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Original Article

Qingkailing injection induces pseudo-allergic reactions via the MRGPRX2 pathway

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Abstract: Objective: Qingkailing Injection (QKLI) is a traditional Chinese medicine injection mainly used for sedation, heat clearing, and other treatments. However, recent clinical studies have shown a risk of pseudo-allergic reactions. The purpose of this study is to elucidate the underlying mechanism of QKLI-induced mast cell degranulation in Laboratory of Allergic Diseases 2 (LAD2) and to validate QKLI-induced activation of guinea pig IgE-independent allergic responses. Methods: Levels of β-hexosaminidase (β-Hex), histamine (His), and complement pathway-related indicators in guinea pigs and LAD2 cells were assayed using the Enzyme-linked Immunosorbent Assay (ELISA). The release rates of β-Hex and His from LAD2 cells were measured using the o-phthalaldehyde (OPA) fluorimetric method. The antagonist for complement component 3a (C3a) receptors, SB290157 and siRNAs were used to inhibit the C3a pathway and the Mas-related G-protein-coupled receptor X2 (MRGPRX2) pathway. The MRGPRX2 pathway and its downstream proteins were detected by Western Blot (WB). Results: The results show that QKLI significantly increased levels of β-Hex, His, C3a, complement component 5a (C5a), and terminal complement complex C5b-9 (SC5b-9) in guinea pigs, while levels of interleukin 4 (IL-4), interleukin 13 (IL-13), and interleukin 6 (IL-6) were unaffected. The C3a receptor inhibitor SB290157 significantly reduced levels of β-Hex and His. In LAD2 cells, QKLI increased the release rates of β-Hex and His in a time-dependent manner and decreased the phosphorylation of Extracellular Signal-regulated Kinase 1/2 (ERK1/2) proteins downstream of the MRGPRX2 pathway. The effective components of QKL, baicalin (BA) and geniposide (GE), individually enhance the allergic responses of LAD2 cells to some extent. However, the use of QKL is significantly superior to the individual use of its components. Conclusions: We found that QKLI induced pseudoanaphylaxis via an IgE-independent response in guinea pigs and through the MRGPRX2 pathway in human LAD2 cells. Among these, the main ingredients causing pseudoallergic reactions in QKLI were BA and GE. Our research contributes to a better understanding of the mechanisms underlying drug hypersensitivity reactions (DHRs).

Keywords: Qingkailing injection, drug hypersensitivity reaction, pseudo-allergic, LAD2 cells, MRGPRX2

Introduction

Qingkailing Injection (QKLI) is a traditional Chinese medicine derived from the ancient Chinese medicine Angong Niuhuang Pill (ANP), which is used to treat fever, encephalitis, and other diseases [1]. QKLI contains baicalin (BA), cholic acid (CA), hyodeoxycholic acid (HDCA), and extracts of *Isatidis radix* [2], *Lonicera japonica Thunb* [3, 4], *Gardenia jasminoides* [5], Nacre [6], and buffalo horn [7]. As a novel preparation of traditional Chinese medicine, QKLI offers a faster and stronger therapeutic effect compared to traditional oral preparations. However, it has also been associated with an increases in cases of drug hypersensitivity reactions (DHR) [8, 9]. QKLI has been controversial in clinical applications in recent years. With the extensive clinical use of traditional Chinese medicine injections, their safety issues have become a significant concern [10].

Although QKLI is notably effective in clinical application, various DHRs have also been reported [11]. Existing studies on the mechanisms of DHRs have revealed limitations and outdated aspects regarding their definition, classification, and clinical diagnosis. Moreover, there are slight variations in the terminology employed across different reviews [12-16]. In

particular, pseudo-allergic reactions have become a focus of attention because they can induce allergy-like symptoms through direct action on blood vessels or nerves without relying on immune mechanisms [17]. Recent research has highlighted the role of the Mas-related G-protein-coupled receptor X2 (MRGPRX2) in mediating pseudo-allergic reactions, making it a critical focus in understanding these responses [18, 19].

MRGPRX2 is a receptor found on mast cells and plays a pivotal role in non-IgE-mediated pseudo-allergic reactions. Its activation by various stimuli can lead to degranulation and the release of mediators responsible for allergy-like symptoms [20, 21]. Experimental research has verified that the hypersensitivity reactions triggered by QKLI were a result of pseudo-allergic hypersensitivity, rather than being mediated by immunoglobulin E (IgE) allergies. Cellular investigations revealed that QKLI possesses the capacity to directly initiate effector cell degranulation [22, 23]. In animal studies, it was observed that QKLI could elicit non-immune-mediated hypersensitivity without causing any alteration in plasma IgE levels [24-26]. QKLI can stimulate a pseudo-allergic reaction through complement component 3a (C3a) stimulation, leading to degranulation [10]. Given its significant role, understanding how QKLI may induce pseudo-allergic reactions through the MRGPRX2 pathway is crucial for improving the safety profile of this widely used medication.

