

Original Article

Effect of Tong Qiao drops on the expression of eotaxin, IL-13 in the nasal mucosa of rats with allergic rhinitis

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Abstract

Background: In recent years, particularly in China, many Chinese medicines and prescriptions for treating allergic rhinitis have been evaluated for their clinical relevance. Studies have found that numerous herbs and their constituent compounds can significantly alleviate allergic symptoms and are effective treatments for allergic rhinitis. The purpose of this study was to examine the modulatory effect of Tong Qiao nose drops on allergy symptoms and the expression of cytokines in the nasal mucosa of rats with ovalbumin-induced allergic rhinitis.

Methods: Sixty healthy male Sprague Dawley rats were randomly divided into three groups ($n = 20$): negative, control, and experimental. Rats in the control or experimental groups were sensitized with ovalbumin to induce allergic rhinitis. The sensitized rats in the experimental group were subsequently exposed to Tong Qiao nose drops, whereas the sensitized control rats were given saline nose drops. Negative control rats were only treated with saline. Allergic symptoms and the pathologic features of the nasal mucosa were observed. The expression of eotaxin in the mucous membrane of rat nasal septums was detected by immunohistochemical staining, and the expression levels of interleukin (IL)-5 and IL-13 were measured by enzyme-linked immunosorbent assay.

Results: The symptom scores for the experimental group were significantly lower than those of control rats ($p < 0.01$). Histopathologic examination revealed pathologic changes of nasal mucosa edema in the experimental group was mild and the infiltration of eosinophils was insubstantial. The expression levels of eotaxin, IL-5, and IL-13 in the nasal mucosa from experimental rats were significantly lower than that of control rats ($p < 0.01$).

Conclusion: Tong Qiao nose drops alleviated the symptoms of allergic rhinitis in a rat model and lowered the expression levels of eotaxin, IL-5, and IL-13.

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Keywords: allergic rhinitis; eotaxin; interleukin; rat model; Tong Qiao nose drops

1. Introduction

Allergic rhinitis (AR) is a type 1 immunoglobulin (Ig) E-mediated hypersensitivity disease that is increasing in incidence.¹ In recent years, particularly in China, many Chinese medicines and prescriptions for treating allergic rhinitis have been evaluated for their clinical relevance. Studies have found that numerous herbs and their constituent compounds can

significantly alleviate allergic symptoms and are effective treatments for allergic rhinitis.^{2,3}

Tong Qiao drops (Chengdu Huashen Zhiyao, Ltd Co., Chenddu, China), a new Chinese therapeutic formula that contains active ingredients from three species of medicinal plants: *Angelica dahurica* (Bai Zhi), *Gleditsia sinensis* Lam (Zao Jia), and *Flos Magnoliae* (Xin Yi). Each agent of the Tong Qiao drops has been demonstrated to have an anti-inflammatory effect, and they can be used effectively to minimize symptoms in many allergic diseases.^{4–6} However, whether Tong Qiao drops have any demonstrable effect in the treatment of allergic rhinitis has yet to be established. This study sought to investigate the effect of Tong Qiao drops on

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experimental allergic rhinitis in rats and to explore its mechanism of action. We used Tong Qiao nasal drops in rats with ovalbumin-induced allergic rhinitis and measured its effect on cytokine levels in the nasal mucosa.

2. Methods

2.1. Reagents

Tong Qiao nasal drops (10 g) *A dahurica* Bai Zhi, 1 g *G sinensis* Lam Zao Jia, 10 g *F Magnoliae* Xin Yi, 80 mL glycerol, and sodium chloride were purchased from Chengdu Huasheng Modern Biology Science & Technology Co (Chengdu, China). Ovalbumin (OVA) was purchased from Sigma (St. Louis, MO, USA), aluminum hydroxide gel (40 mg/mL) was purchased from Gibco (Life Technologies Corp, Grand Island, NY, USA), and rat interleukin (IL)-5 and IL-13 enzyme-linked immunosorbent assay (ELISA) kits were purchased from Shanghai Yi-Li Bio-Technology Co., Ltd. (Shanghai, China). Rabbit antimouse antieotaxin polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and the ready-to-use EnVision reagent (Dako Corporation, Copenhagen, Denmark) were also used.

