Int J Clin Exp Med 2019;12(5):4829-4839
www.ijcem.com /ISSN:1940-5901/IJCEM0087168

Original Article

Effect of Xingbi nasal gel on allergic rhinitis rats based on PLCE1-PKC-NF-κB signal pathway

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Received October 23, 2018; Accepted November 21, 2018; Epub May 15, 2019; Published May 30, 2019

Abstract: Purpose: To observe the effect of traditional Chinese medicine Xingbi nasal gel on the signal pathway of PLCE1-PKC-NF-κB in rats with allergic rhinitis (AR). Methods: Fifty rats were selected and randomly divided into normal group (group A), pseudo model group (group B), model group (group C), Xingbi nasal gel group (group D) and Rhinocort group (group E) with 10 rats in each group. The AR rat model was established by ovalbumin sensitization method and given the intervention therapy respectively with the traditional Chinese medicine Xingbi Nasal Gel and western medicine Rhinocort (as control). HE staining was used to observe the pathological changes of nasal mucosa in AR rats and calculate the number of eosinophils (EOS). The serum levels of IgE, IL-4 and IL-5 were detected by ELISA. Real-time PCR, and Western blot was used to detect the expression changes of mRNA and protein levels in PLCE1-PKC-NF-κB signaling pathway (PLCE1, PKC and NF-κB) in nasal mucosa. Results: ① The replication success rate of the AR rat model was 86.67%. ② The number of inflammatory cells and EOS in the model group was higher than that in the normal group and the pseudo model group. The number of EOS in the treatment group was lower than that in the model group (P<0.01). ③ The concentration levels of IgE, IL-4 and IL-5 in the model group were higher than those in the normal group and the pseudo model group (P<0.01); ④ The mRNA and protein expression levels of PLCE1, PKC and NF-κB in the model group were higher than those of the normal group and the pseudo model group (P<0.01). Conclusion: Xingbi Nasal gel drop may inhibit activation of NF-κB by inhibiting activation of PKC in the nasal epithelial cells of AR rats, or directly inhibit activation of NF-κB, block PLCE1-PKC-NF-κB signaling pathway in the nasal mucosa of AR rats, reduce secretion of Th2 cytokines and decrease the advantage of Th2 immune response so as to reduce the nasal inflammation of AR rats and alleviate the nasal immune response.

Keywords: PLCE1-PKC-NF-κB, signaling pathway, allergic rhinitis rats, influence

Introduction

Allergic rhinitis (AR) refers to the non-infectious inflammation of nasal mucosa with the major presence of type I allergic reaction due to the exposure of atopic individuals to external allergens and is one of the most common allergic diseases in clinical practice. The main clinical manifestations of AR are nasal congestion, nasal itching, sneezing and running at the nose, etc. The chronic AR may also cause complications such as headache and olfactory loss, and even induce bronchial asthma, sinusitis, nasal polyps, otitis media, conjunctivitis and other diseases, which not only affects quality of life of patients badly, but also bring greater economic burden to the society [1]. According to the statistics, the annual investment of US in AR is as high as 6.3-7.9 billion US dollars [2]. In recent years, with the rapid development of industries and the excessive consumption of ecological environment, about 25% to 35% of the population in the world has been plagued by this disease [3], and in some areas the percentage has reached up to 44.2%, and the incidence rates tend to increase annually [4]. Therefore, prevention and treatment of AR has become a global health problem to be solved urgently.

The pathogenesis of AR is quite complicated. The current studies mainly involve a variety of

cytokines and immune mediators, and AR is the result of multiple factors and links. A large amount of research mainly focuses on the Th1/Th2 immune imbalance, immunoglobulin E (IgE) and its receptor FcεRI, eosinophils (EOS), mast cells, neuromodulation and related genes. The Thl/Th2 cytokine network imbalance is an important immunological basis for AR pathogenesis, especially the abnormally high expression of Th2 cytokines plays an important role in the pathogenesis of AR. Protein kinase C (PKC) is a key enzyme in the intracellular biological signaling pathway, which not only is involved in the intracellular signal transduction triggered by many extracellular ligands stimuli related to G protein coupling and antigen receptors but also induces the activation of many transcription factors including nuclear factor-κB (NF-κB) and activator protein-1 (Activator Protein-1, AP-1) [2]. NF-κB is an important transcriptional regulatory protein of inflammatory cytokines and enables to regulate gene expression of a variety of inflammatory cytokines. Phospholipase C epsilon-1 (PLCE1) up-regulates the expressions of interleukin-4 (IL-4) and interleukin-5 (IL-5) via the PKC-NF-κB signal transductional pathway [5]. IL-4 and IL-5, as the Th2 type cytokines, are involved in chemotaxis, recruitment, differentiation activities and active substance release processes of EOS and key factors to form the late phase of AR allergic inflammatory response [6, 7].

AR treatment with traditional Chinese medicine highlights the overall concept based on syndrome differentiation in combination with the modern pharmacological study techniques following the principles of symptom treatment in urgent cases and radical treatment in chronic cases, and has achieved good effect in recent years [8]. Previous studies have displayed that the traditional Chinese medicine Xingbi nasal gel reduces the expression of IL-5, GM-CSF and CCL-1 by inhibiting the activation and nuclear translocation of NF-κB; consequently, it reduces the inflammatory reaction of nasal passage of AR guinea pigs, so as to achieve the purpose of treating AR [9].