Investigating the MRGPRX2 pathway in the context of QKLI-induced pseudo-allergic reactions is of great importance for enhancing its clinical safety. It is not known whether QKLI induces pseudoallergic reactions through the MRGPRX2 pathway. This study aims to explore the mechanism by which QKLI induces pseudo-allergic reactions. Findings will provide valuable insights and recommendations for the clinical application of QKLI.

Materials and methods

Reagents

Dulbecco's Modified Eagle Medium (DMEM, batch number: KGM12800-500) was purchased from KeyGEN BioTECH, Nanjing, China. Fetal bovine serum (FBS, batch number: A5256701) was purchased from Gibco, MA, USA. P-nitrophenyl-N-acetyl-β-D-glucosaminide (NAG, batch number: S10146), histamine (His, batch number: B24434), and o-phthalaldehyde (OPA, batch number: B24663) were purchased from Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China. Compound 48/80 (batch number: C2313-100MG) was purchased from Sigma-Aldrich, Dorset, UK. The SB290157 (batch number: HY-101502A) was purchased from MedChemExpress, Shanghai, China. QKLI (Lot: 220416A2) was purchased from China Shineway Pharmaceutical Group Limited, Shijiazhuang, China.

Cells and animals

Laboratory of Allergic Diseases 2 (LAD2) cells were purchased from the Cell Bank of Chinese Academy of Sciences, located in Shanghai, China, and cultured in complete growth medium containing DMEM supplemented with 10% (v/v) fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were cultured in a CO_2 incubator at 37°C with 5% CO_2 and 95% relative humidity.

All animal experiments were conducted at the Experimental Animal Center of Nanjing University of Chinese Medicine following established protocols [27, 28]. These protocols complied with the laboratory animal management regulations of Jiangsu Province and were approved by the Experimental Animal Center of Nanjing University of Chinese Medicine (Ethics approval lot number No. 202201A011). Male Hartley guinea pigs (300 g ± 50 g) were purchased from Pizhou Dongfang Breeding Co., Ltd., Pizhou, China. The guinea pigs were anesthetized by intraperitoneal injection of ketamine (75 mg/kg) and xylazine (7.5 mg/kg). Drugs from each group were injected into the femoral vein for stimulation. Approximately 1 mL of blood was drawn from the carotid artery 30 minutes after stimulation, anticoagulated with EDTA-2K, and the animals were sacrificed through bloodletting. The blood samples were centrifuged at 2000 r/min for 10 minutes, and the supernatant plasma was taken and frozen at -80°C.

β-Hex assay (OPA fluorimetric method)

Detection of β-hexosaminidase (β-Hex) in LAD2 cells was carried out using the OPA fluorescence method [29]. After cell lysis, the superna-

Table 1. The sequence of MRGPRX2 siRNA

	Sense (5'-3')	Antisense (5'-3')
siRNA1	GUCCUGUCAUCUCUUAACATT	UGUUAAGAGAUGACAGGACTT
siRNA2	GUCAGACAUUUGAUUUCAUTT	AUGAAAUCAAAUGUCUGACTT
siRNA3	CUAGCUUCUUCACCACUGUTT	ACAGUGGUGAAGAAGCUAGTT

tant was collected for further analysis. An OPA fluorescence assay kit from Abcam was used, and the procedures were conducted according to the instructions obtained from the Abcam official website. Following completion of the relevant steps outlined in the manual, the fluorescence signal intensity generated was measured at a wavelength of 405 nm using a fluorescence spectrophotometer. β-Hex assay release rate = $Abs_{supernatant}/(Abs_{supernatant} + Abs_{cell\ lysate})$.

His assay (OPA fluorimetric method)

The release of His was measured using a fluoro spectrophotometry method [30]. Take 100 μL of cell lysate and add 0.5 mol/L NaOH (40 μl) and 2.5 g/L OPA (20 μl), mix well, and incubate the mixture at 37°C for 30 minutes. At the end of the incubation, add 3 mol/L HCl (10 μl). Measure the fluorescence intensity using a fluorescence plate reader at an excitation wavelength of 365 nm and emission wavelength of 465 nm. Use the fluorescence intensity of Tyrode solution with corresponding drugs as blanks; His release rate = $F_{supernatant}/(F_{supernatant} + F_{cell\ lysate})$.

Enzyme Linked Immunosorbent Assay (ELISA)

The concentrations of β-Hex (batch number: ML0380392), His (batch number: MLL03940), complement component 3a (C3a, batch number: ML059693) and SC5b-9 (batch number: MLGR-E40117) were analyzed by ELISA test. The test kits were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China. The interleukin 4 (IL-4, batch number: EGP0031), interleukin 13 (IL-13, batch number: EGP0026), interleukin 6 (IL-6, batch number: EPG0032), complement component 5a (C5a, batch number: EPG0068). ELISA kits were purchased from Wuhan Fine Biotech Co., Ltd., Wuhan, China [31]. The blood plasma samples from guinea pigs were collected 30 min after drug administration into tubes containing EDTA-2K, and then centrifuged at 2000 rpm for 10 min. After separation of the plasma samples, the levels of various markers were measured using a Tecan SPARK 10 M plate reader according to the instructions of the ELISA kit. The C3a receptor antagonist SB290-157 was used as reference in previous studies [32]. Basis on this, a preliminary experiment was conducted, and the results are shown in Supplementary Figure 1.

