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Licochalcone A inhibits IgE-mediated allergic reaction through PLC/ERK/STAT3 pathway

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Abstract

Licochalcone (LicA) is a flavonoid commonly derived from the licorice plant that is reported to have a variety of pharmacological activities. However, few studies have focused on its anti-allergic properties. IgE-mediated passive and systemic anaphylaxis mice models were used to assess the in vivo anti-allergic effect of LicA and its underlying mechanism, while degranulation, cytokines, and chemokines released from laboratory of allergic disease (LAD2) cells were used to assess its in vitro anti-allergic effect. We used western blot analysis to explore the downstream signaling pathway of its anti-allergic effect. We found that in the mouse model, LicA attenuated IgE-mediated paw inflammation, recovered the allergy-induced drop in body temperature, and reduced the concentrations of tumor necrosis factor-alpha and monocyte chemoattractant protein-1 in mouse serum in a dose-dependent manner. LicA inhibited the allergic reaction via inhibition of IgE-mediated LAD2 cell activation through the PLC/ERK/STAT3 pathway.

Keywords

Licochalcone A, IgE, mast cell, STAT3, allergy

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Introduction

Allergic diseases are pathophysiological disorders, characterized as hypersensitivity reactions.[1,2] Type I allergic diseases are very common, and include atopic dermatitis (AD),[3] allergic conjunctivitis,[4] and allergic asthma,[5] which are characterized by immunoglobulin E (IgE)-mediated hypersensitivity, called an acute hypersensitivity reaction.[6] Allergic reactions are mainly mediated by IgE, which is cross-linked with its high-affinity receptor (FcεRI) to activate mast cells (MCs),[7,8] thereby inducing MCs to degranulate and release a variety of prefabricated or newly synthesized inflammatory mediators.[7]

Immunoglobulin E (IgE)-mediated allergy is always triggered by MCs, and can result in many kinds of allergic diseases.[9] IgE-mediated allergic reactions begin with cross-linking of FcεRI receptors on MCs,[10] followed by a rapid increase in intracellular Ca^{2+} concentration,[11] and the release of pre-formed mediators including histamine and

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β-hexosaminidase,[12,13] and several inflammatory mediators, such as cytokines and chemokines from the MCs. These biologically active media then very rapidly cause allergic symptoms that then lead to allergic diseases.[14] At present, it is reported that the IgE-induced MC activation mechanism starts with the activation of Src family proteins,[15] such as Lyn kinases,[16] at the proximal end of the receptor, and then the induced activation of spleen tyrosine kinase (Syk),[17,18] which is a downstream molecule that subsequently adapts to the activation of MC for signal propagation.

Glycyrrhiza glabra, commonly known as licorice, is a leguminous plant that occurs in the Middle East, Southern Europe, and India. As early as 500 BC, licorice was used in medicine. Licorice has several pharmacological effects,[19] such as anti-inflammatory,[20] anti-cancer,[21] antimicrobial, antioxidant, and anti-diabetic effects. The biologically active ingredients of licorice mainly include triterpenes, polysaccharides, and flavonoids, among others. Licochalcone (LicA) is a licorice extract with high flavonoid content.[19] It has known antibacterial, antioxidant, hypoglycemic, anti-inflammatory, and nerve activity effects.[22] It has been widely used in the food and pharmaceutical industries. Although the anti-allergic properties of LicA have been studied,[23] the underlying mechanism remains unclear.

Materials and methods

Chemicals

Licochalcone A (purity >98%) was purchased from Pufeide (Chengdu, Sichuan, China; JOT-10472). Fluo-3 AM ester and Pluronic F-127 were purchased from Biotium (Fremont, CA, USA; 121714-22-5). Evans blue (E2129), anti-2,4-dinitrophenyl (DNP)-specific IgE (D8406), dinitrophenyl-conjugated human serum albumin (DNP-HSA) (A6661), β-hexosaminidase (N9003), OVA (A5503) and fluorescein isothiocyanate (FITC)-avidin (S4762), were purchased from Sigma-Aldrich (St. Louis, MO, USA). All ELISA kits were purchased from Sino Biological (Beijing, China). The antibodies used were phosphorylated PLCγ1 (P-PLCγ1) (8713S), extracellular signal-regulated kinases 1 and 2 (ERK1/2) (4695S), phosphorylated ERK1/2 (9101S), and GAPDH (5174T), all purchased from Cell Signaling (Danvers, MA, USA).

Animals

Sixty male C57BL/6 mice (8 weeks old, 18–22 g) were provided by the Experimental Animal Center of Xi'an Jiaotong University (Xi'an, China). The animals were kept in separate cages in a large colony room, with drinking water freely accessible and standard dry feed twice a day. The breeding environment was 20–25°C, relative humidity was 40%, and the light/dark cycle was 12 h. All experiments involving equal treatment of animals are carried out by experiment is blind to the experimental conditions.