In this study, the AR rats were interfered with traditional Chinese medicine Xingbi Nasal Gel drops to observe the pathological changes of nasal mucosa and the changes in serum inflammatory factors such as IL-4, IL-5 and IgE before and after intervention in AR rats, and western blot, real-time PCR and other methods were used to detect the expression of PLCE1, PKC and NF-κB proteins and genes in nasal mucosa so as to elucidate the correlation between the nasal gel intervention and the PLCE1-PKC-NF-κB signaling pathway in AR rats. The in-vitro study was carried out to observe the signal changes of PLCE1-PKC-NF-κB signaling pathway in epithelial cells of nasal mucosal under the nasal gel intervention, to clarify the mediation route of PLCE1-PKC-NF-κB signaling pathway, and to further elucidate the regulatory mechanism of AR treatment with Xingbi nasal gel, providing new ideas and new methods for prevention and treatment of AR with traditional Chinese medicine.

Materials and methods

Experimental drugs

Traditional Chinese Medicine Xingbi Nasal Gel Drop: prepared by People's Hospital Affiliated to Fujian University of Traditional Chinese Medicine, drug preparation No. Z20110006, concentration: 248 mg/ml, prepared by the preparation laboratory of People's Hospital Affiliated to Fujian University of Traditional Chinese Medicine. Western medicine Budesonide Nasal Spray (trade name: Reynolds): produced by AstraZeneca Pharmaceutical Co., Ltd., national medicine No. J20140048, strength: 1.28 mg/ml, provided by the Pharmacy Department of People's Hospital Affiliated to Fujian University of Traditional Chinese Medicine.

Experimental animal

50 specific pathogen free (SPF) Sprague-Dawley (SD) rats with half males and half females, 2 months old, weighing 200 g to 250 g were selected. The experimental rats were provided by Shanghai Slack Laboratory Animals Center with the license number of SCXK (Hu) 2012-0011. The environment and equipment for the experimental animals were provided by the Animal Laboratory of Fujian University of Traditional Chinese Medicine with the license number of SYXK (Min) 2009-0001.

Principle solution preparation

(1) OVA suspension for sensitization: containing 0.05% OVA + 3% aluminum hydroxide adjuvant.

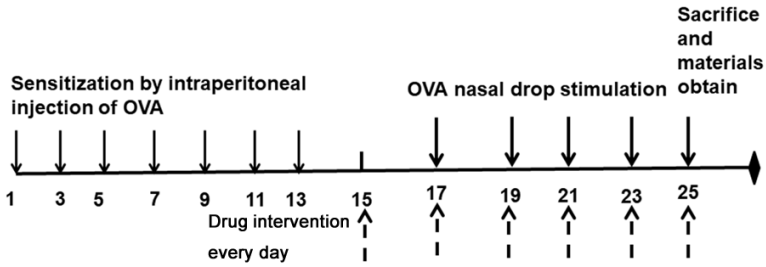

Figure 1. AR rat model replication with OVA sensitization and drug intervention.

OVA (20 mg) and aluminum hydroxide powder (1.2 g) were placed in a 50-ml sterile centrifuge tube, to which 0.9% NS injection of the constant volume 40 ml was added to form the suspension. The suspension was prepared immediately before use.

(2) OVA solution for excitation: 2% OVA solution. OVA (80 mg) was placed in a 15-ml centrifuge tube, to which 0.9% NS injection of the constant volume 4 ml was added and vortex shake to complete dissolution. The solution was prepared immediately before use.

(3) Nasal gel solution: 20 mg of extract for nasal gel was placed in a 5-ml centrifuge tube, to which 4 ml of sterile PBS (0.0067M) was added, vortex shake to complete dissolution so as to prepare the nasal gel solution at a concentration of 5 mg/ml. The solution was stored at 4°C for spare use and would be expired after one month.

(4) Cultivation solution containing 25 μg/ml of Reynolds: 3.91 ml of Reynolds suspension at a concentration of 1.28 mg/ml was absorbed, to which the DMEM/F12 nutrient solution containing 10% FBS was added to constant volume 200 ml and thoroughly mixed. After filtering through sterile filter with 0.22 μm pore size, the solution was stored at 4°C and would be expired in 3 months.

Experimental method

Establishment of AR rat model

The ovalbumin (OVA) sensitization method was applied to replicate the rats in the model establishment group (groups C, D, E) into an AR model; see **Figure 1** for details. 1. Sensitization: each rat was intraperitoneally injected (aseptically) 1 ml of OVA suspension containing 0.5 mg/ml OVA and 30 mg/ml aluminum hydroxide once every other day for 7 times, so that the experimental rats were systemically sensitized. Rats in group B were injected intraperitoneally with the same amount of NS, and the methods and procedures were the same as before. 2. Excitation: on the 4th day after sensitization by intraperitoneal injection, the rats in the model establishment group were placed face upward and excited by dropping 50 μl of 2% OVA solution into each nostril, once every other day for a total of 5 times; the same amount of 0.9% normal saline (NS) replaced OVA in group B, and the method and the procedures were the same as before. 3. Modeling effect evaluation: according to the National Allergic Rhinitis Diagnostic and Efficacy Evaluation Standards revised in 1997, the AR model behavioral rating scale was developed; within 30 minutes after the bilateral nasal stimulation each time, the number of sneezing, nose scratching and nasal discharge in each group were recorded [10]. The total score was calculated by the superposition method. The total score ≥ 5 indicated successful modeling. The scoring method was shown in **Table 1**.

