0.174
	0.0552	0.0592	0.254	0.183
0.625	0.0546	0.0536	0.267	0.172
	0.0495	0.0536	0.252	0.175
	0.0524	0.0538	0.269	0.161
	0.0491	0.0512	0.254	0.172
	0.0534	0.0522	0.251	0.169
1.25	0.0549	0.0535	0.217	0.233
	0.0537	0.0517	0.222	0.161
	0.0522	0.0531	0.208	0.152
	0.0519	0.0512	0.192	0.157
	0.0512	0.0492	0.199	0.153
2.5	0.0559	0.0555	0.169	0.156
	0.0523	0.0507	0.148	0.142
	0.0517	0.0491	0.188	0.157
	0.0516	0.0520	0.153	0.152
	0.0552	0.0592	0.146	0.166

18 年 12 月 1 日		Before Challenge		After Challenge	
PF Hind Paw		温度: °C	相对湿度: %	第	页
		R	L	R	
0.	0.232	0.234	0.286	0.248	
	0.233	0.231	0.283	0.243	
	0.228	0.230	0.278	0.244	
	0.232	0.234	0.279	0.248	
	0.234	0.230	0.282	0.245	
0.625	0.237	0.235	0.256	0.252	
	0.228	0.231	0.251	0.249	
	0.227	0.223	0.249	0.238	
	0.232	0.228	0.255	0.247	
	0.231	0.23	0.259	0.246	
1.25	0.238	0.235	0.263	0.250	
	0.228	0.231	0.254	0.248	
	0.233	0.223	0.254	0.236	
	0.235	0.228	0.258	0.244	
	0.234	0.230	0.264	0.244	
2.5	0.228	0.228	0.247	0.241	
	0.229	0.229	0.249	0.243	
	0.231	0.223	0.246	0.234	
	0.233	0.232	0.251	0.244	
	0.233	0.230	0.251	0.241	

Weight of Hind Paw and OD

18 年 11 月 19 日

温度: °C

相对湿度: %

第 页

足趾重量与 OD

	L	R	L	R
0	0.0523	0.0506	0.294	0.174
	0.0504	0.0485	0.287	0.177
	0.0486	0.0488	0.221	0.192
	0.0532	0.0526	0.253	0.147
	0.0564	0.0547	0.256	0.189
0.625	0.0513	0.0508	0.284	0.172
	0.0534	0.0521	0.280	0.181
	0.0520	0.0508	0.241	0.188
	0.0526	0.0516	0.251	0.182
	0.0548	0.0555	0.237	0.182
1.25	0.0511	0.0504	0.207	0.148
	0.0506	0.0482	0.193	0.158
	0.0542	0.0532	0.211	0.162
	0.0511	0.0489	0.203	0.157
	0.0524	0.0537	0.211	0.161
2.5	0.0523	0.0506	0.133	0.138
	0.0504	0.0485	0.136	0.138
	0.0486	0.0488	0.148	0.141
	0.0532	0.0526	0.145	0.163
	0.0564	0.0547	0.153	0.151

18年 11月 18日

温度: °C

相对湿度: %

第 页

PF Hind Paw

芍药苷足趾

Before Challenge 前

1/3 After Challenge

	L	R	L	R
0	1. 0.236	0.238	0.282	0.252
	2. 0.232	0.234	0.284	0.248
	3. 0.234	0.232	0.285	0.247
	4. 0.233	0.233	0.282	0.248
	5. 0.236	0.236	0.284	0.252
0.625	1. 0.224	0.226	0.250	0.242
	2. 0.23	0.234	0.260	0.251
	3. 0.234	0.232	0.254	0.248
	4. 0.233	0.235	0.256	0.249
	5. 0.241	0.238	0.277	0.254
1.25	1. 0.231	0.226	0.254	0.241
	2. 0.232	0.234	0.252	0.248
	3. 0.228	0.232	0.247	0.246
	4. 0.224	0.235	0.250	0.248
	5. 0.228	0.238	0.247	0.251
2.5	1. 0.228	0.229	0.244	0.241
	2. 0.228	0.23	0.245	0.241
	3. 0.231	0.228	0.248	0.239
	4. 0.235	0.232	0.252	0.244
	5. 0.223	0.228	0.241	0.24

年 月 日 温度: °C 相对湿度: % 第 页

Weight 重量

	L	R	L	R
0	0.0551	0.0542	0.272	0.179
	0.0523	0.0532	0.296	0.188
	0.0511	0.0528	0.258	0.169
	0.0498	0.0512	0.272	0.203
	0.0533	0.0522	0.268	0.167
0.625	0.0477	0.0469	0.258	0.171
	0.0498	0.0502	0.255	0.183
	0.0517	0.0521	0.247	0.172
	0.0508	0.051	0.262	0.166
	0.0511	0.0504	0.264	0.176
1.25	0.0512	0.0515	0.185	0.147
	0.0523	0.0528	0.203	0.154
	0.0485	0.0501	0.182	0.15
	0.0488	0.0472	0.188	0.146
	0.0523	0.0527	0.193	0.241
2.5	0.0551	0.0542	0.162	0.161
	0.0523	0.0532	0.138	0.154
	0.0511	0.0528	~~0.138~~ 0.155	0.156
	0.0498	0.0512	0.162	0.142
	0.0533	0.0522	0.137	0.138