Ethical considerations

This study was performed in strict accordance with the recommendations stated in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The experimental protocols for the animal experiments were approved by the Animal Ethics Committee in Xi'an Hong Hui Hospital, Xi'an, China (Permit Number: 2021-713).

Cell lines

The human mast cell line Laboratory of Allergic Disease 2 (LAD2) used in this study was a generous gift from Mr Daniel Lee (NIH, Bethesda, MD, USA). Cells were cultured in StemPro medium (10639011) supplemented with StemPro nutrient additive (10 mL/L) (A1033201), 100 U penicillin, 100 U streptomycin (15140163), 2 mM L-glutamine (25030081), and 100 ng/mL human stem cell factor (SCF) (PHC2116). StemPro medium and all other reagents/supplements were purchased from Gibco. All cultures were grown at 37°C in incubators maintained with 5% CO_2. During culturing, the medium was replaced every 3 days, and the cells were maintained at 2×10^6 cells/mL.

Bone marrow mast cells were harvested and cultured as previously described.[24]

IgE-mediated passive cutaneous anaphylaxis (PCA)

The 8-week-old mice were randomly allocated into the following groups, with six mice per group: vehicle, negative control, and the LicA group (different doses of 20, 40, and 80 mg/kg, respectively).

Mice in the negative control or LicA groups were sensitized with 0.2 mL of 20 μg/mL OVA twice given by intraperitoneal injection 1 day apart (ref); mice in the vehicle group remained untreated (sham-injected). At 48 h after the second OVA dosing, mice in the LicA groups received a single 0.2 mL gavage that delivered the test agent at a level that achieved body doses of 20, 40, or 80 mg/kg LicA, respectively. All mice received an intraperitoneal injection of 0.2 mL 1% pentobarbital sodium (Sigma, USA; P3761) half an hour after gavage. Then, 15 min later, an intravenous injection of 0.2 mL 0.4% Evans blue solution was administered.

All mice then received a 5 μL injection of 200 μg/mL OVA solution in their left paw, and an equal volume of saline alone was injected into the right paw as a negative control. Paw thickness was measured both before and 15 min after the OVA injection using a Vernier caliper. All

mice were then euthanized and paw tissues removed. The paws were each dried at 50°C for 24 h and then weighed individually to calculate normalization. Paw tissues were cut into pieces and Evans blue dye extravasation was then analyzed by addition of a 500 μL acetone-saline (7:3) mixture. After 2 h, the materials were all centrifuged (1000 × *g*, 15 min, 4°C) and the supernatant recovered. An aliquot (200 μL) of supernatant was transferred to a 96-well plate and sample absorbance was measured at 620 nm in a spectrophotometer (Bio-Rad, city, state). Data were analyzed after normalization for tissue sample weight.

IgE-mediated passive systemic anaphylaxis in vivo

Another set of 8-week-old mice was randomly divided into the following groups, with six mice per group: vehicle, negative control, and the LicA group (different doses of 20, 40, and 80 mg/kg, respectively). All the mice were challenged with 0.2 mL of 20 μg/mL OVA twice as mentioned above. Half an hour before the intravenous injection of 0.2 mL 200 μg/mL OVA, the mice of the LicA group were orally administered different doses of LicA (20, 40, and 80 mg/kg). An animal thermometer probe was inserted into the anus and the body temperature was then measured continuously for 30 min (recorded every 3 min after the OVA challenge).

After 8 h, the blood of the mice was extracted through eyeballs on ice, for the measurement of cytokine release. The samples were centrifuged (4000 × *g*, 15 min, 4°C) and the supernatant was collected and stored at −80°C. The levels of TNF-α, MCP-1, and IL-8 in the serum were detected with Mouse TNF-α, Mouse MCP-1, and Mouse IL-8 ELISA kits (Sino Biological Inc., Beijing, China), respectively, according to provided instructions.

Intracellular Ca^{2+} mobilization assay

BMMCs were generated from bone marrow cell suspensions in the presence of IL-3, IL-4, and SCF as described previously. Calcium imaging buffer (CIB; 125 mM sodium chloride, approximately 3 mM potassium chloride, 2.5 mM, 0.6 mM $MgCl_2$, 10 mM HEPES, 20 mM glucose, 1.2 mM $NaHCO_3$, 20 mM sucrose, pH 7.4) was used for pre-sensitized BMMCs, while for LAD2 cells, pre-incubation buffer (4 μM Fluo-3, 0.1% (v/v) pluronic F-127 CIB) together with LicA (0, 25, 50, 100 μM) was used for 30 min. Then, the cells were washed twice with CIB and stimulated with DNP-HSA (1 μg/mL, for BMMCs) or streptomycin (100 ng/mL, for LAD2; Sigma-Aldrich) for the first 10 s. We used a fluorescence microscope to photograph the cells at ×200 magnification under green light (1 s per photo). The activation status of MCs was determined by the changes in fluorescence intensity.