2.2. Rats

Institutional Review Board approval for the study was obtained through our hospital. Eighty special pathogen free (SPF) male Sprague Dawley (SD) rats, 6–8 weeks old and weighing 180–220 g each, were housed in the First Affiliated Hospital, Zhejiang University School of Medicine Laboratory Animal Center. Using a random number table the rats were divided into experimental, control, negative, and positive control groups ($n = 20$ per group).

2.3. Induction of allergic rhinitis in rats

Rats were sensitized following a revised version of the protocol published by An *et al.*⁷ The sensitizing solution was prepared by dissolving 0.3 mg OVA into 1 mL saline using 30 mg aluminum hydroxide as an adjuvant (negative control rats were given 1 mL saline plus 30 mg aluminum hydroxide). Rats were injected intraperitoneally every other day for 14 days (for a total of seven injections per rat). Starting on Day 14, the rats were treated with 50 mL 2% OVA-saline solution in the form of intranasal drops on each side of the nose, once a day for 7 days (negative control rats were given saline drops). After the development of allergic rhinitis (Day 21), experimental rats were given Tong Qiao intranasal drops, two or three drops (10 μ L/kg/nostril) per treatment, three times per day for 7 or 15 days. Control rats were given saline nose drops. Positive control rats were given mometasone furoate nasal spray was administered topically at a volume of 10 μ L into the bilateral nasal cavities by micropipette 1 hour before the nasal antigen challenge (according to previous studies with modifications^{8,9}).

Adhering to previous allergic rhinitis model scoring criteria,⁴ each animal was observed after nasal provocation for nose scratching, sneezing, nasal discharge, and feeding behavior.

Animal behavior was observed 30 minutes after the last nasal provocation and was scored using the superposition and quantitative method,⁴ in which a total score more than five points indicates successful induction of allergic rhinitis. Symptoms were scored as follows: nasal itch, 1 = scratching the nose lightly one to two times, 2 = scratching the nose and face constantly; sneeze, 1 = one to three times, 2 = four to 10 times, 3 = 11 or more times; nasal discharge, 1 = secretions flow to the anterior nostril, 2 = secretions surpass the anterior nostril, 3 = secretions cover the face.

2.4. Nasal mucosa tissue collection

Following 30 minutes after the last nasal provocation, all animals were anesthetized with 1% pentobarbital sodium (50 mg/kg body weight), which was administered by intraperitoneal injection. The nasal septum mucosa of each rat was harvested, and a portion of each sample was fixed in 10% formaldehyde. Additional portions of each sample were immediately stored in liquid nitrogen for future use.

2.5. Pathologic observation

After fixing in formaldehyde, the nasal septum mucosa specimens were embedded in paraffin, sliced into 4 mm-thick sections, and then stained by hematoxylin and eosin. Samples were observed under an optical microscope.

2.6. Immunohistochemical staining for eotaxin in nasal mucosa

Cryopreserved nasal mucosa tissue samples were thawed, rehydrated, embedded in paraffin, and sliced into 4 μ m-thick sections. Samples were deparaffinized using a baking sheet and water, washed with phosphate-buffered saline (PBS) for 3 minutes (two times), then subjected to two rounds of submersion in 0.3% hydrogen peroxide for 10 minutes, followed by washing in PBS for 3 minutes (two times) to block endogenous peroxidase activity. Rabbit anti-mouse antieotaxin polyclonal antibody was added to the samples at a 1:100 dilution at 37°C for 2 hours, followed by the addition of the ready-to-use EnVision reagent. After incubation at 37°C for 30 minutes, samples were washed in PBS for 3 minutes (three times) before staining with diaminobenzidine for 1–3 minutes, as well as hematoxylin. Sections were dried, mounted with neutral resin, and observed under an optical microscope. The nuclei stained blue, while eotaxin staining appeared as yellow or brown granules in the cytoplasm. Positive staining was defined visually, using the semi-quantitative staining intensity method: negative (–), not stained or appeared as background; weakly positive (+), few small granules; strongly positive (+++), numerous coarse granules; positive staining ranged between (+) and (+++).

