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Berberine suppresses mast cell-mediated allergic responses *via* regulating FcεRI-mediated and MAPK signaling

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ABSTRACT

The anti-allergic effect of berberine was evaluated in cellular and animal models of allergic responses. In this study, the results of the *in vitro* model of immunoglobulin (Ig) *E*-mediated mast cell degranulation showed that berberine significantly inhibited the release of *β*-hexosaminidase (*β*-HEX), histamine, IL-4 and TNF-*α* in rat basophilic leukemia cells (RBL-2H3 cells). Pretreatment with berberine prevented morphological changes in IgE-stimulated RBL-2H3 cells such as the recovery of an elongated shape. Pretreatment with berberine also suppressed the phosphorylation of antigen-induced Lyn, Syk, and Gab2, thus suppressing the downstream MAPK pathways. In the *in vivo* model of allergic responses, administration of berberine inhibited passive cutaneous anaphylaxis (PCA) in mice. The above results indicate berberine could suppress mast cell activation and allergic responses.

1. Introduction

Anaphylaxis is defined as a serious allergic hypersensitivity reaction that is rapid in onset and can be life-threatening and even fatal, and it is rapidly increasing worldwide [1–3]. Among allergic diseases, type I hypersensitivity reactions include mast cell-mediated allergic diseases such as allergic rhinitis, asthma and atopic eczema [4–6]. Type I hypersensitivity is caused by many environmental factors including certain foods, dust, mites and pollen, which evoke the production of antigen-specific IgE antibodies that bind to high-affinity IgE receptors (FcεRI) on the surface of mast cells or basophils [7–9].

Mast cells are one kind of key effector cells in IgE-mediated allergic responses [10]. After IgE binds to the FcεRI, mast cells can be activated by crosslinking of the FcεRIs with multivalent antigens, resulting in the degranulation response and secretion of preformed mediators such as *β*-hexosaminidase (*β*-HEX) and histamine in early phase, as well as newly inflammatory mediators including prostaglandins, interleukins and other proinflammatory cytokines in the late phase of allergic responses [11,12].

FcεRI cross-linking initiates mast cell activation through Src tyrosine kinase families, such as Lck/Yes novel tyrosine kinase (Lyn) and spleen tyrosine kinase (Syk) [13]. Lyn activates Syk, which plays a critical role in mast cell activation *via* activating downstream molecules, such as GRB2-associated binding protein 2 (Gab2), thus regulating inflammatory cytokine production [14]. FcεRI-mediated signaling an lso induces the activation of mitogen-activated protein kinases (MAPKs) to induce proinflammatory genes, which potentiate inflammatory immune responses *via* the secretion of cytokines [15].

Recently, antihistamines, antileukotrienes, mast cell stabilizers, and steroids have been broadly used for the treatment of allergic diseases [6,16]. However, the prolonged used of these drugs results in undesirable side effects, including drowsiness, dry mouth and upset stomach [17–20]. As such, natural products have drawn attention due to their unique effects in various diseases. Berberine is an isoquinoline alkaloid that exists in many medicinal plants such as *Coptis chinensis*, *Phellodendron japonicum*, and *Berberis aquifolium* [21]. Berberine has biological activities including antimicrobial, antitumor, antidiabetic, and anti-inflammatory. Berberine is widely used in the treatment of diarrhea, bacterial gastroenteritis, dysentery, and other digestive diseases [22,23]. Previous studies have demonstrated that berberine can attenuate 2,4-dinitrofluorobenzene (DNFB)-induced allergic contact dermatitis, which belongs to the T-cell-mediated delayed-type hypersensitivity (DTH) response [24]. FAHF-2 (a berberine-containing food allergy herbal formula-2) was also found to persistently protect against peanut anaphylaxis [25]. These findings suggest that berberine has anti-allergic properties. The beneficial effects of berberine both on dextran sulfate sodium (DSS)- and trinitrobenzene sulfonic acid (TNBS)-induced colitis in mice were also reported [26,27]. These results showed that berberine significantly reduced the inflammation in mice. Hence, we evaluated the anti-allergic effect of berberine on IgE-

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mediated mast cells degranulation, histamine-induced rise of vascular permeability in mice and rats, and passive cutaneous anaphylaxis in mice.