Cell transfection

Culture 1 × 10^5 LAD2 cells in a 48-well plate for 24 hours until the cell density reaches 70% to 90%. First, add 0.5 μL of Lipo2000 to 25 μL of OPTI-MEM and incubate for 5 minutes. Next, add 0.5 μL of siRNA (50 nM) to 25 μL of OPTI-MEM and incubate for 5 minutes. Mix the two solutions and incubate for 20 minutes, followed by adding 150 μL of OPTI-MEM to the mixture. Transfect the cells with the transfection mixture for 6 hours. After 6 h of transfection, remove the transfection mixture for drug stimulation. The siRNAs were purchased from Shanghai Jima Pharmaceutical Technology Co., Ltd. Details of the RNA sequence numbers are provided in **Table 1**.

WB analysis

After extracting proteins from LAD2 cells according to different groups, use the Bicinchoninic Acid Assay (BCA) Protein Assay Kit (Thermo, Reagent A - 23221, Reagent B - 1859078) to determine the protein concentration and set the sample loading amount. Take an appropriate amount of the protein sample for SDS-PAGE electrophoresis, using Tris-Glycine as the running buffer. Then transfer the proteins to a membrane and block them. Next, incubate the membrane with the primary antibody overnight. Subsequently, the membrane incubate with the HRP-conjugated secondary antibody (1:10,000). Use the chemiluminescent substrate (Tanon High Sig ECL Western Blotting Substrate, 180-5001W, 180-5001B) for developing, expose the membrane using an imaging system, and finally use ImageJ software for protein quantification analysis. Details of all antibodies used are shown in **Table 2**.

Table 2. Information about the antibodies used in this study

Antibody	Dilution ratio	Art.NO.
ERK1/2	1:8000	11257-1-AP
p-ERK1/2	1:3000	28733-1-AP
MRGPRX2	1:1000	NB110-75035
β-actin	1:1000	66009-1-Ig

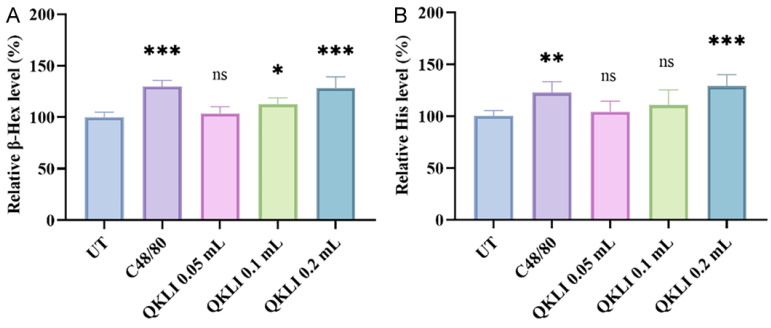

Figure 1. Related β-hexosaminidase (β-Hex) and histamine (His) levels in guinea pigs. A. Relative levels of β-Hex. B. Relative levels of His. Eight guinea pigs per group were respectively treated with 0.2 ml/300 g 0.9% NaCl (the UT group), 0.75 mg/kg compound 48/80 (C48/80), and 0.05-0.2 ml/300 g Qingkailing Injection (QKLI) for 30 min. Values indicate mean ± SD (N = 6). Unpaired student t-tests were employed between each group. *ns, P > 0.05; *, P < 0.05; **, P < 0.005; ***, P < 0.0005;* compared to the UT group.

High Performance Liquid Chromatography (HPLC)

HPLC was conducted using a Waters E2695 separations module with a Hypersil ODS-2 C18 column (4.6 mm × 250 mm, 5 μm). The mobile phases consisted of methanol (A) and 0.1% formic acid in water (B). The gradient elution program was as follows: 0 to 5 minutes: 5% A, 95% B; 5 to 10 minutes: 25% A, 75% B; 10 to 40 minutes: 60% A, 40% B; 40 to 50 minutes: 80% A, 20% B; 50 to 60 minutes: 90% A, 10% B. The flow rate was set to 1 mL/min, and the column temperature was maintained at 35°C. The effluent was detected at 254 nm using a Waters 2998 PDA detector and simultaneously analyzed using an Alltech 2000ES Evaporative Light-Scattering Detector (ELSD) detector, with a drift tube temperature of 120°C and a gas flow rate of 2.8 L/min. All standard samples, including BA, HDCA, geniposide (GE), caffeic acid (CAA), chlorogenic acid (CHA), and adenosine (Ado), were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd., Shanghai, China.

Statistical analysis

Data analysis was conducted using GraphPad Prism version 9.3.1 (GraphPad Software, La Jolla, CA, USA). Statistical comparisons were performed using one-way analysis of variance (ANOVA) and t-tests. Results were considered significant when *p*-values were less than 0.05. Mean ± SEM (standard error) is used in cell experiments to estimate the mean of repeated experiments to clarify how close the mean of the sample is to the true mean (i.e., the population mean). Mean ± SD (standard deviation) is used in animal experiments for multiple samples in one experiment to reveal the degree of dispersion of individual data for different guinea pigs.