2.7. Measurement of IL-5 and IL-13 in nasal mucosa tissue

Approximately 200 mg of nasal mucosa tissue was homogenized in saline solution on ice. Samples were centrifuged at

4000 rpm for 20 minutes, and the supernatants were collected. The levels of IL-5 and IL-13 were measured in the supernatants by ready-made ELISA kits, according to manufacturer instructions. Plates were read at 490 nm using a microplate reader (ELx808 Super microplate reader; Shanghai Bio-Technology Co., Ltd. Shanghai, China). All specimens were run simultaneously.

2.8. Statistical analysis

Measurement data are expressed as the mean ± standard deviation (SD). Group differences of continuous variables were compared by analysis of variance (ANOVA) using SPSS 16.0 for Windows (SPSS, Chicago, IL USA). A *p* value less than 0.05 was considered statistically significant.

3. Results

3.1. Allergic rhinitis symptoms

In the control group, 19 rats were sensitized with OVA and presented with typical symptoms of allergic rhinitis which included abundant, clear nasal discharge, frequent sneezing, severe scratching, and gasping (Table 1). Of the experimental rats, prior to treatment, 18 presented with typical allergic rhinitis symptoms. Seven days after treatment, the total symptom scores of allergic rhinitis in the negative, control, experimental, and positive control group rats were 1.7, 22.0, 17.5, and 16.9, respectively. The scores of the control group, experimental group and positive control group were not significant different (*p* > 0.05). Fifteen days after treatment, the total symptom scores of allergic rhinitis in the negative, control, experimental, and positive control group rats were 1.6, 22.0, 7.8, and 6.9, respectively. The score of the control group was significantly higher than the experimental group (*p* < 0.01). The signs and symptoms of the experimental group were significantly reduced as compared with the control group (*p* < 0.01), with mild sneezing and nasal gasping movements. The signs and symptoms of the experimental group were not significant different as compared with the positive control (*p* > 0.05). Rats in the negative group were asymptomatic.

3.2. Histopathologic changes

In the control rats, nasal mucosa edema, vasodilation, glandular hyperplasia, and eosinophil infiltration were seen in the lamina propria (Fig. 1). In the experimental group and positive control rats, the nasal mucosa edema was mild and the

infiltration of eosinophils was insubstantial. In the negative group, no mucosal edema or eosinophil infiltration was observed.

3.3. Eotaxin expression in the nasal mucosa

Using immunohistochemical staining, eotaxin was detected within epithelial cells, glandular cells, fibroblasts, macrophages, endothelial cells, and other inflammatory cells (Fig. 2). In the control group, eotaxin-positive cells were detected around the epithelial cells and glandular cells of the nasal mucosa; two rats were positive, and eight rats were strongly positive. In the negative group, no significant eotaxin staining was seen in the nasal mucosa, although eight rats had weakly positive staining. In the experimental group, eotaxin-positive cells were substantially reduced; one case was positive, and eight cases were weakly positive. In the positive control group, eotaxin-positive cells were substantially reduced; two cases were positive, and six cases were weakly positive.

