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Effect of nose sensitive pill (NSP) on serum IFN-γ and il-4 levels in allergic rhinitis using rats model

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Abstract: This study was conducted to investigate the changes of serum interleukin-4 (IL-4), interferon-γ (IFN-γ), interleukin-17 (IL-17) and interleukin-10 (IL-10) in allergic rhinitis model rats after using the traditional Chinese nose sensitive pill (NSP) and its possible mechanism to treat allergic rhinitis. Forty Sprague Dawley (SD) rats were randomly divided into 4 groups of 10 rats each i.e. blank control group, model group, nose sensitive pill group and loratadine group. Allergic rhinitis was induced in all three groups (except blank control group) using ovalbumin as allergen. After successful induction of allergic rhinitis, intragastric administration of 0.9% NaCl solution, NSP or loratadine solution was carried-out, respectively. The behavior of rats was observed before administration and then after 1, 3 and 5 weeks. Enzyme linked immunosorbent assay (ELISA) was used to detect the levels of 4 cytokines in each group after 5 weeks. After 5 weeks study period, nasal symptoms of NSP group and loratadine group were significantly ($P<0.01$) lower than those of model group. Compared with blank control group, levels of IL-4 and IL-17 in model group increased, and levels of IFN- γ and IL-10 decreased significantly ($P<0.01$). Compared with model group, levels of IFN-γ and IL-10 increased but levels of IL-4 and IL-17 decreased significantly ($P<0.01$) in NSP and loratadine group. On the basis of findings of this study, NSP is an effective prescription to treat allergic rhinitis. One of its therapeutic mechanisms is to regulate balance between Th1/Th2 and Th17/Treg cells by influencing the levels of IL-4, IFN-γ, IL-17 and IL-10.

Keywords: Nose sensitive pill, Allergic rhinitis, IL-4, IFN-γ, IL-17, IL-10.

INTRODUCTION

Allergic rhinitis (AR), which is called "sneeze" or "epistaxis" by Chinese medicine, is an allergic disease that occurs in nasal mucosa. The clinical symptoms include nasal itching, sneezing, hyperactivity of nasal secretion, nasal mucous swelling, etc. It is often divided into perennial and seasonal, which often exists with bronchial asthma, that is, "an airway disease". In study of its pathogenesis (Wheatley & Togias, 2015), Th1/Th2 and Th17/Treg cell immune balance are always the hot spots. Th1 cells produce interferon-γ (IFN-γ) and other cytokines, mainly mediate cellular immune responses; Th2 cells secrete interleukin-4 (IL-4) and other cytokines, which stimulate humoral immune response; Interleukin-17 (IL-17) is an important effector cytokine mainly secreted by Th17 cells; Interleukin-10 (IL-10) is an anti-inflammatory cytokine secreted by Tr1 cells, a subset of Treg cells. In normal population, a homeostasis is maintained between Th1 and Th2, which can be reflected by the levels of IFN-γ and IL-4. When imbalance occurs, Th2 cells increase, and the number of cytokines represented by IL- 4 is increased (Brozek *et al.,* 2017, Olivieri *et al.,* 2015).

Allergic rhinitis (AR) is a common clinical manifestation of ear nose throat (ENT) disease with a prevalence rate of 8%-21.4% (Kim *et al.,* 2008). AR has recurrent attacks and a long resistance to treatment, seriously affects patient's life, study and work, brings great pain and inconvenience to patients, is one of the urgent problems of modern medicine. Loratidine is considered as the model drug for AR in allopathic system of medicine however more than one antihistamine drug is trying often in perennial allergic rhinitis as the first fails mostly, therefore limitations of current treatment of AR in Western medicine, more patients choose traditional Chinese medicine therapy to control their symptoms (Wang *et al.,* 2102). The traditional Chinese medicine nose sensitive pill is composed of Astragalus, Codonopsis, Atractylodes, Poria, Schisandra chinensis, Magnolia, Licorice, Cortex Moutan, Angelica, Radix Saposhnikoviae, Radix Paeoniae alba, Fructus Mume, Fructus Xanthii (fried), Platycodon root, Cassia twig, which is able to disperse wind cold, free nasal orifices, and exerts anti-inflammatory, anti-allergy and other effects. However, there are few reports about mechanism of nose sensitive pill in allergic rhinitis treatment. The aim of this study is to observe the changes of IL-4, IFN-γ, IL-17 and IL-10 cytokines in serum of AR rats and to explore the possible mechanism of nose sensitive pill in AR treatment.