2. Materials and methods

2.1. Animals

Male BALB/c mice weighing 18–22 g were obtained from the Laboratory Animal Center of Jilin University. The mice were fed standard laboratory chow and water *ad libitum* and were housed under 12-hour light/12-hour dark conditions in an air-conditioned room (temperature 25 ± 2 °C, relative humidity 55 ± 5%). All animals in this study were performed in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Jilin University.

2.2. Reagents

The following reagents were used in this study: berberine, ketotifen, palmatine, and jatrorrhizine (purity ≥98%) (Sigma, St. Louis, MO, USA); Evans blue (Unchem, Shanghai, China); histamine, TNF-α and IL-4 ELISA Kits (R & D Systems, Inc., Minneapolis, MN, USA). The following kits were used in this study: RIPA Lysis Buffer (GenStar, Beijing, China); BeyoECL Star Kit (Beyotime, Shanghai, China); Color Prestained Protein Marker (GenStar, Beijing, China); and BCA Protein Assay Kit (Beyotime, Shanghai, China). Anti-Syk, anti-phospho-Syk, anti-Lyn, anti-phospho-Lyn, Anti-Gab2, anti-phospho-Gab2, anti-JNK, anti-phospho-JNK, anti-ERK, anti-phospho-ERK, anti-P38, and anti-phospho-P38 antibodies were purchased from Bioss (Beijing, China). Bay 61-3606, specific inhibitor of Lyn/Syk pathway, was purchased from Selleck (Shanghai, China); Goat anti-mouse IgG and goat anti-rabbit IgG antibodies were purchased from Life Science (Santa Cruz, CA, USA).

2.3. Cell culture

The RBL-2H3 cell line (ATCC#CRL-2256™) was obtained from the National Infrastructure of Cell Line Resource (Shanghai, China) and cultured in MEM supplemented with 15% fetal bovine serum (FBS), 100 U/mL penicillin and 100 ng/mL streptomycin at 37 °C in a 5% CO_2 humidified incubator.

2.4. Cell activity assay

To confirm that the effect of berberine on the decrease of mast cell degranulation was not due to its cytotoxicity on RBL-2H3 cells, the effect of berberine, ketotifen, palmatine, jatrorrhizine on the cell viability was checked by Thiazolyl blue tetrazolium bromide (MTT). The MTT results showed that the cell viability of RBL-2H3 cells was not affected by berberine, ketotifen, palmatine, jatrorrhizine at 30 μM.

2.5. β-HEX and histamine release assay

To evaluate the anti-allergic effect of berberine, the release of β-hexosaminidase and histamine, two indicators of degranulation, were examined. RBL-2H3 cells (6×10^5 cells/mL) were seeded into a 48-well plate and sensitized with 100 ng/mL of DNP-IgE overnight. The next day, the RBL-2H3 cells were pretreated with 30 μM of berberine, palmatine, jatrorrhizine, and berberine (0.3, 3 or 30 μM) for 1 h. The sensitized cells were triple washed with modified Tyrode's buffer [126 mM NaCl, 5.6 mM glucose, 4.0 mM KCl, 0.6 mM KH_2PO_4, 10.0 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.6 mM $MgCl_2/6H_2O$, 1.0 mM $CaCl_2$ and 0.1% bovine serum albumin (BSA)], 1 mL/times. The pretreated cells were then challenged with 250 ng/mL of DNP-HSA for 1 h. After the DNP-HSA challenge, the culture supernatants from each group were incubated in an ice bath for 10 min and then centrifuged (300 × *g*, 10 min) at 4 °C. Culture supernatants (50 μL) were transferred into separate wells, mixed with 50 μL of substrate solution (1 mM *p*-nitrophenyl *N*-acetyl-β-D-gluco-samine in 0.1 M Citric acid), and were then incubated for 1.5 h at 37 °C. The reaction was terminated by adding 200 μL of stop solution (0.1 M Na_2CO_3/$NaHCO_3$, pH 10.0) to each well. The absorbance was recorded at 405 nm with a microplate reader (Biotek, Vermont, USA).