3.4. Expression of IL-5 and IL-13 in the nasal mucosa

The levels of IL-5 in the nasal mucosa of the negative, control, and experimental rats were 7.1 ± 1.6 pg/mL, 13.4 ± 1.9 pg/mL, and 8.2 ± 1.0 pg/mL, respectively, whereas the levels of IL-13 were 23.7 ± 2.1 pg/mL, 33.6 ± 2.9 pg/mL, and 22.0 ± 1.1 pg/mL, respectively (Table 2). After treatment with Tong Qiao nasal drops, the levels of IL-5 and IL-13 in the nasal mucosa of experimental rats were significantly lower than in the control group. The difference was statistically significant (*p* < 0.01), though they were still lower compared to the negative group, and were higher compared with the positive control group where there was no statistical difference at all (*p* > 0.05).

4. Discussion

Allergic rhinitis is also referred to as “congested nose tears” in traditional Chinese medicine. Its etiology and pathogenesis is mainly ascribed to lung qi deficiency. In other words, body fluid accumulates in the lungs and nasal passages congest, thus causing sneezing and runny nose. The nose is considered the open mouth of the lung, so nasal discharge originates in the lung as a way to clear yang qi from the spleen and kidney. Because nutrition is absorbed by the kidney, this disease is considered related to spleen and kidney deficiencies. Often, treatments target symptoms, while, according to traditional

Table 1
Results of symptoms in different groups.

Group	<i>n</i>	Nose scratch	Discharge	Sneeze	Gasps	Total score
Negative	20	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	1.6
Control	19	5.0 ± 1.3 ^Δ	2.5 ± 0.6 ^Δ	13.0 ± 2.9 ^Δ	1.5 ± 0.5 ^Δ	22.0
Experimental	18	2.2 ± 1.0*	0.9 ± 0.3*	4.3 ± 1.5*	0.4 ± 0.2*	7.8*
Positive control	20	2.0 ± 0.9	0.8 ± 0.3	4.0 ± 1.0	0.4 ± 0.1	6.9

^Δ compared between control group and negative group, *P* < 0.01; *comparison between experimental group and control group.