Animal feeding and grouping

50 SD rats were housed and fed in the laboratory with SPF class laminar flow of Experimental Animal Center of Fujian University of Traditional Chinese Medicine. The room of the laboratory was in the brightness or darkness in turn for 12 hours respectively, and kept at the room temperature of 25±1°C and the relative humidity of 40%-60%. The rats were not restricted from activities or eating; the diet was normal organic feed; the drinking water was distilled water after high temperature sterilization. After 1 week of adaptive feeding, the rats were grouped as normal group (group A) including 10 rats, pseudo model group (group B) including 10 rats and model establishment group including 30 rats according to the random number table. Afterwards, the rats after systemic sensitization were divided as the layer randomization method into a model group (group C) and two

Table 1. AR Behavioral Scoring Method

Symptoms Scores	Sneeze (unit)	Nose itching/nose scratching (times)	Nose discharge
1 point	3~9	2~3	Running to the nostril
2 point	10~14	4~5	Running beyond the anterior naris
3 point	≥ 15	≥ 5	Running in all the faces

Table 2. Primer sequences of PLCE1, PKC, NF-κB and other genes

Primer	Sequence	Length of the product (bp)
GAPDH	F: 5'-TGCCACTCAGAAGACTGTGG-3'	129
	R: 5'-TTCAGCTCTGGGATGACCTT-3'	
PLCE1	F: 5'-TGCTTTTCAGTGGATTGCTG-3'	119
	R: 5'-CCGACCATCCTCCTGATAGA-3'	
PKC	F: 5'-CCCATTCCAGAAGGAGATGA-3'	212
	R: 5'-TTCCTGTCAGCAAGCATCAC-3'	
NF-κB	F: 5'-GGGCTGACCTGAGTCTTCTG-3'	119
	R: 5'-GATAAGGAGTGCTGCCTTGC-3'	

treatment groups (group D and group E), with 10 rats in each group.

Drug intervention and materials obtain

Drug intervention: the drug intervention started on the second day after the 7th intraperitoneal injection of OVA suspension for sensitization. The rats were placed in the face upward position, and the drugs were dropped on both sides of the nose three times daily with a micro-sampler for a total of 11 days. The rats in group D were given 50 μl of xingbi nasal gel drop bilaterally; Group E was given 50 μl of Rhinocort bilaterally; the rats in group B and group C were given the same amount of NS replacement liquid with the same method and procedures as before.

Materials obtain and specimen treatment: after the end of drug administration on the 11th day, the rats in each group were fasted (water drinking was allowed) for 12 hours and anesthetized by intraperitoneal injection of 2% sodium pentobarbital at 50 mg/kg. The rats were placed in the supine position; the abdominal cavity was opened; the tissue was bluntly separated; the abdominal aorta was exposed; and the whole blood was collected in the abdominal aorta with disposal blood collection needles. After standing at room temperature for 30 min, whole blood was centrifuged at 3500 rpm for 10 min; the supernatant was taken, separately filled and stored at -80°C for the detection of various protein indicators with ELISA method afterwards. The nasal cavity was opened; the nasal septum was removed and the nasal mucosa on both sides of the nasal septum were peeled off, within which the right mucosa was fixed in 4% paraformaldehyde for morphological observation while the left mucosa was temporarily placed in the liquid nitrogen for rapid freezing, and then stored at -80°C for subsequent detection of biochemical indicators.

Detection of PLCE1-PKC-NF-κB signaling pathway related mRNA content in rat nasal mucosa by real-time PCR method

Total RNA was extracted by the Trizol method, and reversely transcribed according to the instructions on the reverse transcription kits. The fluorescent quantitative PCR amplification and the primer design and synthesis were performed: the NCBI gene library was used to find the full sequence of target genes, and Primer Premier 5.0 software was used to design each primer sequence which was synthesized by Shanghai Biosune Technology Co., Ltd. According to the instruction of PCR kits, 10 ul of amplification reaction system was prepared, fully mixed and centrifuged to remove bubbles. GAPDH was used as internal reference to calculate the target gene 2-ΔΔCt; the relative expression level of mRNA of each target gene was compared and analyzed. Each sample was repeated in 3 replicate wells to obtain the average value. ΔΔCt = (Ct value of the target gene in the experimental group - Ct value of the internal reference) - (Ct value of the target gene in the control group - Ct value of the internal reference). The primers were shown in **Table 2**.

Detection of expression levels of PLCE1-PKC-NF-κB signaling pathway-related proteins in rat nasal mucosa by western blot

The weight of the nasal mucosa tissue was measured and the tissue was fully lysed by RIPA and centrifuged at 12,000 rpm for 20 min

Table 3. Behavioral Score of AR Rats

Groups	Times of Excitation					Remarks
	First time	Second time	Third time	Fourth time	Fifth time	
Normal group	0	0	0	0	0*	
Pseudo model group	0.5±0.707	0.7±0.675	1.0±0.943	1.6±1.174	1.8±1.476*	1 rat was excluded
Model group	2.556±0.726	3.889±0.601	5.444±0.726	6.222±0.667	6.778±1.202	1 rat was dead and 1 rat was excluded
Nasal gel group	2.5±0.527	3.7±0.823	5.1±0.738	5.1±1.197	5.7±0.823☆	1 rat was excluded
Rhinocort group	2.4±0.699	3.8±0.632	5.2±0.632	5.2±0.789	5.3±0.823*	1 rat was excluded

Note: the rank sum test was used. At the 5th times, Group A, B, D, E compared with the group C, ☆P<0.05, *P<0.01.