After being sensitized with DNP-IgE, the culture supernatant was transferred and centrifuged (10,000 × *g*, 10 min) at 4 °C. The level of histamine in the culture supernatant was measured using the histamine ELISA kit according to the manufacturer's instructions.

2.6. Inflammatory cytokines assay

Allergic responses have been considered to be highly associated with mast cell degranulation and released inflammatory mediators such as IL-4 and TNF-α. After being sensitized with DNP-IgE, the culture supernatant was transferred and centrifuged (10,000 × *g*, 10 min) at 4 °C. Levels of TNF-α and IL-4 were determined by ELISA kits according to the manufacturer's instructions.

2.7. Toluidine blue staining

Toluidine blue is a synthetic basic dye, it can combine with the acidic material and heparin, histamine and other heterochromatic substances in mast cell, then shows different colors. After being sensitized with DNP-IgE, the cells were washed with phosphate buffered saline (PBS) and then incubated with 250 μL of 4% paraformaldehyde/PBS for 30 min at room temperature (RT). Cells were then stained with toluidine blue dye (0.1% *w/v* in 0.9% NaCl solution, pH 2.5) for 30 min. Images of the stained cells were then examined and captured using an inverted microscope.

2.8. Western blot

The proteins were isolated from treated RBL-2H3 cells. The protein concentrations were measured using the BCA Protein Assay Kit. Proteins were then separated using 10% SDS-PAGE and transferred to PVDF membranes. After blockage in 5% skim milk for 2 h, the blocked membrane was incubated with primary antibodies at 4 °C overnight. The secondary antibodies for 1 h at RT. Signals were visualized using an enhanced chemiluminescence reagent.

2.9. Animals and experimental protocol

After adaptive breeding for one week, all the animals were randomly divided into different groups: the control group, PCA group, chlorpheniramine (Chol.) group and berberine group. All the mice were orally administration at a dose of berberine (10 or 20 mg/kg) and chlorpheniramine (16 mg/kg), for 7 days, control and PCA groups received distilled water alone on the same schedule.

After IgE binds to the FcεRI, mast cells can be activated by cross-linking of the FcεRIs with specific antigen, which can result in the PCA reaction. Mice were sensitized by 100 ng DNP-IgE on the right ear, after 24 h, 0.5% Evans blue containing DNP-HSA was intravenously injected into tail vein. After 30 min of the challenge, all the ear was collected, and was soaked in acetone: saline (7:3) for 24 h. The absorbance was recorded at 610 nm with microplate reader.

2.10. Statistical analysis

All data were expressed as the mean ± standard error (SE). Statistical analyses were performed by one-way analysis of variance (ANOVA) using SPSS statistical software. When $p < 0.05$, $p < 0.01$ or $p < 0.001$, the differences between the two groups were considered

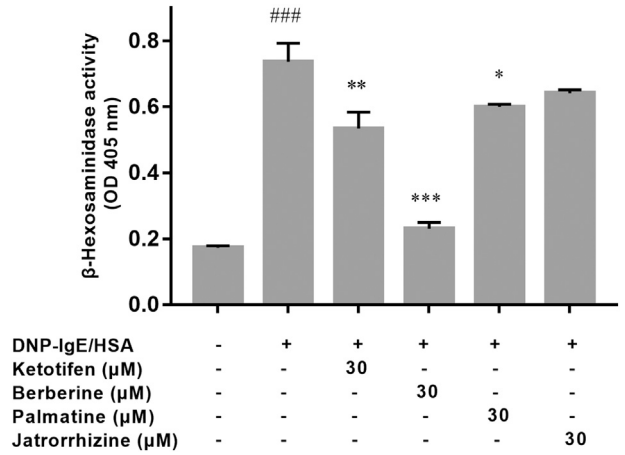

Fig. 1. Effect of berberine, palmatine, and jatrorrhizine on *β-HEX* release in IgE stimulated RBL-2H3 cells. The data were expressed as the mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, in comparison with DNP-IgE/HSA group; $^{\#\#\#}p < 0.001$ in comparison with control group.

significant.