Results

QKLI induces allergic reactions in guinea pigs

To verify whether QKLI can induce pseudo-allergic reactions in guinea pigs and to determine the eliciting dose, we conducted the following validation. Based on previous studies [27, 28], we used 0.75 mg/kg of compound 48/80 and 0.05 to 0.2 mL/300 g QKLI to stimulate pseudo-allergic hypersensitivity. The results showed that 0.2 mL/300 g QKLI significantly increased the β-Hex and His the levels in guinea pigs (**Figure 1**).

QKLI induces pseudo-allergic reactions in guinea pigs through the complement pathway

As shown in **Figure 2A-F**, to investigate the mechanism by which QKLI induces allergic reactions in guinea pigs, we used ELISA to detect the levels of IL-4, IL-13, C3a, C5a, SC5b-9, and IL-6 in the serum of guinea pigs. The results showed that, compared to the UT group, the levels of C3a, C5a, and SC5b-9 were significantly elevated in the QKLI group, while the levels of IL-4, IL-13, and IL-6 showed no significant

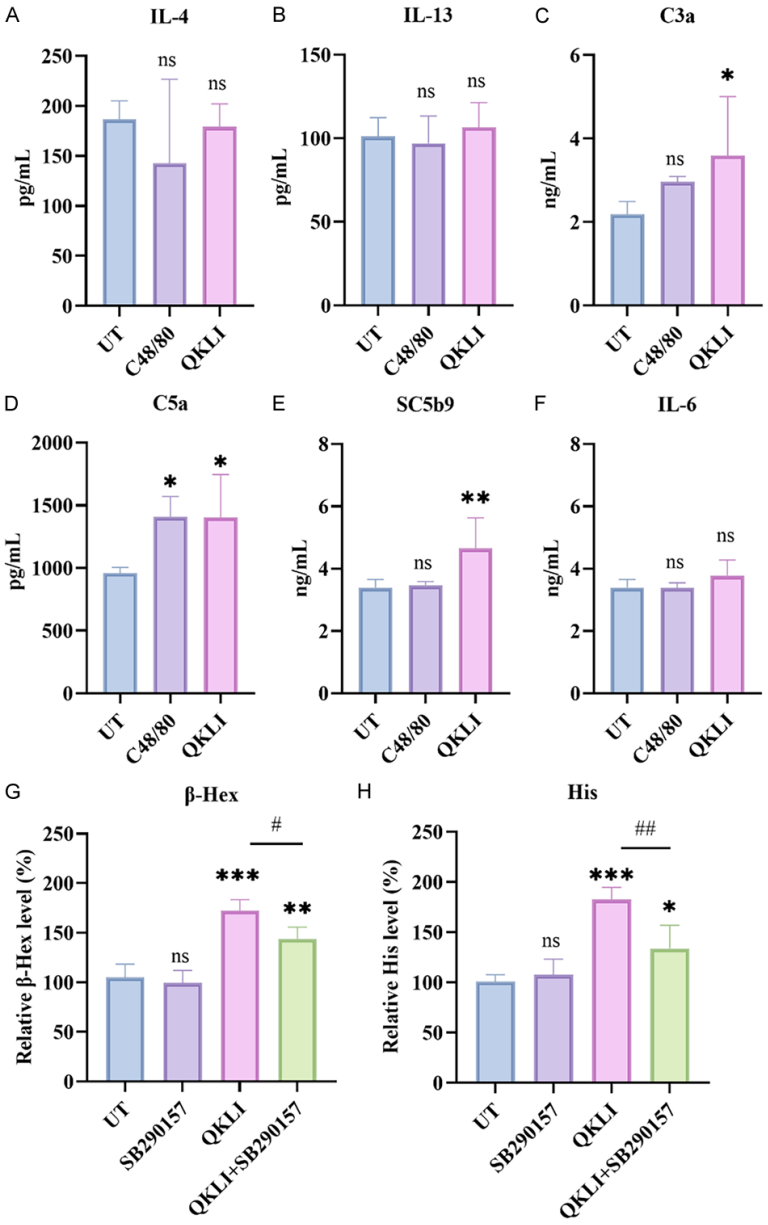

Figure 2. Effect of QKLI on cytokine concentrations in guinea pig plasma. A-F. Relative levels of interleukin 4 (IL-4), interleukin 13 (IL-13), complement component 3a (C3a), complement component 5a (C5a), SC5b-9, and interleukin 6 (IL-6), respectively (N = 5). G, H. C3a receptor antagonists (SB290157) were used to detect the relative levels of β-Hex and His in different groups (N = 4). Guinea pigs per group were treated with 0.9% NaCl (the UT group), 0.75 mg/kg C48/80, and 0.2 ml/300 g QKLI for 30 min. Values indicate mean ± SD (N = 6). *ns, P > 0.05; */#, P < 0.05; **/##, P < 0.005; ***, P < 0.0005.* The control for "*" is the UT group, and the control for "#" is the C48/80-treated group.

changes. To demonstrate that Qingkailing induces pseudo-allergic reactions in guinea pigs through the activation of the C3a pathway, we used the C3a receptor antagonist SB290157 for verification. We measured two indicators, β-Hex and His (**Figure 2G**, **2H**), for this purpose. The results showed that pseudo-allergic reactions induced by Qingkailing through the C3a pathway could be inhibited by SB290157, as evidenced by a significant decrease in the β-Hex and His levels due to the action of SB290157.