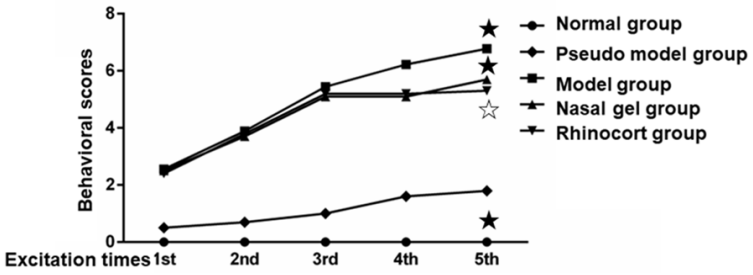

Figure 2. Effect of Nasal Gel Drop on Behavioral Scores of AR Rats. Column shows the behavioral Score of AR Rats. Note: At the 5th times compared with the group C, ☆P<0.05, *P<0.01.

at 4°C. The supernatant was absorbed for protein denaturation. 5 μl of pre-stained protein and 30 μg of protein sample to be tested in each group were sequentially added to the gel sampling well; the parameters for electrophoresis were set as followings: pre-running for 20 min at a constant voltage of 20 V; 60 to 100 V for 50 min, and 100 to 120 V for 60 to 80 min. When electrophoresis was finished, the gel glass plate was peeled off; the target protein gel was cut off from the respective corresponding position, transformed in the semi-dry transfer film groove; an appropriate amount of transfer liquid was dropped to keep the moisture and avoid the bubble generation. According to the molecular weight of each target protein, the membrane transfer time was set as follows: β-actin (45 kd) for 5 min, NF-κB (65 kd) for 6 min, PKC (80 kd) for 8min and PLCE1 (123 kd) for 12 min; (1) The PVDF membrane was transferred to the blocking solution and incubated on a horizontal shaker for 1 to 2 hours at room temperature; (2) The membrane was washed for 5 minutes with TBST; (3) Each PVDF membrane was transferred to the corresponding specific antibody (primary antibody) (β-actin 1:1000 dilution, PLCE1 1:200 dilution, PKC 1:1000 dilution, NF-κB 1:1000 dilution) and incubated on the shaker overnight at 4°C; (4) The membrane was washed thoroughly with TBST on the next day for 5 min * 3 times; (5) PVDF membrane was transferred to the HRP-labeled sheep anti-rabbit IgG (secondary antibody) (1:5000 dilution) and incubated for 1 to 2 h at room temperature on a horizontal shaker; (6) The membrane was washed thoroughly with TBST for 5 min * 3 times. According to the size and quantity of PVDF membranes, the ECL coloring solution was prepared in the dark and uniformly dropped to each PVDF membrane in order. After 1 min of standing and reaction, the image was developed on a chemiluminescence imager. The gray value of each strip was read by Image Lab 4.0 software, and the expression level of each target protein was semi-quantitatively analyzed with β-actin as the internal reference.

Statistical analysis

The experimental data was processed by SPSS statistical software, version 20.0. Measurement data are expressed with mean ± standard deviation ($\bar{x} \pm s$). The one-way ANOVA was used for comparison between groups. The Kruskal-Wallis Test rank sum test was used for those dis-complying with the normal distribution. P<0.05 implied a statistically significant difference; P<0.01 indicated a remarkably significant statistical difference.

Results

AR rat behavioral scoring

30 rats in the model establishment group were replicated into AR model by OVA sensitization. In the modeling period, one rat died and the