Measurement of β-hexosaminidase

DNP-IgE (1 μg/mL) was used to sensitize BMMCs, and biotinylated human IgE (100 ng/mL, Raleigh, NC, USA) was used to sensitize LAD2 cells for 12 h. Cells were incubated with LicA (25, 50, and 100 μM) which was dissolved in Tyrode's solution (TM buffer: 6.954 g/L sodium chloride, 0.353 g/L potassium chloride, 0.282 g/L calcium chloride, 0.143 g/L $MgSO_4$, 0.162 g/L KH_2PO_4, 2.383 g/L informed, 1 g/L bovine serum albumin (BSA), and 0.991 g/L glucose, pH 7.4) for 30 min. After that, 100 μL DNP-HSA (1 μg/mL, resolved in TM buffer) and 100 ng/mL streptomycin were used to stimulate BMMCs and LAD2 cells, respectively, for 30 min. Cells were then lysed with 0.1% (v/v) Triton x-100 for 5 min to get intracellular β-hexosaminidase. The supernatant was collected and incubated with 1 mM β-hexosaminidase in 0.1 M citric acid/sodium citrate buffer (pH 4.5) for 90 min at 37°C. The hydrolysis reaction was terminated with 150 μL stop buffer (0.1 M Na_2CO_3/$NaHCO_3$, pH 11), and optical density (OD) at 405 nm was detected with a microplate reader (Bio-Rad, CA, USA). The percentage of β-hexosaminidase release was calculated as follows: the extracellular supernatant absorbance × 100%/the sum of the absorbances of the extracellular supernatant and the intracellular supernatant.

Chemokine release assay

DNP-IgE (1 μg/mL) were used to sensitize BMMCs in a 96-well plate (1×10^6 cells/well). The cells were incubated with DNP-IgE for 24 h at 37°C under 5% CO_2. The culture medium was then removed, and the cells were incubated with LicA (50, 100, 200 μM) and HSA for 6 h at 37°C. TNF-α, MCP-1, and IL-8 concentrations in the cell supernatant were detected with ELISA kits, according to provided instructions.

The LAD2 cells were plated in a 96-well plate (1×10^6 cells/well) and incubated with 100 ng/mL biotinylated human IgE for 24 h at 37°C. Cells were treated with LicA for 30 min, and then stimulated with 100 ng/mL streptavidin. After another 6 h of incubation, human MCP-1 and TNF-α in the supernatant were detected using ELISA kits according to the provided instructions.

Western blot analysis

BMMCs were seeded into a 6-well plate (1×10^7 cells/well) and then sensitized with DNP-IgE for 24 h. Cells were then incubated with different concentrations of LicA for 30 min followed by HSA for stimulation for 30 min, and collection of total proteins using RIPA lysis buffer containing 10% protease inhibitor (Roche Diagnostics) and phosphatase inhibitor cocktail (Roche Diagnostics) in ice-cold conditions. The protein concentration was determined using Bio-Rad protein analysis reagent. Proteins were dissolved

in 5× loading sample buffer and boiled for 5 min, then equal quantities of protein were separated using SDS-PAGE. The proteins were then transferred onto a polyvinylidene fluoride membrane (Millipore, MA, USA; HVLP04700), then blocked in a blocking solution (5% skim milk) for 1 h at 37°C, followed by incubation with primary antibody (1:1000) for 12 h at 4°C, and washed with Tween 20/Tris buffered saline (TBST) three times. The secondary antibody (1:20000) conjugated with horseradish peroxidase was incubated for 2 h at room temperature. The blot was washed three times with TBST and then visualized with an enhanced chemiluminescence (ECL) kit. The images of the developed blots were captured by a Lane 1DTM transilluminator (Beijing Creation Science Co., Ltd., Beijing, China) and were analyzed by Image-Pro Plus 5.1 software (Media Cybernetics, Inc., Rockville, MD, USA).

Statistical analyses

All experiments were repeated at least three times and calculated as the mean ± SD. Statistical significance between groups was determined using independent sample variance analysis. Differences were considered statistically significant at $*p < 0.05$. All statistical analyses were performed with SPSS software.

Theory

Licorice plays an important role in traditional Chinese medicine owing to its numerous active components and a variety of pharmacological activities. LicA has a variety of pharmacological activities, but its anti-allergic activity has not been studied. In this study, LAD2 cells and mouse primary BMMC cells were used to construct an in vitro allergy model. OVA induced local and systemic allergy mouse models were used to evaluate the anti-allergic activity of LicA. Calcium influx into mast cells is the key signal of cell activation, and the degree of activation was quantified by the degrees of degranulation and cytokine release. Subsequently, the degree of PLC/ERK/STAT3 phosphorylation, which is closely related to calcium release and cytokine synthesis, was studied to identify the presence of mast cell signaling pathway inhibition by LicA at the molecular level. The findings of this study provide a mechanism that can be used for the modernization of traditional Chinese medicine and the preparation of anti-allergic drugs.