QKLI induces pseudo-allergic reactions in LAD2 cells

To investigate the concentration of QKLI that induces pseudo-allergic reactions in mast cells, we treated LAD2 cells with QKLI at concentrations ranging from 2.5% to 20%. The results shown in **Figure 3** illustrate that with the increase in the concentration of QKLI, the release rates of β-Hex and His accelerated. We selected a concentration of 10% QKLI for further experiments.

QKLI induces pseudo-allergic reaction by the MRGPRX2 pathway in LAD2 cells

As shown in **Figure 4**, to investigate whether QKLI induces pseudo-allergic reactions in LAD2 cells via the Mas-related G-protein-coupled receptor X2 (MRGPRX2) pathway, we transfected LAD2 cells with three different siRNAs and compared the release rates of β-Hex and His in LAD2 cells treated with C48/80. The results showed that inhibiting the MRGPRX2 gene significantly reduced the release rates of both enzymes. Subsequently, we compared the QKLI group with the QKLI + siRNAs group, revealing no significant difference between the QKLI + siRNA2 group and the QKLI group, but significant differences were found in the other two groups. Furthermore, we examined the protein expres-

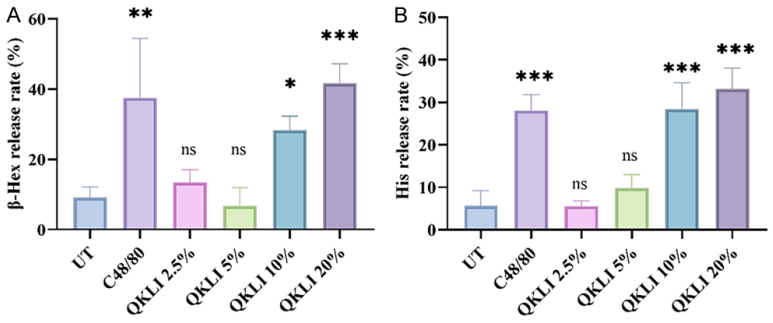

Figure 3. Effect of different concentrations of QKLI on the relative release rate of β-Hex and His in Laboratory of Allergic Diseases 2 (LAD2) cells. A. The release rate of β-Hex in different groups. B. The release rate of His in different groups. LAD2 cells were treated with H_2O (the UT group), 10 μg/mL C48/80, and 2.5%-20% QKLI for 30 min, respectively. The biological repeats were 3. Values indicate mean ± SEM. *ns, P > 0.05; *, P < 0.05; **, P < 0.005; ***, P < 0.0005,* compared to the UT group.

sion of MRGPRX2 in different groups and found that QKLI did not affect the protein expression of MRGPRX2 but directly influenced the phosphorylation of the downstream protein Extracellular Signal-regulated Kinase 1/2 (ERK1/2) with clear temporal dynamics.

BA and GE are involved in QKLI-induced pseudo-allergic hypersensitivity reactions

We determined the main ingredients in the QKLI used in this study using HPLC (**Figure 5A**, **5B**). The concentration of the main components of QKLI is as follows: BA, 6.5353 mg/mL; CA, 7.1264 mg/mL; HDCA, 9.2325 mg/mL; GE, 0.3050 mg/mL; CAA, 0.0159 mg/mL; CHA, 0.0097 mg/ml; Ado, 0.0170 mg/ml. Then, we used equal doses of BA, CA, GE, and CHA in QKLI to treat LAD2 cells and determined the release rates of β-Hex and His. The results indicate that while BA and GE individually enhance the hypersensitivity response of LAD2 cells to some extent, their effects is not as significant as that of QKL. Therefore, we further clarified the strong inductive effect of QKL on the hypersensitivity response of LAD2 cells (**Figure 5C**, **5D**).

Discussion

QKLI, as a compound traditional Chinese medicine formulation, has been widely used for its remarkable therapeutic effects in treating various diseases. QKLI was developed in the 1970s and has been utilized since the 1990s, and it provides potent therapeutic effects while also inducing severe DHRs [8, 9]. These severe DHRs have restricted the drug's usage and prompted research into the underlying mechanisms [10]. Pseudo-allergic reactions are triggered by inflammatory or anaphylactic mechanisms that are independent of antigen-specific immune responses, potentially accounting for up to two-thirds of all immediate hypersensitivity reactions and leading to significant morbidity and healthcare costs. The agents can be categorized into three types: the first type consists of direct mast cell activators, which can activate mast cells in an IgE-independent manner; the second type consists of complement activators; and the third type consists of nonsteroidal anti-inflammatory drugs [33]. In our study, we measured the concentrations of β-Hex and His to verify whether QKLI indeed can induce allergic reactions in guinea pigs, confirming that it can induce pseudo-allergic reactions.