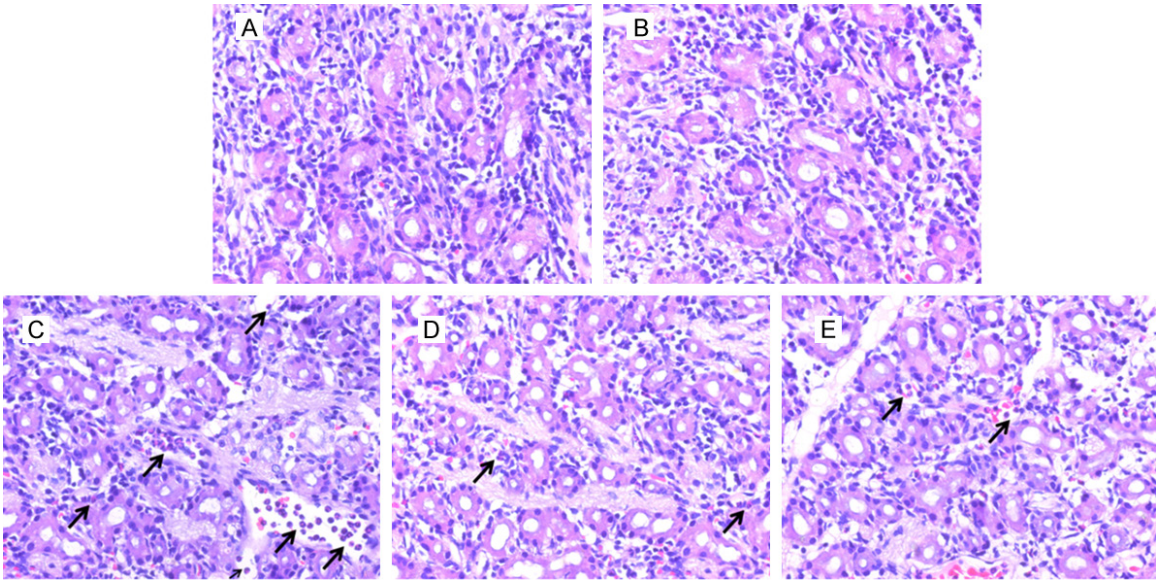

Figure 3. HE staining of pathological sections of AR rat nasal mucosa (×400). Note: as shown in the above figure, (A) is the rat nasal mucosa of the normal group; (B) is the rat nasal mucosa of the pseudo model group; (C) is the rat nasal mucosa of the model group; (D) is the rat nasal mucosa of the xingbi nasal gel drop group; (E) is the rat nasal mucosa in the Rhinocort group.

Table 4. Eosinophil count in pathological sections of AR rat nasal mucosa

Groups	*n*	EOS quantity (unit/section)
Normal group	10	0.111±0.323★
Pseudo model group	10	0.333±0.594★
Model group	10	23.889±5.769
Xingbi nasal gel group	10	10.222±4.558★
Rhinocort group	10	12.278±4.812★

Note: the rank sum test was adopted for the comparison. Compared with group C, ☆P<0.05, ★P<0.01.

behavioral scoring after excitation showed that 26 AR rats were successfully replicated including 8 in group C, 9 in group C and group D respectively with a success rate of 86.67%. After the last excitation, the scores in group A, B, D and E were significantly lower than those of group C (compared with group C, P of group A, B and E were <0.01, P of group D was <0.05); the scores in group D and group E were not significantly different (P>0.05) (**Table 3** and **Figure 2**).

Morphology observation and analysis of nasal mucosa in AR rats

The HE staining of rat nasal mucosa in each group showed that in group A and group B, the nasal mucosa tissues were complete in structure, and the epithelial and goblet cells were neatly arranged without obvious inflammatory cell infiltration and vascular changes observed; in group C, the nasal mucosa edema was observed, the glandular hyperplasia was obvious, and the interstitial vascular congestion and expansion were found with a large number of eosinophils and mast cells infiltration in the lamina propria; In group D and group E, the traditional Chinese medicine Xingbi nasal gel and western medicine Rhinocort were applied respectively for intervention treatment, and the congestion and edema were still observed in the nasal mucosa, which was significantly improved and the inflammatory cell infiltration was significantly reduced compared with that of the group C (**Figure 3** and **Table 4**). The EOS counting displayed that ① compared with group A, the number of EOS in group B was not significant different (P>0.05); ② the number of EOS in group A, B, D and E was significantly less than that in group C (compared with group C, P<0.01); ③ there was no significant difference in the number of EOS between group D and the group E (P>0.05).

Serum IgE, IL-4 and IL-5 levels in AR rats

The results showed that ① compared with group A, the concentration levels of IgE, IL-4

Table 5. Serum IgE, IL-4 and IL-5 in AR Rats

Groups	IgE (ng/ml)	IL-4 (ng/ml)	IL-5 (ng/ml)
Normal group	153.465±67.359★	4.282±0.946★	11.708±2.750★
Pseudo model group	174.421±84.992★	4.710±1.472★	11.569±2.470★
Model group	931.810±37.852	17.959±3.617	26.099±2.200
Xingbi nasal gel group	595.507±117.361★	7.645±1.644★	18.600±2.505★
Rhinocort group	647.221±123.681★	8.035±1.164★	17.408±2.598★

Note: One-way ANOVA was adopted. Compared with the group C, ☆P<0.05, ★P<0.01. The below picture is the in the same case.

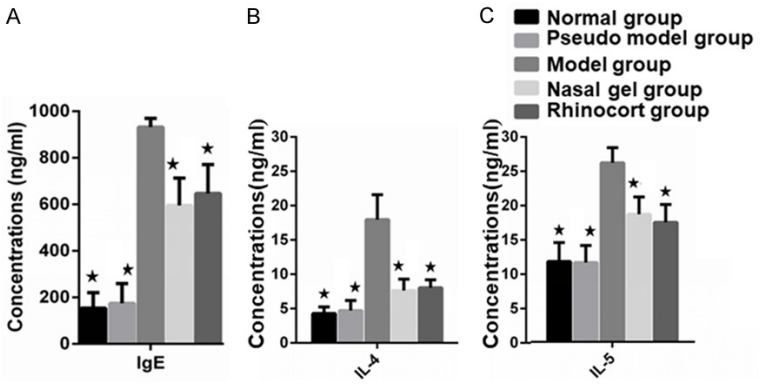

Figure 4. Effect of Xingbi Nasal Gel Drop on Serum IgE, IL-4 and IL-5 in AR Rats. A. Serum IgE level in AR rats. B. Serum IL-4 level in AR rats. C. Serum IL-5 level in AR rats. Note: One-way ANOVA was adopted. Compared with the rat nasal mucosa of the model group, ☆P<0.05, ★P<0.01. The picture below was in the same case.