3. Results

3.1. Effect of berberine, palmatine, and jatrorrhizine on β-HEX release in IgE stimulated RBL-2H3 cells

To evaluate whether berberine, palmatine, and jatrorrhizine inhibit mast cell degranulation, the levels of *β*-HEX release from RBL-2H3 cells were determined in all groups at 1 h after the DNP-HSA challenge. The levels of *β*-HEX after pretreatment with berberine were significantly lower than pretreatment with palmatine or jatrorrhizine. Furthermore, berberine at the concentration of 30 μM showed a similar effort as control group of releasing *β*-HEX level (Fig. 1). Therefore, we chose berberine for the follow-up experiment.

3.2. Effect of berberine on β-HEX and histamine release in IgE stimulated RBL-2H3 cells

β-HEX and histamine were two important markers of mast cell degranulation in RBL-2H3 [28]. To evaluate whether berberine treatment inhibits mast cell degranulation, the levels of *β*-HEX and histamine in RBL-2H3 cells were determined. Berberine and ketotifen fumarate both inhibited the *β*-HEX and histamine release of DNP-IgE/HSA-stimulated RBL-2H3 cells (Fig. 2A, B).

3.3. Effect of berberine on the levels of IL-4, TNF-α in IgE stimulated RBL-2H3 cells

IL-4 and TNF-*α* are major key proinflammatory cytokines during mast cell degranulation. In our present study, pretreatment with berberine and ketotifen fumarate markedly inhibited the overproduction of IL-4 and TNF-*α* (Fig. 3A, B).

3.4. Effect of berberine on toluidine blue staining in RBL-2H3 cells

Mast cell granules can be induced naturally by metachromatic staining such as toluidine blue [29]. We observed clear structure of an elongated shape and numerous intracytoplasmic granule content in normal RBL-2H3 cells (Fig. 4A). In contrast, after the challenge of DNP-HSA, the cells showed typical degranulation morphology including irregular shapes and poor and disorganized intracytoplasmic granule content. This indicated that mast cells were undergoing a degranulation process (Fig. 4B). Pretreatment with berberine and ketotifen fumarate restored elongated shape and intracytoplasmic granule content increase of IgE-sensitized RBL-2H3 cells (Fig. 4C–F).

3.5. Effect of berberine on FcεRI-mediated signaling in RBL-2H3 cells

Antigen binding to IgE in mast cells induced the cross-linking of FcεRI and activated the Lyn/Syk pathway that lead to calcium influx [30]. The phosphorylation of Lyn and Syk, key proteins of the signaling pathway, were markedly attenuated by berberine. Berberine or Bay 61-3606 also downregulated Gab2 (Fig. 5).

3.6. Effect of berberine on MAPK signaling in RBL-2H3 cells

It is well known that the production of proinflammatory cytokines such as IL-4 and TNF-*α* is influenced by the activation of MAPKs including JNK, ERK, and P38 MAPK, as well as their upstream kinases [13]. The phosphorylation of JNK, ERK and P38 in MAPK pathways was increased significantly in the DNP-IgE/HSA group compared to in the control group. However, these proteins were suppressed by berberine or Bay 61-3606 (Fig. 6).