MATERIALS AND METHODS

Animals

Forty PF-class SD rats, aged 3 months and weighing

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about 180-230g, were all female and were provided by Nanjing Junke Bioengineering Co., Ltd.

Chemicals and reagents

Ovalbumin is purchased from Aladdin Industrial Corporation. Aluminum hydroxide is purchased from Shanghai Ling Feng chemical reagents Co., Ltd. IL-17 and IL-10 kit was purchased from Nanjing Kenji Biotech Development Co., Ltd. Loratadine tablet was purchased from Shanghai Schering Plough Pharmaceutical Co., Ltd. Chinese medicine nose sensitive pill (NSP) was purchased from Qianjiang Yongan Pharmaceutical Co., Ltd.

Instruments

Desktop constant temperature oscillator, Shanghai Jinghong Experimental Equipment Co., Ltd. THZ-312; Refrigerator, Haier BCD-258A/C; Enzyme labeling instrument, SpectraMaxM3, MD, USA.

Grouping, modeling and administration

Forty SD female rats were sorted according to body weight and randomly divided into 4 groups: blank control group, model group, nose sensitive pill group, loratadine group. Every group was comprised of 10 rats. Animals were handled as per international ethical guidelines.

The blank control group was reared normally without modeling and administration. Establishment of allergic rhinitis (AR) rat model: 0.3 mg ovalbumin as antigen, aluminum hydroxide 30 mg as adjuvant, plus 0.9% NaCl solution 1 ml was taken and injected intraperitoneally once every other day, and repeated 8 times as basal sensitization. Then 50μl 0.25% ovalbumin saline solution was dripped on nasal side, 1 time a day for 1 week. Symptoms were observed and scored after 1 week of sensitization (before administration). Nasal symptoms score criteria were: (1) nasal itching: mild is grazing nose gently for several times, 1 point; severe is scratching nose continually, 2 points; (2) continuous sneeze: 1 to 3 times for 1 point, 4 to 10 times for 2 points, more than 11 times for 3 points; (3) runny nose: flow to the former nostril for 1 point, over the former nostril for 2 points, runny nose covered all face for 3 points. Points were recorded by superposition, and the total points of more than 5 points indicated modeling success.

After modeling success, each group was given the corresponding solution per os and the dosage was made equivalent to routine dosage of adults. Loratadine group was given loratadine solution ($1.67\mu g \cdot g^{-1} \cdot d^{-1}$), and NSP group was given the aqueous extract of nasal sensitive pill ($10g \cdot kg^{-1} \cdot d^{-1}$). Blank group and model group were given same volume of 0.9% NaCl solution as nose sensitive pill. Successive medication was given for 5 weeks.

Draw materials and index detection

Nasal symptom score

According to the above-mentioned rat nasal symptom score standards, grazing nose times, sneezing times and runny nose degrees of rats in each group were recorded respectively before administration and 1, 3 and 5 weeks after it, which were also scored and compared. The standard procedure was as follows: After intranasal administration of 0.25% ovalbumin physiological saline solution (50μl on each side) the number of snuff nose, sneezing and runny nose over 30 min was observed and behavioral scores were calculated.

ELISA detection of 04 cytokines

Five weeks after administration, five animals in each group were selected and blood was collected from posterior capsular vein. After centrifugation, serum was collected and stored at -8°C in a refrigerator. Levels of IL-4, IFN-γ, IL-17 and IL-10 were measured according to ELISA kit instruction, and the concentration of each sample was calculated according to a standard curve.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS 21.0 software. Measurement data was used ($\bar{x} \pm s$). Comparison between groups and differences before and after treatment were tested by Student's t-test (if data obeyed normal distribution) or nonparametric test (if data did not obey normal distribution or variance of variance). A p-value of <0.05 was considered statistically significant.