Table 6. mRNA Expression of PLCE1, PKC and NF-κB in Nasal Mucosa of AR Rats

Groups	PLCE1	PKC	NFκB
Normal group	1.004±0.102★	1.008±0.146★	1.012±0.182★
Pseudo control group	1.387±0.327★	1.086±0.355★	1.306±0.232★
Model group	4.767±0.617	3.426±0.786	3.052±0.619
Nasal gel group	1.771±0.481★	1.568±0.525★	2.098±0.564☆
Rhinocort group	1.855±0.427★	1.747±0.446★	1.717±0.636★

Note: One-way ANOVA was adopted. Group A, B, D, E compared with group C, ☆P<0.05, ★P<0.01.

and IL-5 in group B were not significantly different from those in group B (P>0.05); ② the concentration levels of inflammatory factors in group C were significantly higher than those in group A and group B (compared with group C, P in group A and group B were both <0.01); the concentration of each inflammatory factor in group D and group E were significantly reduced after intervention treatment respectively with traditional Chinese medicine and western medicine; ③ comparing between group D and group E, the expression level of each inflammatory factor was not significant different (P>0.05) (**Table 5** and **Figure 4**).

mRNA expression levels of PLCE1, PKC and NF-κB in nasal mucosa of AR rats

The results displayed that ① compared with group A, the mRNA expression levels of PLCE1, PKC and NF-κB were not significantly different from those in group B (P>0.05); ② the mRNA expression level of each target gene in group C was significantly higher than that in group A and Group B (compared with group C, P in group A and group B were both <0.01); after intervention treatment of traditional Chinese medicine and western medicine respectively in group D and group E, the mRNA level of each target gene was significantly reduced (compared with group C, P in group E was <0.01; P in group D was <0.01 in terms of PLCE1 and PKC expression, P was <0.05 regarding the NF-κB); ③ compared with group D and group E, the mRNA level of each target gene was not significantly different (P>0.05) (**Table 6** and **Figure 5**).

PLCE1, PKC and NF-κB protein expression levels in nasal mucosa of AR rats

The results showed that ① compared with group A, the protein expression levels of PLCE1, PKC and NF-κB in group B were not significantly different (P>0.05); ② the expression level of each target protein in group C was significantly higher than that in group A and group B (com-

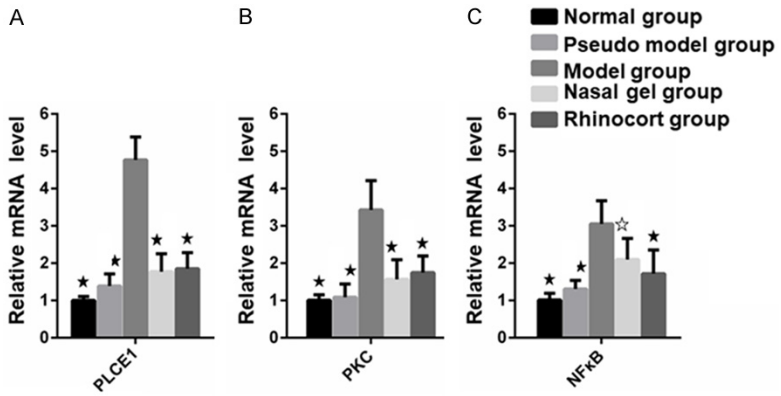

Figure 5. Effect of Xingbi Nasal Gel on mRNA expression of PLCE1, PKC and NF-κB in nasal mucosa of AR rats. A. mRNA expression level of PLCE1 in nasal mucosa of AR rats; B. mRNA expression level of PKC in nasal mucosa of AR rats; C. mRNA expression level of NF-κB in nasal mucosa of AR rats. Note: One-way ANOVA was adopted; compared with the the rat nasal mucosa of the model group, ☆P<0.05, ★P<0.01. The picture below was in the same case.

Table 7. Protein Expression of PLCE1, PKC and NF-κB in Nasal Mucosa of AR Rats

Groups	PLCE1	PKC	NFκB
Normal group	0.240±0.157★	0.291±0.060★	0.114±0.053★
Pseudo model group	0.306±0.232★	0.404±0.261★	0.161±0.053★
Model group	0.943±0.100	1.087±0.163	0.525±0.083
Nasal gel group	0.683±0.102☆	0.430±0.102★	0.217±0.066★
Rhinocort group	0.514±0.182★	0.587±0.140★	0.290±0.080★

Note: One-way ANOVA was adopted. Compared with the group C, ☆P<0.05, ★P<0.01. The picture below was in the same case.

pared with the group C, P in group A and group B were both <0.01); the expression level of each target protein in group D and group E after drug interventions of traditional Chinese medicine and western medicine respectively was significantly reduced (compared with group C, P in group E was <0.01, P of the PKC and NF-κB expression in group D was <0.01 and P of PLCE1 in group D was <0.05; ③ compared with group D and group E, the expression level of each target protein was not significantly different (P>0.05) (**Table 7** and **Figure 6**).

Discussion

The etiology of AR is complicated, and its pathogenesis is multiple factors related. At present, the research hotspots for AR mainly focus on immune imbalance, environmental pollution, family genetic mechanisms and health hypothesis, etc. Clinically, there are two main protocols for prevention and treatment of allergic diseases: one is to block the action of mechanism and effect system of allergy, and the other is immunotherapy. Currently, the drug therapy is still a preferred treatment for AR. The primary drugs for the AR treatment include 6 kinds, that is, glucocorticosteroids, antihistamines, decongestants, anticholinergics, antileukotrienes and mast cell membrane stabilizers.