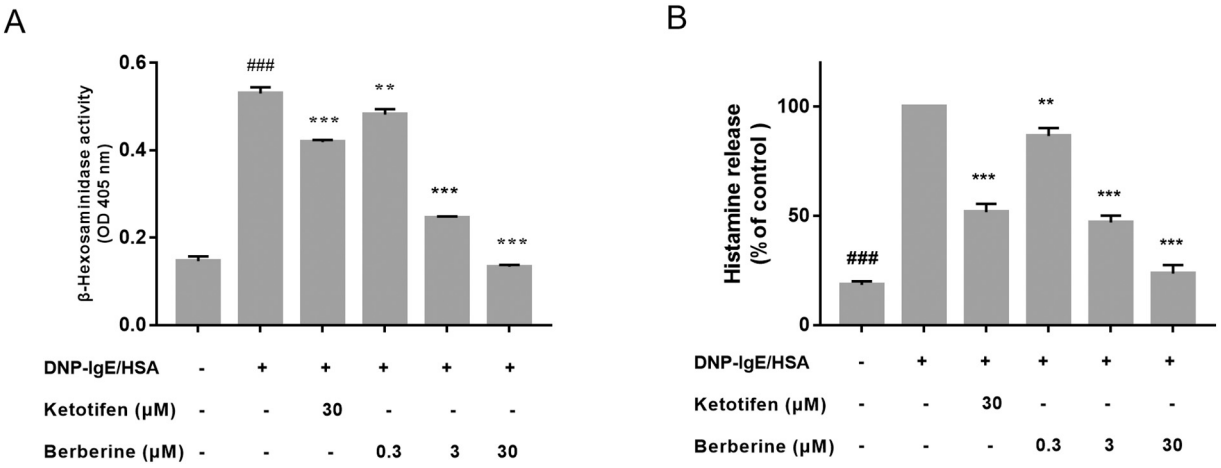

Fig. 2. Effect of berberine on *β*-HEX and histamine release in IgE stimulated RBL-2H3 cells. A: The level of *β*-HEX of berberine pretreated (0.3, 3 or 30 μM) in IgE sensitized RBL-2H3 cells. B: The level of histamine of berberine pretreated (0.3, 3 or 30 μM) in IgE sensitized RBL-2H3 cells. The data were expressed as the mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, in comparison with DNP-IgE/HSA group; $^{\#\#\#}p < 0.001$ in comparison with control group in panel A, $^{\#\#\#}p < 0.001$ in comparison with DNP-IgE/HSA group in panel B.

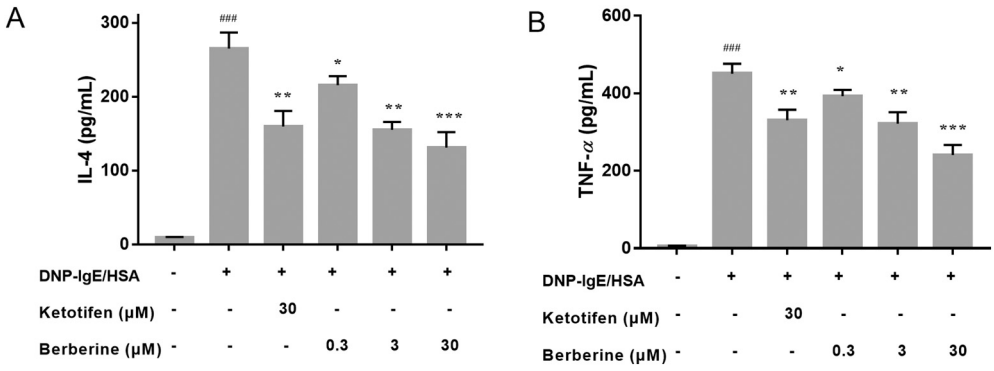

Fig. 3. Effect of berberine on the levels of IL-4, TNF-α in IgE stimulated RBL-2H3 cells. A: The level of IL-4 of berberine pretreated (0.3, 3 or 30 μM) in IgE stimulated RBL-2H3 cells. B: The level of TNF-α of berberine pretreated (0.3, 3 or 30 μM) in IgE stimulated RBL-2H3 cells. The data were expressed as the mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, in comparison with DNP-IgE/HSA group; $^{###}p < 0.001$ in comparison with control group.