RESULTS

Comparison of nasal symptoms in each group

Before administration, all rats in model, NSP and loratadine groups were induced typical symptoms of allergic rhinitis, such as severe scratching of nasal face, continuous sneeze and a large number of continuous itching outflows of anterior nostrils. The scores of each group were more than 5 points after 1 week of stimulation (before treatment), which was significantly different from that of blank control group ($P<0.01$), indicating that the model was successful.

One week after administration, compared with model group, the difference between NSP and loratadine group was statistically significant ($P<0.05$, $P<0.01$). There was no significant difference between NSP and loratadine group ($P>0.05$).

Three weeks after administration, compared with model group, the difference between NSP and loratadine group was statistically significant ($P<0.01$). There was no significant difference between NSP and loratadine group ($P>0.05$).

Five weeks after administration, compared with model group, sneezing of NSP and loratadine group decreased obviously, and symptoms of occasional runny nose and

Table 1: Comparison of nasal symptoms in each group（n=10，$\bar{x} \pm s$，score）

Group	Before administration	1 week after administration	3 weeks after administration	5 weeks after administration
Blank control group	0.37±0.12	0.24±0.13	0.24±0.15	0.37±0.12
Model group	5.84±0.52**	3.89±0.67	3.61±0.91	3.49±0.64
Nose sensitive pill group	5.84±0.52**	2.65±0.42$^{\#\#}$	1.72±0.37$^{\#\#}$	0.87±0.25$^{\#\#\Delta}$
Loratadine group	5.72±0.46**	3.14±0.33$^{\#}$	2.24±0.31$^{\#\#}$	1.61±0.34$^{\#\#}$

Table 2: Comparison of serum IL-4 and IFN-γlevels in each group（n=5 $\bar{x} \pm s$ ng/L）

Group	IFN-γ	IL-4
Blank control group	180.71±27.16	47.11±7.49
Model group	72.90±24.39**	221.08±42.35**
Nose sensitive pill group	121.73±28.64$^{\#\Delta}$	102.12±7.10$^{\#\#\Delta\Delta}$
Loratadine group	112.96±6.69$^{\#}$	197.03±8.39

Table 3: Comparison of serum IL-17 and IL-10 levels in each group (n=5 $\bar{x} \pm s$ ng/L)

Group	IL-17	IL-10
Blank control group	18.35±0.81	137.84±4.29
Model group	96.00±2.47**	42.62±1.87**
Nose sensitive pill group	44.53±18.27$^{\#\#\Delta}$	122.59±2.38$^{\#\#\Delta\Delta}$
Loratadine group	64.02±2.42$^{\#\#}$	66.36±1.26$^{\#\#}$

PS: Compared with blank control group，$^{**}P$＜0.01; Compared with model group，$^{\#}P$＜0.05, $^{\#\#}P$＜0.01: Compared with loratadine group, ΔP＜0.05.

scratching nose disappeared, the differences were statistically significant (P<0.01). There was a significant difference between NSP and loratadine group (P<0.05) as shown in table 1.

Comparison of serum IL-4 and IFN-γ levels in each group

Compared with blank control group, level of serum IFN-γ in model group was significantly decreased, and level of serum IL-4 was significantly increased (P<0.01). Compared with model group, level of serum IFN-γ increased and level of serum IL-4 decreased in NSP group (P<0.05, P<0.01); level of serum IFN-γ increased in loratadine group (P<0.05). Compared with loratadine group, levels of IL-4 and IFN-γ in NSP group were significantly different (P<0.01, P<0.05) as shown in table 2.

Comparison of serum IL-17 and IL-10 levels in each group

Compared with blank control group, level of serum IL-10 in model group decreased obviously, level of serum IL-17 increased significantly (P<0.01). Compared with model group, level of serum IL-10 in nose sensitive pill group and loratadine group was significantly increased, while level of serum IL-17 was significantly decreased (P<0.01). The difference of IL-17 and IL-10 levels in nose sensitive pill group and loratadine group was statistically significant (P<0.05, P<0.01) as shown in table 3.