Animal models are important tools to study the human diseases. Establishing an effective and stable animal model is the premise of reliability of medical research. The higher the similarity between animal models and human diseases in clinical features and pathological mechanisms is, the greater the guidance significance for the medical study will be. The AR animal model was firstly established successfully in 1988 by Tanaka et al. through the application of 2,4-toluene diisocyanate (TDI) [11]. The rats are rich in complement, capable to produce stable specific antibody IgE and IgG with high potency, and easy to induce rapid phase reaction and late phase reaction. The rats are rich in sources, have highly developed immune system and are sensitive to allergic substances. Currently, the research on mouse genes and immune system has been relatively perfected, which brings convenience to the development of multiple experimental techniques including gene recombination and in situ hybridization with the advantages to further study the AR pathogenesis from the molecular biological level, which not only benefits the study of molecular mechanisms, but also stabilizes in genetic traits and is easy to sensitize. Therefore, the SD rats were selected as the objects of the study with the advantages of easy operation during modeling, easy observation of behavioral changes and high survival rates.

There are many types of allergens in AR animal models, including chemical drugs such as TDI and OVA, and biosensitizers such as pollen,

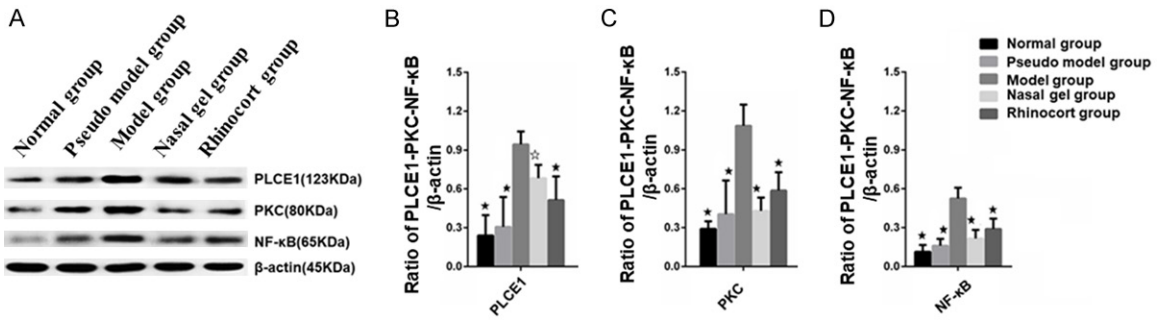

Figure 6. Effect of xingbi nasal gel on the protein expression of PLCE1, PKC and NF-κB in nasal mucosa of AR rats. A. Western blot result of PLCE1, PKC and NF-κB in different groups. B. Statistical analysis results of PLCE1. C. Statistical analysis results of PKC. D. Statistical analysis results of NF-κB. Note: As shown in the figure above, A is the protein imaging, and B is the protein gray value analysis result of PLCE1, PKC and NF-κB.

fungi and dust mites. The immunoadjuvants often used in combination include BCG, aluminum hydroxide and lipopolysaccharide, etc. Among them, aluminum hydroxide is widely used because of its non-toxicity and good adsorption ability as well as its capacity to protect the antigen at the injection site from being removed by the body. Dong et al. studied the AR guinea pig model established with different sensitizers (OVA, TDI and fungi), and the results suggested that the OVA modeling was more in line with the clinical manifestations of AR, and it was simple and easy in operation with high model survival rates and 100% lesion rate based on the comprehensive evaluation results of behavioral evaluation, serum biochemical index detection and tissue morphometric analysis [12]. Li et al. found that the AR rat model was successfully established by intraperitoneal injection of 0.3 mg OVA + 30 mg aluminum hydroxide as adjuvant [13]. Zeng summarized the literature on AR guinea pig model published at home and abroad in 2010-2015, and the results demonstrated that 63% of the AR model was established by using OVA combined with aluminum hydroxide, which induced less specific lung stimulation and was safer to the experiment operator compared with the earliest applied TDI [14]. Therefore, this study selected OVA combined with aluminum hydroxide sensitization + OVA nasal drop excitation to establish AR rat model and as the behavioral scoring, the success rate of modeling was higher than 85%.

In this experiment, the AR rat model was replicated by OVA sensitization method, and treated with interventions of traditional Chinese medicine Xingbi nasal gel drop and western medicine Rhinocort respectively. The behavioral scoring, pathological section observation and serum inflammatory factors detection showed that the Xingbi nasal gel drop enabled to significantly improve the allergic symptoms in AR rats, reduce eosinophil accumulation in nasal mucosa, and to reduce the levels of IgE, IL-4 and IL-5 in peripheral serum, which suggests that the nasal gel drops may inhibit the synthesis of IgE antibody and block the binding of IgE to target cells in rats by inhibiting the secretion of IL-4 and IL-5 of Th2 cells, thereby inhibiting the release of inflammatory mediators, hindering the accumulation of EOS in nasal mucosa, and playing the roles of reducing inflammation of the nasal passages of AR rats and alleviating the symptoms of nasal allergy.