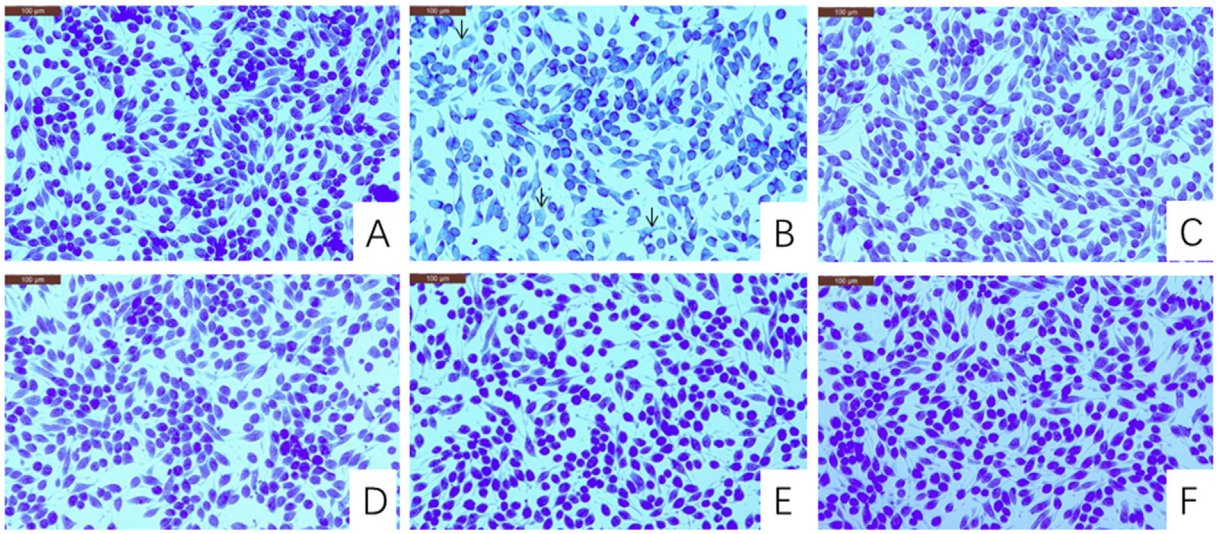

Fig. 4. Effect of berberine on toluidine blue staining in RBL-2H3 cells. A: Normal RBL-2H3 cells; B: IgE sensitized RBL-2H3 cells; C: Effect of pretreatment with ketotifen fumarate IgE sensitized RBL-2H3 cells; D-F: Effect of pretreatment with berberine (0.3, 3 or 30 μM) IgE sensitized RBL-2H3 cells. Arrows in B indicate that the cells morphology became irregular, and purple granules were released outside of the cells. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

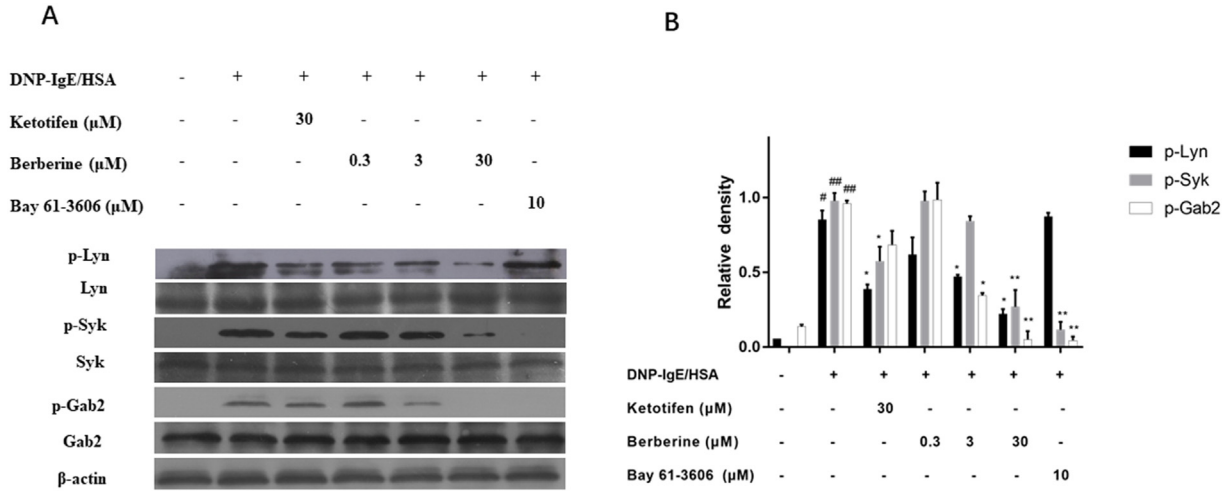

Fig. 5. Effect of berberine on FcεRI-mediated signaling in RBL-2H3 cells. A: Lyn, Syk, Gab2, and phosphorylation of Lyn, Syk, Gab2 were determined with western blot. B: Densitometric analysis. The data were expressed as the mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, in comparison with DNP-IgE/HSA group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ in comparison with control group.