DISCUSSION

The traditional Chinese medicine nose sensitive pill is composed of Astragalus, Codonopsis, Atractylodes, Poria, Schisandra chinensis, Magnolia, Licorice, Cortex Moutan, Angelica, Radix Saposhnikoviae, Radix Paeoniae alba, Fructus Mume, Fructus Xanthii (fried), Platycodon root, cassia twig. Among them, Astragalus is the monarch drug, which has effect of replenishing and restoring pulmonary Qi and kidney Qi. Schisandra has effect of collecting lung. Flos Magnoliae is the official medicine with effect of diverging wind and cold and free nasal orifices. Magnolin as the main active ingredient of Flos Magnoliae, possesses anti-inflammatory and anti-allergic effect. Modern pharmacological studies have shown that most of the prescription drugs have anti-inflammatory, anti-allergic and immunomodulatory effects, combined with pathogenesis of allergic rhinitis; nose sensitive pill has high value of new drug research and development (Abbas *et al.,* 2004, Dong *et al.,* 2002).

The occurrence of allergic rhinitis is that antigen-presenting cell takes allergen and processes it as allergenic peptide, and passes it to Th cells (Kim *et al.,* 2016). The cells progressively differentiate into Th2 cells to express Th2 responses and Th2 cells induce B cells to release IgE. When the same allergen re-enters the body, it binds IgE and releases inflammatory mediators such as histamine, leukotriene and prostaglandin after degranulation of mast cells, causing telangiectasia,

increased permeability, increased gland secretion and acid granulocyte infiltration, thereby causing clinical symptoms of AR (Tasaniyananda *et al.,* 2016). Immune imbalance of Th1 / Th2 cells has been considered as an important pathogenesis of AR (Xia *et al.,* 2006). That is, the occurrence of AR originates from differentiation of allergen specific Th cells, which ultimately causes the imbalance of Th1 and Th2 *in vivo* and is manifested as inflammatory diseases mainly based on Th2. However, Th17/Treg cell balance theory suggests that Th17 mediates inflammatory responses and belongs to "proinflammatory cells", whereas Tregs mediate immune tolerance and belong to "anti-inflammatory cells" (Joller *et al.,* 2014). As both function and differentiation against each other, maintain balance between the two is conducive to maintaining a stable immune state.

IL-4, IFN-γ is a common indicator of allergic rhinitis, mainly reflecting the balance between Th1 and Th2 cells (Keswani *et al.,* 2016). IL-17 is produced by Th17 cells, which stimulates a variety of cells to produce inflammatory cytokines and chemokines and has a pro-inflammatory effect. Therefore, relationship between IL-17 and the occurrence and development of anaphylaxis is very close (Ciprandi *et al.,* 2009). Studies have shown that the number of IL-17 positive cells in nasal mucosa tissue of AR patients is significantly higher than that of normal persons. The serum level of IL-17 in patients is positively correlated with the severity of AR clinical symptoms. IL-10 is secreted by Trl cells, which is the predominant type of Treg cells. IL-10 is an anti-inflammatory factor that inhibits synthesis of proinflammatory cytokines and inhibits proinflammatory effects of eosinophils, basophils and mast cells (Wang *et al.,* 2012; Zhang *et al.,* 2012).

CONCLUSION

After 5 weeks, nasal symptoms of NSP and loratadine group were significantly lower than those of model group. Compared with blank control group, levels of IL-4 and IL-17 in model group increased, and levels of IFN-γ and IL-10 decreased. Compared with model group, levels of IFN-γ and IL-10 increased while levels of IL-4 and IL-17 decreased in nose sensitive pill group and loratadine group. This indicates NSP is an effective prescription to treat allergic rhinitis. One of its therapeutic mechanisms is to regulate balance between Th1/Th2 and Th17/Treg cells by influencing the levels of IL-4, IFN-γ, IL-17 and IL-10. AR rats and plays an anti-allergic rhinitis role.

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