Results

LicA inhibited IgE-mediated PCA

In the PCA model, the degree of edema was measured as the changes in mice paw thickness and the amount of Evans blue dye exuded. The typical image of Evans blue leakage from the mice paw is shown in Figure 1(a). We further measured the changes in paw thickness and Evans blue leakage in mice after each different treatment. As shown in Figure 1(b), in the control group (OVA administration without LicA pretreatment), paw thickness increased by 14.54 ± 8.11%, and the absorbance of exuded Evans blue dye was 6.32 ± 1.45 (Figure 1(c)). However, after pretreatment with 20 mg/kg LicA, the edema reaction was reduced, and the degree of edema decreased to 11.02 ± 4.76%, but without a significant difference compared with the non-administered group, while the exudation of Evans blue was 4.83 ± 0.116%. When at least 40 mg/kg was administered, the degree of edema dropped to 7.40 ± 3.60%, with an exudation of 2.90 ± 1.33%. After the administration of 60 mg/kg, the edema was only 5.82 ± 1.64%, with an exudation of 2.73 ± 1.79%. The above results confirmed that LicA had significant anti-mouse PCA ability.

LicA inhibited IgE-mediated systematic allergy

In the systematic allergy model, the anti-allergic effect of LicA was measured by detecting changes in body temperature and the concentration of inflammatory mediators in the serum after the stimulation. As shown in Figure 2(a), without LicA administration, the body temperature of the mice decreased significantly within 3 min post-stimulation, and decreased by at least 3°C by 6 min. There was no sign of recovery within 30 min. For the mice in the LicA group (20 mg/kg), their body temperature recovered by 6 min post-stimulation. With increasing dose, body temperature recovered more significantly, confirming that LicA can inhibit allergic hypothermia. We further detected changes in the concentration of inflammatory factors in the serum. As shown in Figure 2(b)–(d), both TNF-α and MCP-1 release increased significantly after the challenge, and after the administration of LicA, the concentrations of these factors in the serum significantly reduced in a dose-dependent manner.

LicA inhibited IgE-mediated Ca^{2+} influx in MCs

The degranulation of MCs depends on the influx of intracellular calcium. We detected Ca^{2+} influx change in BMMCs and LAD2 cells. As shown in Figure 3(a) and (b), the vehicle group of BMMCs and LAD2 cells was used as a positive control and showed almost no Ca^{2+} influx. After being stimulated with IgE, the Ca^{2+} concentration in the cells increased significantly. When the LicA concentration reached 25 μM, the intracellular Ca^{2+} concentration increased less compared to that in the untreated group. The IgE-induced Ca^{2+} influx in MCs was inhibited by LicA in a dose-dependent manner.