IgE-mediated activation of bone marrow-derived mast cells (BMMCs) enhances the expression and secretion of IL-4, IL-6, TNF-α, and IL-13 [34]. In studies of acute cow's milk allergy in children, two forms have been identified: IgE-mediated and non-IgE-mediated. Specifically, children with non-IgE-mediated cow's milk allergy exhibited minimal or negligible production of IL-13 and cell proliferation. Additionally, IL-4 was undetectable in all children studied [35]. C3a, C5a, and SC5b-9 are biomarkers of complement system activation, and their increased levels may influence liver damage following ischemia-reperfusion injury (IRI) [36]. Based on previous studies [10, 22, 23, 37, 38], QKLI induces pseudo-allergic reactions via the complement pathway without affecting IgE generation. The immune response triggered by QKLI is non-IgE-mediated. To explore the in vivo mechanisms, we used ELISA to measure the levels of IL-4, IL-13, C3a, C5a, SC5b-9, and IL-6 in guinea pig serum. The results showed that QKLI could induce pseudo-allergic reactions in guinea pigs by activating the complement pathway, thereby validating the findings of Gao et al.

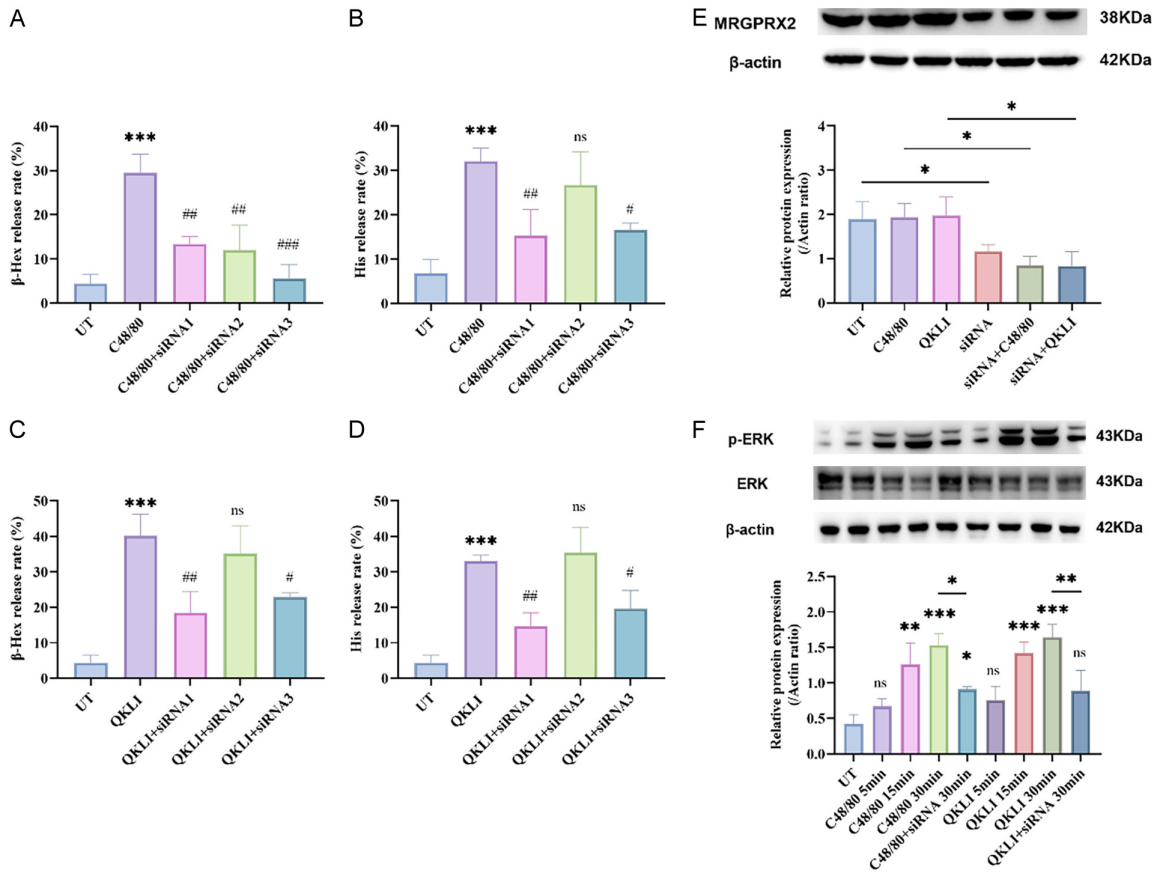

Figure 4. QKLI induces pseudo-allergic reaction by Mas-related G-protein-coupled receptor X2 (MRGPRX2) pathway in LAD2 cells. A. The effect of three different siRNAs on the rate of β-Hex release in C48/80-treated LAD2 cells. B. The effect of three different siRNAs on the rate of His release in C48/80-treated LAD2 cells. C. The effect of three different siRNAs on the rate of β-Hex release in QKLI-treated LAD2 cells. D. The effect of three different siRNAs on the rate of His release in QKLI-treated LAD2 cells. E. Protein bands and visualization analysis of MRGPRX2 under different treatment groups. F. Protein bands and visualization analysis of Extracellular Signal-regulated Kinase 1/2 (ERK 1/2) and p-ERK 1/2 under different treatment groups. The β-Hex and His release rates of LAD2 cells treated H_2O (the UT group), 10 μg/mL C48/80, and 10% QKLI for 30 min. The siRNAs were used as methods mentioned. The biological repeats were 3. Values indicate mean ± SEM. **/#, P < 0.05; **/##, P < 0.005; ***/###, P < 0.0005.* The control for "*" is the UT group, and the control for "#" is the C48/80-treated group.

MRGPRX2 is a newly discovered G protein-coupled receptor primarily expressed in mast cells and basophils. Activation of MRGPRX2 can lead to mast cell degranulation and the release of inflammatory mediators, which play a crucial role in non-IgE-mediated drug allergic reactions [21, 39]. The ERK1/2 pathway is significantly involved in the downstream signaling of MRGPRX2, promoting pseudo-allergic reactions. The activations of MRGPRX2 and the ERK1/2 pathway are a key factor in causing allergic reactions. Studies have proposed potential strategies for alleviating and treating allergic reactions by blocking MRGPRX2 or its downstream pathways, such as ERK1/2 [40, 41]. In our study, we found that QKLI triggers pseudo-allergic reactions in LAD2 cells through the MRGPRX2 pathway, resulting in increased release of β-Hex, His, and ERK1/2 phosphorylation, which is distinct from complement-mediated mechanisms.

In their study of QKLI components, Yan et al. used three different methods - HPLC, ELSD, and Diode - Array Detector (DAD)-to simultaneously determine nine components in QKLI and applied these methods for quality control [42]. The research indicated that BA, Jasminoidin (JA), and CHA exhibit synergistic effects and patterns in the combined treatment of ischemic stroke in a mouse model [43]. Additionally, Qin et al. demonstrated that CHA significantly