PLCE1 is a phospholipase C isoenzyme encoded by PLCE1 gene, which regulates cell proliferation, differentiation, apoptosis and migration. The CD^{4+} T cell receptor (TCR) in AR nasal mucosa activates the PLCE1 after activation and PLCE1 catalyzes the hydrolysis of phosphatidylinositol (4, 5) bisphosphate (PIP2) to inositol-1,4,5-triphosphate (IP3) and second messengers such as diacylglycerol (DAG), and DAG further activates PKC. As a key enzyme in the intracellular biosignaling pathway, PKC not only participates in the intracellular signal transduction triggered by many extracellular ligands involved in G protein coupling and antigen receptors, but also induces activation of multiple transcription factors such as NF-κB and AP-1. NF-κB is the most important nuclear transcription factor for mediating intracellular signaling transferring and also an important

transcriptional regulatory protein of inflammatory factors. It plays a pivotal role in inflammation and immune response by regulating the expressions of immune and inflammation-related factors and inflammatory mediators. Recent studies have shown that activation of NF-κB may be a central link in the complex "network structure" of allergic diseases occurrence [15]. The subunit p65 of NF-κB can directly act on transcriptional elements and activate the transcription process, thereby initiating a series of immune-related genes, such as the transcriptional programs of pro-inflammatory mediators, adhesion molecules and chemokines, etc., promoting the infiltration of a large number of inflammatory cells at the inflammation site and consequently causing or further aggravating the inflammatory response [16-18]. PLCE1 participates in differentiation of Th2 cells in the nasal mucosa through the PKC-NF-κB signal transduction pathway, while the PKC-NF-κB signaling pathway plays an essential role in the IL-2 and IL-5-mediated Th2 cellular immune responses [19-21]. The results of this study also showed that the genes and protein levels of PLCE1, PKC and NF-κB in the nasal mucosa of AR rats were all highly expressed, which proves that the PLCE1-PKC-NF-κB signaling pathway was closely related to the pathogenesis of AR.

This experimental study demonstrated that the gene and protein levels of PLCE1, PKC and NF-κB in nasal mucosa of AR rats were significantly decreased after treatment with Xingbi Nasal Gel Drops. The pathological section observation and serum inflammatory factor detection suggested that the Xingbi nasal gel drop may inhibit secretion and synthesis of IgE, IL-4 and IL-5 *in vivo* by down-regulating the PLCE1-PKC-NF-κB signaling pathway, and correct the Th1/Th2 immune imbalance, so as to achieve the objective of alleviating the immune reaction of AR rats. At the same time, there was no significant difference in the expression levels of each target gene and protein between the Xingbi nasal gel group and the western medicine control group, which suggests that the Xingbi nasal gel drop and the western medicine Rhinocort were similar in the regulatory pathways intervention of immune response in AR rats.

In this experiment, the *in vivo* study was carried out in AR rats by using Xingbi Nasal Gel Drops as the drug intervention, and the results showed that the gene and protein levels of PLCE1, PKC and NF-κB in AR rats were highly expressed. The intervention treatment of Xingbi nasal gel drop significantly improved the allergic symptoms of AR rats, reduced the EOS accumulation in small amount of nasal mucosa, decreased the levels of IgE, IL-4 and IL-5 in peripheral serum and down-regulated the gene and protein levels of PLCE1, PKC and NF-κB, which verifies that the PLCE1-PKC-NF-κB signaling pathway was closely related to the pathogenesis of AR and suggests that the Xingbi nasal gel drop may inhibit secretion of IL-4 and IL-5 in Th2 cells, correct Th1/Th2 immune imbalance, reduce IgE synthesis or decreases IgE levels, and block the binding of IgE antibodies to target cells in rats by down-regulating the PLCE1-PKC-NF-κB signaling pathway. As a result, the drug treatment could inhibit the release of inflammatory mediators, hinder the accumulation of EOS in the nasal mucosa, reduce inflammation in the nasal passages of AR rats and alleviate the symptoms of nasal allergy.

Conclusions

In this study, the *in vivo* study of AR rats was carried out by using Nasal Gel Nasal Drops. The results showed that the mRNA and protein levels of PLCE1, PKC and NF-κB in AR rats were highly expressed by the intervention by the Nasal Gel Nasal Drops. After treatment, it can significantly improve allergic symptoms in AR rats, reduce EOS aggregation in nasal mucosa, reduce the levels of IgE, IL-4 and IL-5 in peripheral blood, and down-regulate the mRNA and protein levels of PLCE1, PKC and NF-κB. It was verified that the PLCE1-PKC-NF-κB signaling pathway is closely related to the pathogenesis of AR; it is suggested that the Nasal Gel nasal drops may inhibit the secretion of IL-4 by Th2 cells by down-regulating the PLCE1-PKC-NF-κB signaling pathway. IL-5, corrects Th1/Th2 immune imbalance, reduces IgE synthesis or decreases IgE levels, blocks the binding of IgE antibodies to target cells in rats, thereby inhibiting the release of inflammatory mediators, hindering the accumulation of EOS in the nasal mucosa, reducing AR Inflammation of the nasal passages of the mouse, and alleviating the symptoms of nasal allergy.

Acknowledgements

This work was supported by the National Natural Science Foundation of China [grant number 81373820], and the Health and Family

Planning Project of Fujian Province [grant number 2016-ZQN-71].

Disclosure of conflict of interest

None.

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