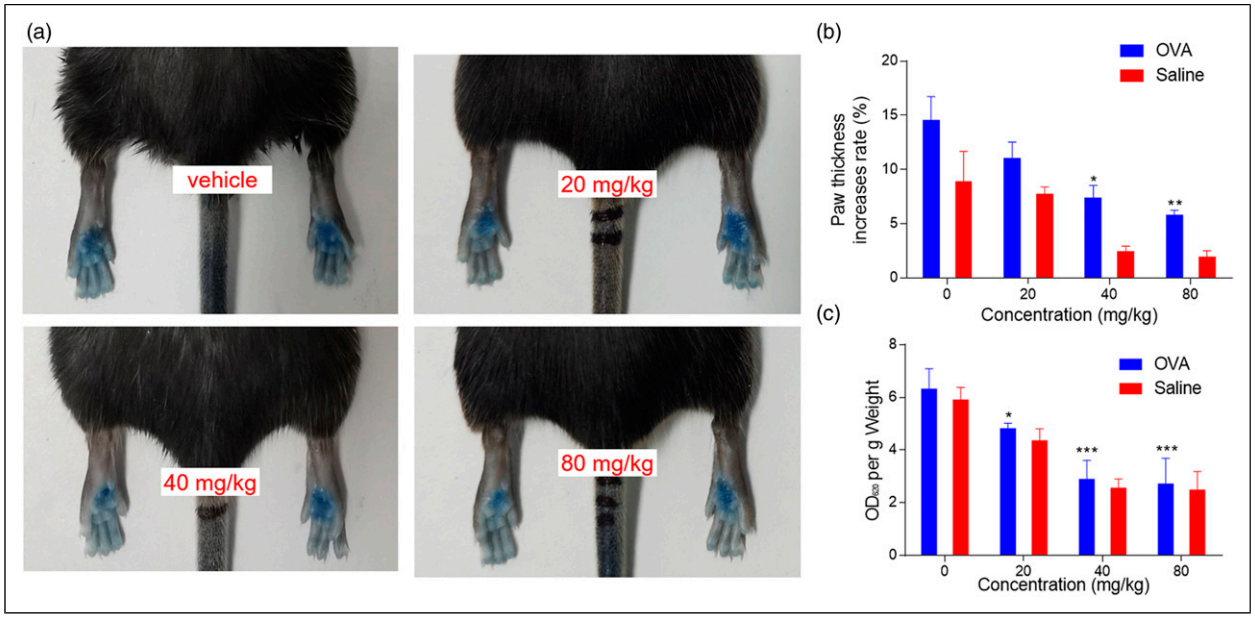

Figure 1. Effect of Licochalcone (LicA) on immunoglobulin E (IgE)-mediated local anaphylaxis in C57BL/6 mice. (a) Photos of mice paws after challenged with OVA from different groups; (b) Quantification of paw thickness changes by cutaneous allergy reactions triggered by OVA/IgE with pretreatment of LicA (0, 20, 40, and 80 mg/kg); (c) Evans blue exudation. The data represent the mean ± SD, $n = 6$. The experiments were repeated three times. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus vehicle group.

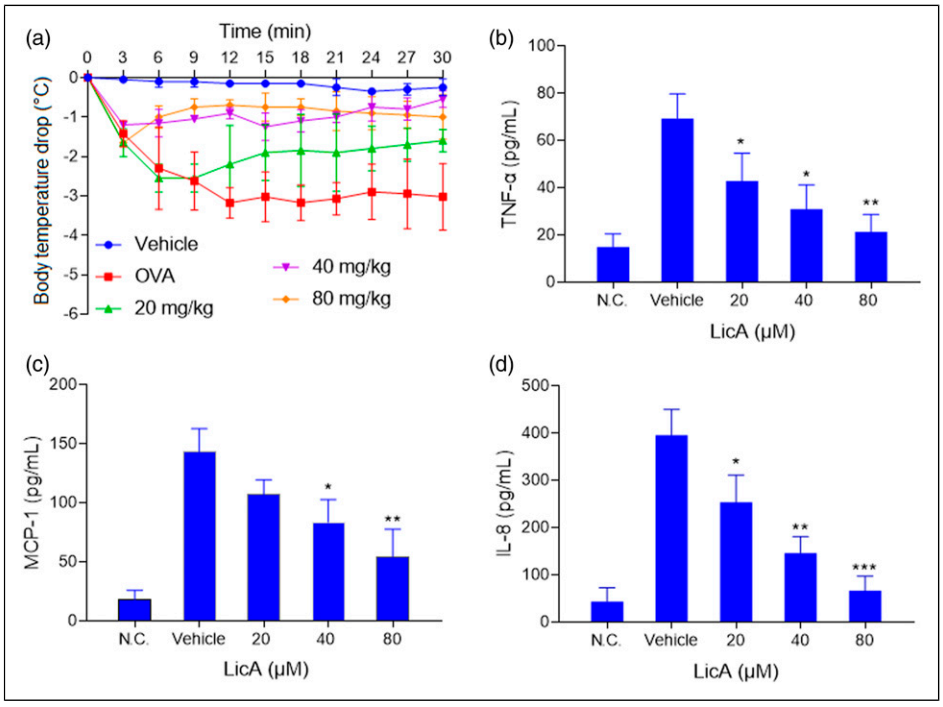

Figure 2. Effect of LicA on IgE-mediated systemic allergic reactions in C57BL/6 mice. (a) Mice were pretreated with LicA (0, 20, 40, and 80 mg/kg), the changes in body temperature were recorded 60 min after OVA injection; (b–d) The effect of LicA pretreatment on OVA/IgE-induced serum levels of TNF-α (b), MCP-1 (c), and IL-8 (d). The data represent the mean ± SD, $n = 6$. The experiments were repeated three times. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus vehicle.