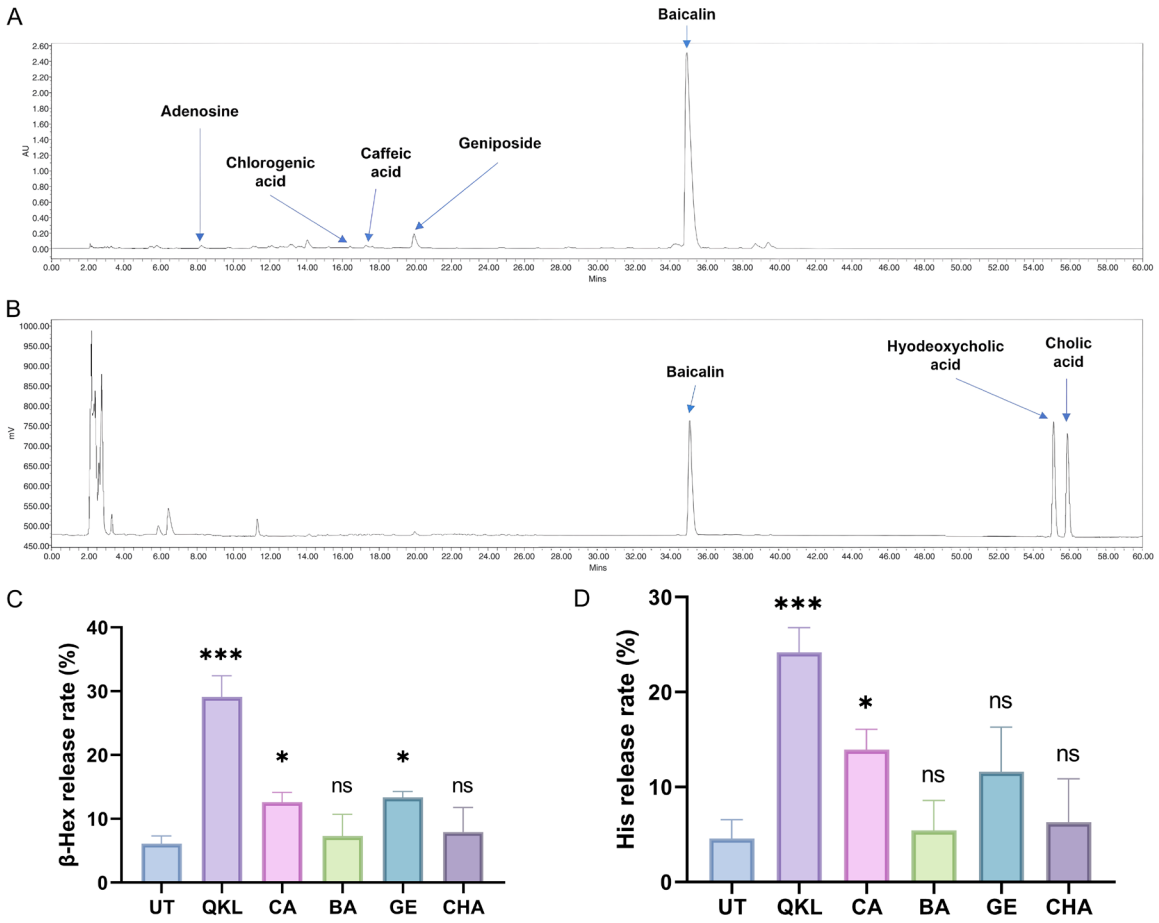

Figure 5. Baicalin (BA) and geniposide (GE) are involved in QKLI-induced pseudo-allergic reactions. (A, B) Determination of components of QKLI by High Performance Liquid Chromatography (HPLC): (A) UV absorption at 254 nm; Evaporative Light - Scattering Detector (ELSD). Column: Hypersil ODS-2 C18 column (4.6 mm × 250 mm, 5 μm). Mobile phases: methanol (A) and 0.1% formic acid in water. The gradient was as follows: 0-5 min: 5% A, 95% B; 5-10 min: 25% A, 75% B; 10-40 min: 60% A, 40% B; 40-50 min: 80% A, 20% B; 50-60 min: 90% A, 10% B. Solvent flow rate of 1 mL/min. Temperature: 35 °C. (C, D) The β-Hex and His release rates in LAD2 cells treated with H_2O (the UT group), 10% QKLI, 0.65 mg/mL BA, 0.712 mg/mL cholic acid (CA), 0.03 mg/mL GE, 0.00097 mg/ml chlorogenic acid (CHA), Values indicate mean ± SEM. *ns, P > 0.05; *, P < 0.05; ***, P < 0.0005,* compared to the UT group.

affects mast cell-dependent allergic reactions [44]. We measured the concentrations of different components in QKLI and tested their hypernasality response in LAD2 cells. We found that both BA and GE, when used individually, induced pseudo-allergic hypersensitivity in cells, suggesting that these two effective components may be involved in QKLI-induced pseudo-allergic hypersensitivity reactions. It is noteworthy that CHA has been mentioned multiple times in various studies on DHRs in herbal injections, including QKLI. In our results, the low levels of CHA in QKLI did not stimulate mast cell degranulation.