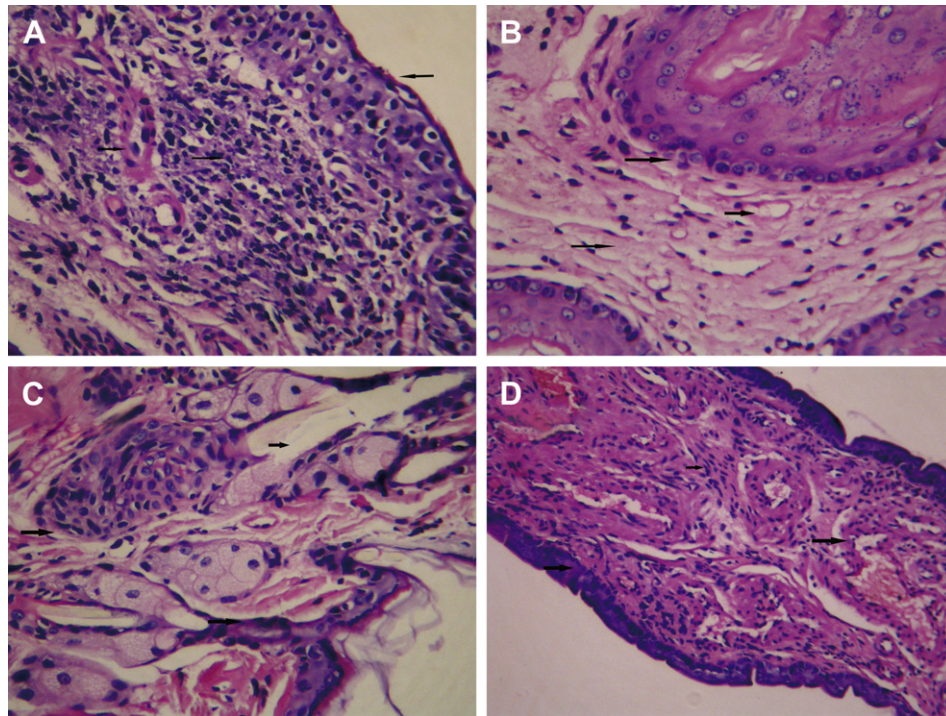


Fig. 1. Histopathological changes in allergic rhinitis with or without treatment. (A) In sensitized control rats, nasal mucosa edema, vasodilation, glandular hyperplasia, and substantial eosinophil infiltration (arrow) were observed in the lamina propria; (B) negative control rats had no mucosal edema or eosinophil infiltration (arrow); (C) after treatment with Tong Qiao drops, the nasal mucosa edema was less severe, with less eosinophil infiltration (arrow); (D) positive control rats had less mucosal edema or eosinophil infiltration. Hematoxylin and eosin staining (arrow). Magnification, $\times 200$.

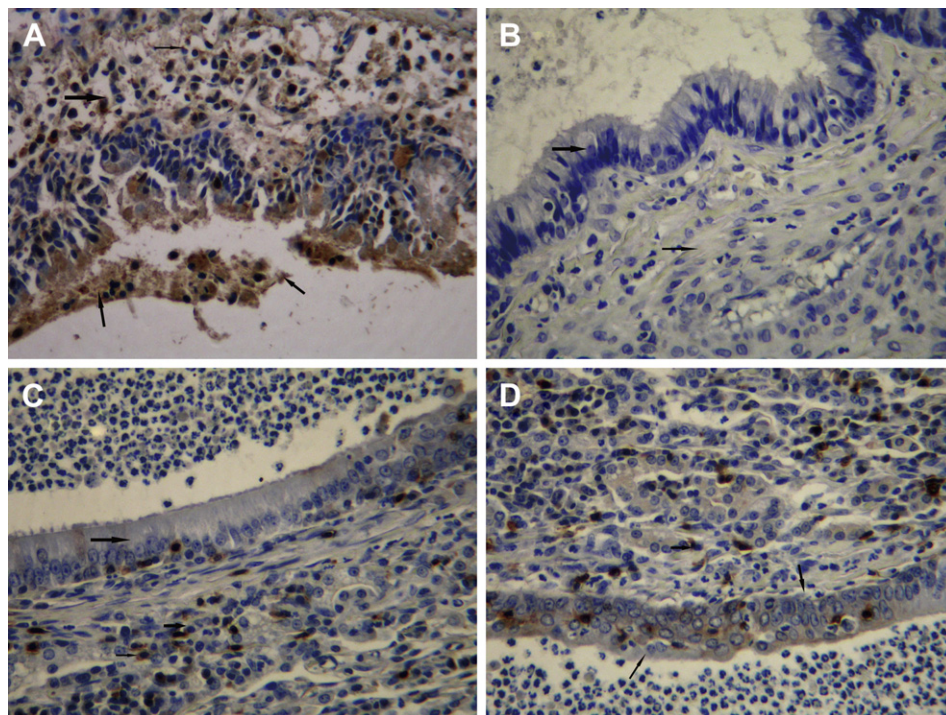


Fig. 2. Eotaxin expression in nasal mucosa of rats with allergic rhinitis, with or without treatment. Nasal mucosa tissue (arrow) from (A) control (allergic rhinitis); (B) negative (healthy); (C) experimental (allergic rhinitis treated with Tong Qiao drops); and (D) positive control (allergic rhinitis treated with mometasone furoate) rats were subject to immunohistochemical staining for eotaxin (brown staining). Magnification, $\times 200$.

Table 2
Expression of IL-5 and IL-13 in the rat nasal mucosa in different groups.

Group	N	IL-5 (pg/ml)	IL-13 (pg/ml)
Negative	20	7.1 ± 1.6	23.4 ± 2.1
Control	19	13.4 ± 1.9 ^Δ	33.6 ± 2.88 ^Δ
Experimental	18	8.2 ± 1.0*	22.0 ± 1.1*
Positive control	20	7.5 ± 0.8	20.2 ± 0.7

^Δ compared between control group and negative group, $P < 0.01$; *comparison between experimental group and control group; IL = interleukin.