3.7. Effect of berberine on PCA in mice

PCA in a mice model was used to confirm the inhibitory effect of berberine *in vivo*. Mice were sensitized with DNP-IgE and intravenously challenged with the DNP-HSA development PCA, which increased vascular permeability of skin. Oral administration with berberine and chlorpheniramine significantly suppressed PCA as observed by impaired extravasation of Evans blue dye in the skin (Fig. 7).

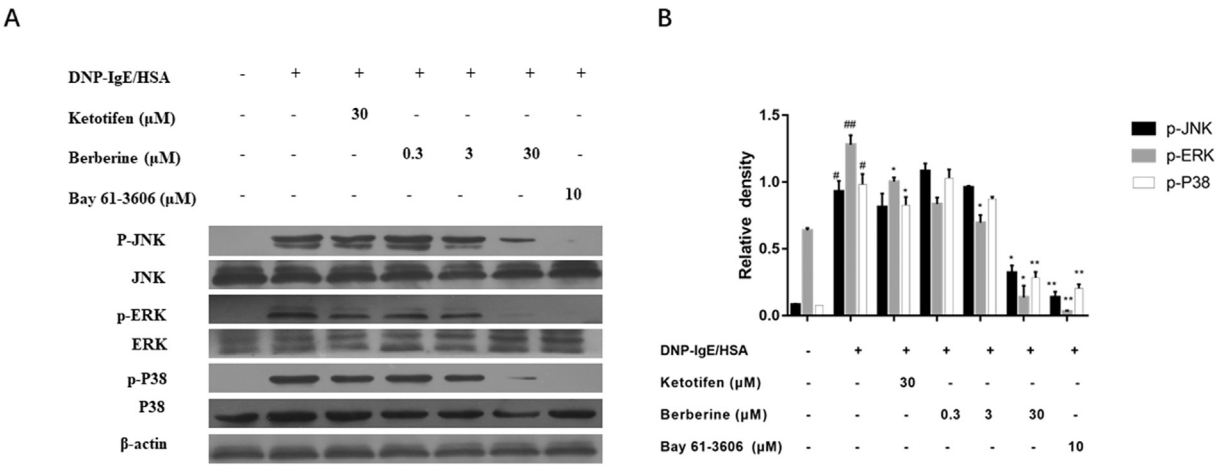

Fig. 6. Effect of berberine on MAPK signaling in RBL-2H3 cells. A: JNK, ERK, P38, and phosphorylation of JNK, ERK, P38 were determined with western blot. B: Densitometric analysis. The data were expressed as the mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, in comparison with DNP-IgE/HSA group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ in comparison with control group.

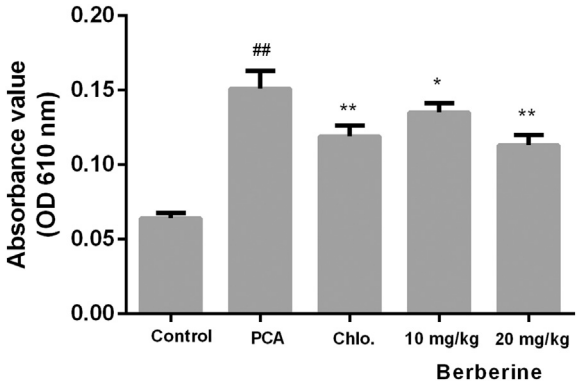

Fig. 7. Effect of berberine on PCA in mice. The data were expressed as the mean ± SE. $^{*}p < 0.05$, $^{**}p < 0.01$, in comparison with anti-serum group; $^{\#}p < 0.05$ in comparison with control group, $n = 10$/per group.