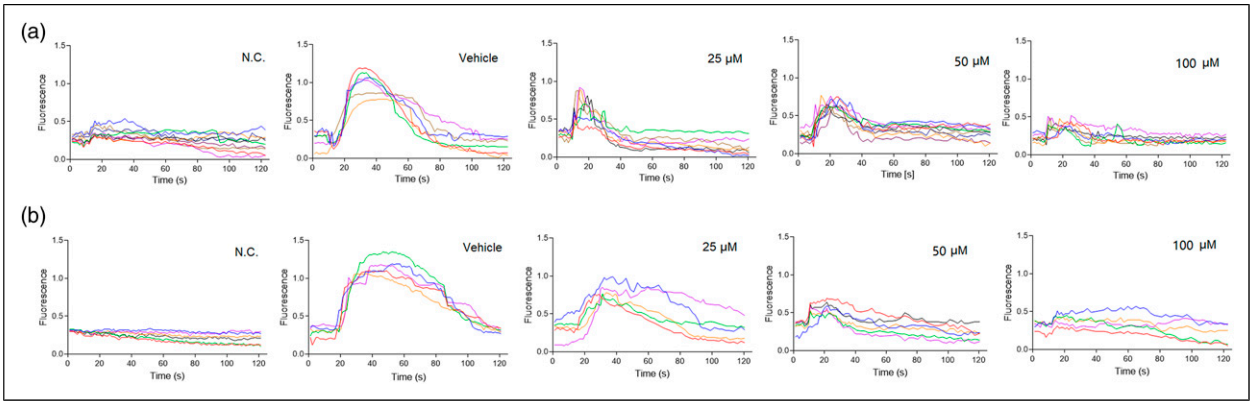

Figure 3. Effect of LicA on IgE-induced intracellular Ca^{2+} mobilization in BMMCs and LAD2 cells. Intracellular Ca^{2+} influx in IgE-sensitized BMMCs and LAD2 cells in response to LicA (0, 25, 50, and 100 μM) pretreatment followed by DNP-HSA (1 μg/mL for BMMC) or streptavidin (for LAD2) stimulation. (a) BMMC; (b) LAD2. Each line represents the fluorescence change of a single cell. Data represent the mean ± SD, $n = 3$. The experiments were repeated three times.

LicA inhibited IgE-mediated MCs degranulation and inflammatory mediator release

The inhibition effect of LicA on IgE-mediated BMMC and LAD2 degranulation was evaluated by detecting the release rate of allergic mediators. We examined the effect of LicA on IgE/DNP-HSA-induced cell degranulation. LicA attenuated IgE/DNP-HAS-induced β-hexosaminidase release (52.11 ± 1.97% release rate in BMMC and 66.17 ± 2.65% release rate in LAD2, Figure 4(a)) in a dose-dependent manner. When the dosage of LicA was at 100 μM, the release rate of β-hexosaminidase was reduced to 21.56 ± 2.01% (BMMC) and 21.32 ± 2.32% (LAD2; Figure 4(b)), respectively. Similarly, 100 μM LicA pretreatment inhibited the MCP-1 and TNF-α release in IgE-sensitized BMMC and LAD2 cells (Figure 4(c) and (d)) in a dose-dependent manner.

LicA downregulated PLC/ERK/STAT3 photoporation in LAD2 cells

Since PLC/ERK/STAT3 pathways are involved in FcεRI signaling, we evaluated whether LicA could influence those pathways. Figure 5 shows the degree of phosphorylation of those proteins in BMMCs. The phosphorylation of PLC, ERK, and STAT3 was significantly increased in the vehicle group, compared with those in the negative control group. When the dosage of LicA treatment reached 100 μM, the phosphorylation of PLC, ERK and STAT3 was reduced significantly. We performed the same experiment under external Ca^{2+} free conditions. Briefly, EDTA was used to abolish Ca^{2+}. The results showed that PLC phosphorylation was still inhibited by LicA, which indicating that the suppressive effect of LicA was still present under external Ca^{2+} free conditions (Figure 5(c)). However, ERK and STAT3 phosphorylation was not occurred.

Discussion

Allergy is one of the most common diseases in the world,[25] the incidence of which is rapidly increasing annually. The World Allergy Organization (WAO) estimates a prevalence rate between 10% and 40%, depending on the country.[26] Mast cells are the key effector cells of allergic reactions.[27] After activation, mast cells can release histamine, 5-HT, TNF-a, IL-8, and other sensitizing mediators and inflammatory factors to induce allergies.[9] LicA is a flavonoid compound widely found in a variety of plants that has a variety of pharmacological activities.[23] This study verified the anti-allergic effect of LicA, and a potential mechanism through in vitro and in vivo experiments, suggesting that it could be developed for use in the treatment of allergies. We observed that LicA has a good anti-allergy effect. LicA inhibits degranulation of MCs, Ca^{2+} influx, and cytokine release in vitro, and inhibits PLC/STAT3/ERK phosphorylation. Our in vivo experiments confirmed that LicA effectively alleviated PCA and systematic allergy in a mouse model.

Ca^{2+} is an important cellular messenger that mediates a variety of cell signals.[28] After the MC activation, intracellular Ca^{2+} influx raises rapidly, triggering an intracellular cascade reaction.[29] The increase in intracellular Ca^{2+} concentration in MCs provides an indispensable and ubiquitous mechanism for the extracellular release of FcεRI proteins during aggregation.[30] This study found that, after the BMMC and LAD2 cells were stimulated by IgE, their intracellular Ca^{2+} concentration increased significantly. When pretreated with LicA, the increase of intracellular Ca^{2+} was significantly inhibited. Meanwhile, the