Chinese medicine, the goal should be to expel the wind and cold from the lungs to warm the yang.¹⁰ The use of Chinese medicine can significantly reduce the financial burden of patients and the state. Studies have shown that *F magnoliae* has reliable anti-inflammatory effects for some acute and chronic inflammatory diseases in animal models. For example, it can significantly reduce congestion, edema, necrosis, inflammatory cell infiltration, and other inflammatory reactions, and can significantly suppress the adhesion of eosinophils, neutrophils, and vascular endothelial cells.¹¹ *A dahurica* and *G sinensis Lam* also have anti-inflammatory and anti-allergic effects.^{3–6,11–14} Kung et al.⁴ suggested that adding single herbal drugs into Chinese herbal formula in one prescription could enhance the therapeutic effect or address certain allergic rhinitis-related symptoms. In this study, we analyzed the expression of eotaxin, IL-5, and IL-13 in the nasal mucosa of rats with allergic rhinitis. *F magnoliae*, *A dahurica*, and *G sinensis Lam* were incorporated into the Tong Qiao nasal drops, which were used to treat the rats; we observed the effect of these drops as a treatment for OVA-induced allergic rhinitis.

To elucidate the mechanism of eotaxin, IL-5, and IL-13 in the pathogenesis of allergic rhinitis, this study used OVA sensitization to establish a rat model of allergic rhinitis. Our results suggested that eotaxin, IL-5, and IL-13 are involved in the pathogenesis of allergic rhinitis. Eotaxin is considered to be an important eosinophil chemotactic factor.¹⁸ Azazi et al.¹⁵ found that eotaxin level was higher in the patients with allergic rhinitis. Erin et al.¹⁶ found elevated levels of IL-3, IL-5, and eotaxin in the nasal lavage of seasonal AR patients. Pagani et al.¹⁷ also confirmed that IL-3, IL-5, and eotaxin might play a role in the pathophysiology of allergies. They detected the IL-3, IL-5, and eotaxin levels in the nasal lavage of AR patients using the quantitative sandwich enzyme immunoassay technique. They found that the IL-3, IL-5, and eotaxin levels were statistically significant higher than those in the healthy controls before treatment.¹⁷

This study determined that in OVA-induced allergic rhinitis, treatment with Tong Qiao nasal drops lowered symptom scores significantly as compared with no treatment, and similar as compared to treatment with mometasone furoate, a proven medicine for allergic rhinitis.¹⁸ Yet, despite this result, symptom scores were still higher after treatment than in the negative group, suggesting that Tong Qiao nasal drops can relieve symptoms of allergic rhinitis, but they do not prevent them. These experiments also showed that the number of inflammatory cells that infiltrated the nasal mucosa after

treatment with Tong Qiao nasal drops was less than without treatment. Edema and hyperemia were also less severe upon treatment and, in fact, were not significantly different as compared with the negative control group. Therefore, we demonstrated that Tong Qiao nasal drops may inhibit the inflammatory response in the mucosa of rats with allergic rhinitis, thus leading to a reduction in local allergic reactions. In addition, Tong Qiao nasal drops significantly reduced the expression of eotaxin, IL-5, and IL-13 in the nasal mucosa of these rats as compared with untreated rats. Other studies have demonstrated that some herbals may regulate endocrine-immune system and can down-regulate the Th2 cells and their cytokines IL-4 and IL-5,¹⁹ IL-6 and IL-13,²⁰ and eotaxin.²¹ Our data suggest that the antiallergic rhinitis mechanism of Tong Qiao nasal drops involves the reduction of eotaxin, IL-5, and IL-13 expression levels in the nasal mucosa tissue as well as the inhibition of eosinophil infiltration.

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