4. Discussion

Cellular models could provide an effective way for testing potential drugs that have been developed for studying the molecular basis of allergies [28]. RBL-2H3 cells have similar characteristics as mast cells, which can express the FcεRI and release a range of preformed and newly synthesized mediators that evoke type I hypersensitivity reaction. Therefore, we investigated the effect of berberine on the model of IgE-stimulated RBL-2H3 cell degranulation.

Allergic responses have been considered to be highly associated with mast cell degranulation and released inflammatory mediators such as histamine, IL-4 and TNF-*α* [31,32]. *β*-HEX and histamine were two important markers of mast cell degranulation in RBL-2H3 cells. In our study, berberine attenuated *β*-HEX and histamine release. IL-4 could promote Th2 cellular response development and promote B cells to produce IgE [33]. TNF-*α* could promote inflammation, leukocyte infiltration, and chemotaxis of both neutrophils and T cells [6,34]. Our research data showed that berberine reduced the levels of IL-4 and TNF-*α*. Furthermore, pretreatment with berberine also prevented morphological changes in IgE-stimulated RBL-2H3 cells such as the recovery of an elongated shape and an increase in intracytoplasmic granule content after toluidine blue staining. These findings suggest that berberine possesses an anti-allergic effect by inhibiting the levels of *β*-HEX, histamine, IL-4 and TNF-*α*, thus suppresses mast cell activation *in vitro*.

Furthermore, we have studied the FcεRI-mediated signaling in RBL-2H3 cells, which plays vital roles in mast cell activation. Therefore, the Lyn, Syk and Gab2 proteins were selected to evaluate how berberine suppressed mast cell activation. Among them, Lyn activated Syk and downstream signals, such as Gab2. Berberine suppressed mast cell activation through FcεRI-mediated signaling, which is located upstream of the MAPK pathways. Since berberine markedly inhibited the levels of IL-4 and TNF-*α*, we further researched how berberine affected MAPK pathways. Our results revealed that antigen-induced phosphorylation of MAPKs were inhibited by berberine. Overall, berberine suppressed mast cell activation *via* regulating FcεRI-mediated and MAPK signaling in RBL-2H3 cells. These results are consistent with those of the pervious study [14].

After IgE binds to the FcεRI, mast cells can be activated by cross-linking of the FcεRIs with specific antigen, which can result in the PCA reaction [30]. We found that oral administration with berberine significantly suppressed IgE-mediated PCA as observed by impaired extravasation of Evans blue dye in the skin. It suggested that berberine could be beneficial for inhibiting allergic responses *in vivo*.

Various studies of alkaloids in allergic models such as allergic asthma demonstrated their beneficial effect. One such study showed that curine, a bisbenzylisoquinnoline alkaloid, inhibited the scratching behavior, paw edema and systemic anaphylaxis induced by either OVA in sensitized animals or compound 48/80. This result occurred through mechanisms of mast cell stabilization and inhibition of mast cell activation to generate lipid mediators [35]. MHTP [2-methoxy-4-(7-methoxy-1,2,3,4-tetrahydroisoquinolin-1-yl) phenol] has been shown to have an anti-allergic effect in a mouse model of OVA-induced pulmonary allergy and immunomodulatory effects. This anti-allergic effect is dependent on a Th1-skewed cytokine production that ameliorates the pulmonary allergic inflammation [36].

In our study, the anti-allergic effect of berberine, an isoquinoline alkaloid, was studied *in vitro* and *in vivo*. Our findings demonstrate that berberine suppresses mast cell-mediated allergic responses *via* regulating FcεRI-mediated and MAPK signaling. This study is meaningful for the further development of berberine. Furthermore, the therapeutic effect of berberine in treating allergic diseases *in vivo* deserves further research.

Acknowledgements

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Author contributions

Tie Hong, Shuilian Fu, Saihong Ni, Danni Wang and Meng Fu conceptualized and designed this study and wrote the paper. Tie Hong, Shuilian Fu performed most of the experiments. Saihong Ni, Danni Wang, and Meng Fu analyzed the data. All authors read and approved the final manuscript.

Notes

The authors declare no competing interest.

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