In conclusion, our study reveals that QKLI induces pseudo-allergic reactions via the complement pathway in guinea pigs and the MRGPRX2 pathway in human LAD2 cells. Additionally, BA and GE, as the effective components of QKLI, may play a facilitative role in the pseudo-allergic hypersensitivity induced by QKL in vitro. These findings underscore the complexity of the mechanisms underlying pseudo-allergic reactions to herbal injections and contribute to a better understanding of DHRs and the mechanisms.

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Disclosure of conflict of interest

None.

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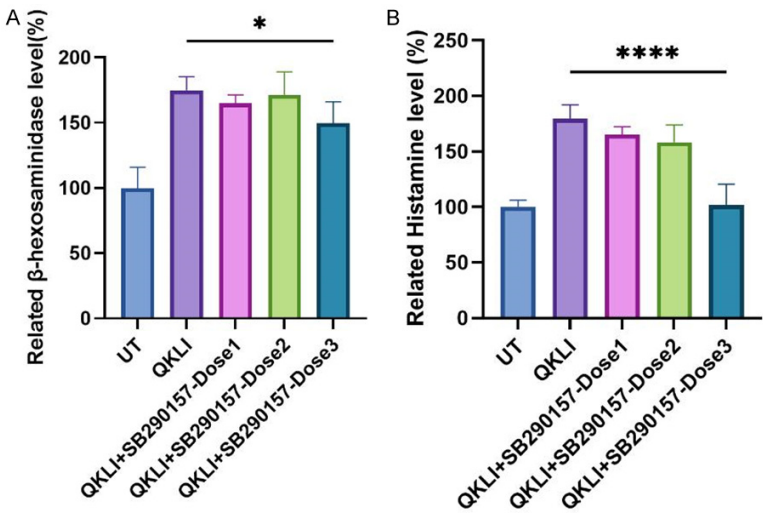

Supplementary Figure 1. Related β-hexosaminidase (β-Hex) and histamine (His) levels in guinea pigs. A. Relative levels of β-Hex. B. Relative levels of His. Eight guinea pigs per group were respectively treated with 0.2 ml/300 g 0.9% NaCl (untreated group, the UT group), 0.2 ml/300 g Qingkailing Injection (QKLI) for 30 min. For, SB290157, the guineapigs were Guinea pigs were injected with 2 mg/kg (Dose1), 10 mg/kg (Dose2), 30 mg/kg (Dose3) SB290157 for 3 h. Values indicate mean ± SD (N = 5). One-way ANOVA was employed. *ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0005$*; compared to the UT group.