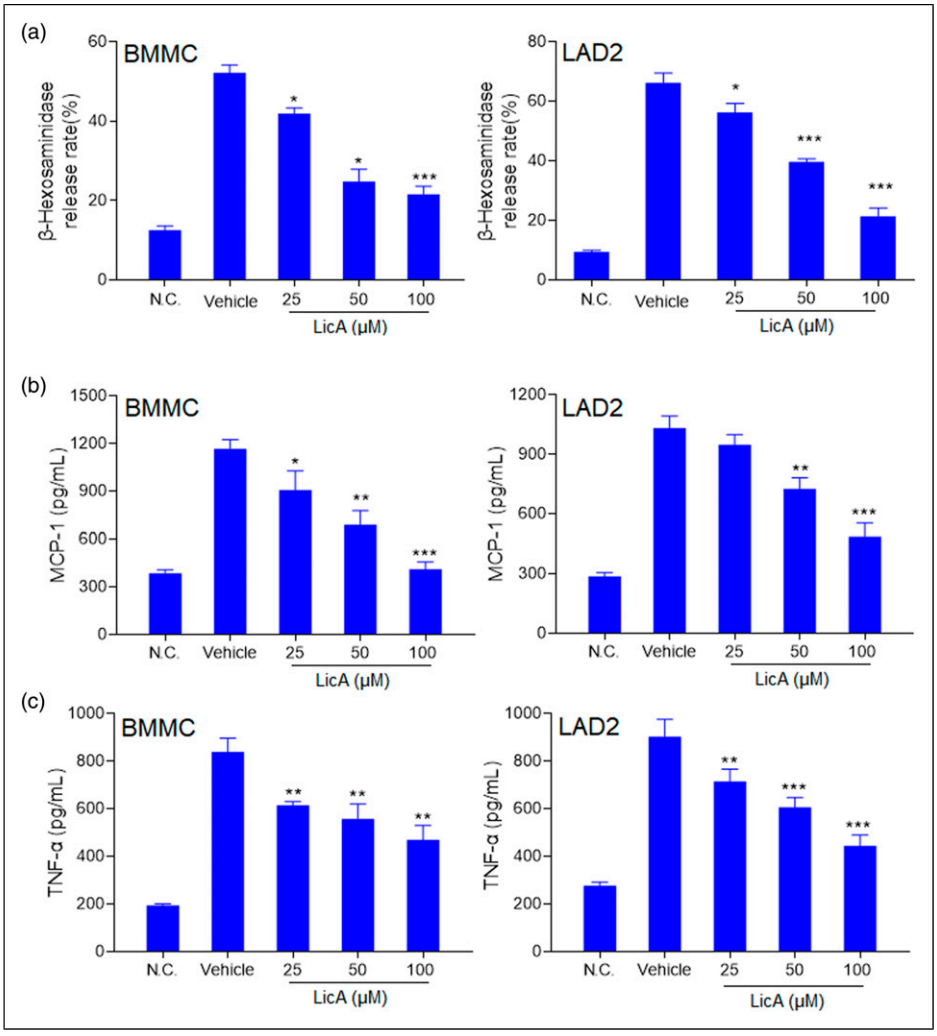

Figure 4. Effect of LicA on the degranulation and secretion of proinflammatory mediators in BMMCs and LAD2 cells. (a) β-hexosaminidase release rate; (b) MCP-1; (c) TNF-α. Data represent the mean ± SD, $n = 3$. The experiments were repeated three times. $^{**}p < 0.01$, $^{***}p < 0.001$ versus vehicle group.

release of β-hexosaminidase was also effectively controlled, indicating that LicA can inhibit IgE-induced MC activation. The combination of IP3 and its receptor IP3R induces Ca^{2+} release from the endoplasmic reticulum storage and then induces long-term Ca^{2+} outflow from the endoplasmic reticulum. Further Ca^{2+} influx is also promoted by the activity of the calcium-activated plasma membrane calcium modulator 1 (ORAI-1. Ca^{2+} influx can then mediate the production of eicosanoids and lipid mediators.[31] Syk tyrosine kinase can be activated instantaneously under the stimulation of FcεRI, which then rapidly phosphorylates PLC. PLC plays an important role in the production of IP3 and the subsequent increase of cytoplasmic Ca^{2+} levels.[32] We assumed that the inhibition of intracellular Ca^{2+} release led to extracellular Ca^{2+} influx. As it was widely reported, the Ca^{2+} influx of mast cell activation was store-operated calcium entry (SOCE) type.[33] The release of intracellular Ca^{2+} from endoplasmic reticulum was the signal of following Ca^{2+} influx. From our results, we found that LicA inhibited the phosphorylation of PLC, which modulated the intracellular Ca^{2+} release. In this study, we observed that DNP-HSA induces the rapid phosphorylation of PLC in BMMC and LAD2 cells, and that LicA can significantly block this phosphorylation. From the results that LicA inhibited PLC phosphorylation under both Ca^{2+} free and not free conditions, we concluded that LicA showed an inhibitory effect on store-operated calcium channels, which resulted in the inhibition of Ca^{2+} influx and intracellular cascade.

Various inflammatory mediators work together to produce complex physiological effects. In response to stimulation, members of the STAT family are phosphorylated

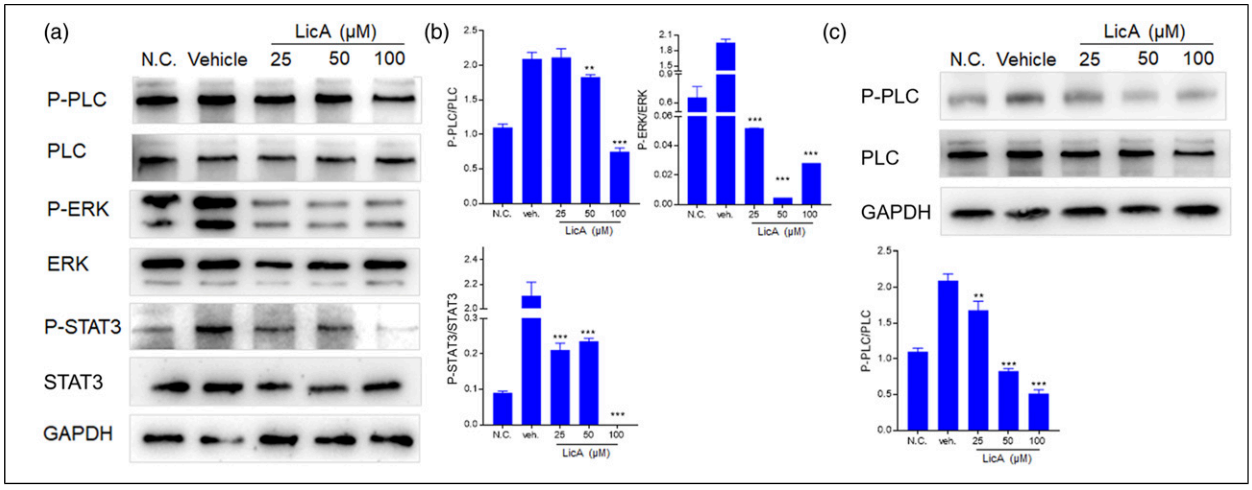

Figure 5. Effect of LicA on the PLC/ERK/STAT3 pathway in FcεRI signaling of BMMCs. (a) Representative western blot images of PLC, ERK, and STAT3; (b) Quantification of the proteins in A by densitometric analyses; (c) Representative western blot images and quantification of the proteins of PLC under external Ca^{2+} free conditions. The data represent the mean ± SD, $n = 3$. The experiments were repeated three times. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus vehicle.

by receptor-associated kinases and then form homodimers or heterodimers that translocate to the nucleus where they act as transcriptional activators. STAT is activated by responding to the phosphorylation of various cytokines and growth factors,[34] including IFN, EGF, IL5, IL6, HGF, LIF, and BMP2. It has been observed that allergic diseases are diminished in patients with STAT3 mutations.[35] ERK/STAT3 pathways are crucial to cytokine release in cancers and inflammation. STAT3 not only controls the expression of cytokines, but also modulates metabolism, proliferation, and other life activities.[36] 100 μM LicA completely inhibited P-ERK, it was much lower than that of NC. We assumed that the phosphorylation of ERK was compensatory expressed and maintained the normal physiological activities of the cells. In this study, both ERK and STAT3 phosphorylation was upregulated after the challenge, then decreased remarkably after treatment with LicA.

Systemic allergic reactions are often accompanied by a significant drop in body temperature, and therefore, changes in body temperature can be an obvious indicator of allergic reactions.[37] In this study, the body temperature of the mice decreased significantly after the systemic allergic reaction occurred. After the administration of LicA, the reduction in the body temperature of the mice was prevented in a dose-dependent manner. Anal temperature measure did not affect mice, as there was no obvious abnormality in mice of the Vehicle group. At the same time, serum concentration of TNF-α and MCP-1 both decreased significantly. The above results indicated that LicA has an inhibitory effect on the systemic allergic reaction in mice. Although BALB/c mice were selected conventionally, C57BL/6 male mice were also used in the relevant studies.[38] Power analysis for sample size calculation was lack and was a limitation of this study.

Conclusion

Therefore, LicA has significant anti-allergic effects both in vitro and in vivo. LicA inhibits the occurrence of the allergic reaction by inhibiting Ca^{2+} influx and inflammation mediator production through the PLC/ERK/STAT3 pathway. The findings of this study provide a novel mechanism that could be used for the modernization of traditional Chinese medicine and the preparation of anti-allergic drugs.

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Author contributions

J.S.: Conceptualization, Methodology, Writing- Original draft preparation. X.C.: Methodology, Data curation, Writing-original draft preparation. X.L.: Visualization, Software. W.Y.: Investigation, Data Curation. W.Z.: Software, Validation. X.H.: Data Curation, Software. C.L.: Supervision, Resources, Conceptualization, Writing- Reviewing and Editing.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

Ethical approval for this study was obtained from Animal Ethics Committee in Xi'an Hong Hui Hospital, Xi'an, China (Permit Number: 2021-713).

Animal welfare

The present study followed Guide for the Care and Use of Laboratory Animals of the National Institutes of Health for humane animal treatment and complied with relevant legislation.

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Appendix 1

Abbreviations

LicA	Licochalcone
LAD2	laboratory of allergic disease 2