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Impact of Yupingfengsan Oral Liquid on the Expression of IL-4, IFN-γ and NF-κB of Allergic Rhinitis Mice

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Abstract

To observe the impact of Yupingfengsan Oral Liquid on the expression of IL-4 and IFN-γ in AR mice's serum and expression level of nuclear factor—κB (NF-κB) protein and gene in nasal mucosa. **Method:** Forty BALB/c mice were randomly divided into the normal group, model group, Yupingfengsan Oral Liquid group (6 g/kg) and Loratadine group, with 10 mice per group. AR mice model was established by OVA, and IL-4 and IFN-γ contents can be measured with ELISA. The morphological changes of nasal mucosa were observed by hematoxylin eosin (HE) staining and NF-κB expression in the nasal mucosa of mice was tested with Real-Time PCR and Western blot. **Results:** Compared with the model group, the nasal symptoms in the Yupingfengsan Oral Liquid group and Loratadine group were obviously relieved. HE staining showed that there was a little inflammatory cell infiltration in the nasal mucosa of Yupingfengsan Oral Liquid group and Loratadine group and it was significantly reduced when compared with the model group. IL-4 level in the serum and expression of NF-κB protein and gene in the nasal mucosa was consistent and it was decreased when compared with the model group ($P < 0.01$), but the IFN-γ level in the serum was increased ($P < 0.01$). **Conclusion:** Yupingfengsan Oral Liquid can improve the clinical symptoms and histopathological manifestations of AR mice sensitized by OVA, inhibit the NF-κB expression, balance the percentage of Th1/Th2 cells, increase the IFN-γ level in the serum and decrease the IL-4 level.

Keywords

Allergic Rhinitis (AR), Yupingfeng Powder Oral Solution, Nuclear Factor-κB (NF-κB), Interleukin-4 (IL-4), γ-Interferon (INF-γ)

1. Introduction

Allergic rhinitis (AR) is a common and frequently-occurring disease in the otorhinolaryngology head and neck surgery department, and it is a type-I allergic disease resealed by the IgE-mediated mediators, and involved with multiple immune cells and cytokines after the body is exposed to the allergen [1]. Its pathogenesis is complex and is not clear, wherein, the balance disorder of Th1/Th2 cytokines is the key for the development of allergic diseases [2]. In recent years, the studies have shown that NF-κB can adjust the release of Th cytokine and interfere the balance of Th1/Th2 cytokine after activation, so as to be involved in the occurrence and development of immunity and inflammation [3]. Yupinfengsan Oral Liquid, which is originated from Yupinfengsan, is a classic prescription for reinforcing the vital energy and consolidating the constitution, and it is functioned with treatment, prevention and good immune regulation [4]. By establishing the mice AR model and detecting the cytokine contents of IL-4 and IFN-γ in serum, and NF-κB protein and gene expression level in the nasal mucosa, this experiment discusses the regulatory role of Yupinfengsan Oral Liquid on the expression of related cytokines of the mice AR model.

2. Main Experimental Materials

Experimental animals: 40 clean-grade BALB/c mice, female, age of 5 - 6 weeks, weight of 18 - 20 g, were not fed by OVA-containing food and came from the animal center of China Three Gorges University with the production certificate No. of the experimental animal of SYXK (E) 2019-0083. They were raised in the SPF-level environment of Hubei College of Chinese Medicine.

Hubei Dongxin Pharmaceutical Co., Ltd., Loratadine Tablets were purchased from Hainan Hishen Tongzhou Pharmacy Co., Ltd, IL-4, IFN-γ test reagents were purchased from Ruixin Biotech. Primer was purchased from Wuhan GeneCreate Biological Engineering Co., Ltd. Trizol was purchased from Tiangen Biochemical Technology Co., Ltd.

Experimental institution: High speed freezing centrifuge (Eppendorf company, Germany), paraffin embedding center, tissue freezing machine, paraffin slicing machine, microscope (Leica company, Germany), microplate reader (DR-200Bs, Diatek company), fluorescence quantitative PCR instrument (Shanghai Hongshi Medical Technology Co., Ltd.).

2.1. Model Preparation

This research adopted OVA to establish the AR model of mice. Except the normal group, other groups were injected with 0.05 mg OVA and 5 mg $AL(OH)_3$ intraperitoneally and 1 ml normal saline was added for mixing once every other day; after the basic sensitization, the mice received the nasal sensitization on the 15th day, and the nasal cavity was dripped with 5% ova solution, with 0.02 ml/side, once/day and consecutive 7 days [5]. The normal group received the intraperitoneal injection and nasal drip with the equal volume of normal saline.

The successful judgement method of the model is subject to the literature [6]. After the last nasal provocation, the uninformed personnel recorded the times of sneezing, nose scratch and nasal mucus volume of each group in 30 min. One point shall be recorded for 1 - 3 times of sneezing/1 - 2 times of nose scratch/nasal discharge to anterior nostril, respectively, two points shall be recorded for 4 - 10 times of sneezing/rubbing between the two nostrils/nasal discharge from anterior nostril, respectively and three points are recorded for sneezing > 10 times/rubbing around the nose/covering tears on the face, respectively. If the total score is > 5 points, it showed that the model was successfully established.

2.2. Experimental Grouping and Intervention

Forty BALB/c mice were selected and divided into four groups based on the random number method: normal group, model group, Yupinfengsan Oral Liquid group (6 g/kg), Loratadine group (3 mg/kg), with 10 mice per group. The normal group and model group received the intragastric administration with the equal volume of normal saline, and the Yupinfengsan Oral Liquid group and Loratadine group received the intragastric administration with Yupinfengsan Oral Liquid and Loratadine, respectively. The body mass of the Yupinfengsan Oral Liquid group and Loratadine group was 6 g/kg and 3 mg/kg, respectively (Clinical commonly-used dose was chosen and the intragastric dose in mice was gotten in accordance with the dose conversion method of experimental zoology), with once a day for 7 consecutive days.

2.3. IL-4 and IFN-γ Levels of Serum in the Four Groups of Mice Were Detected According to the ELISA Method

After the last administration, the blood was collected after removing the eyeball, and placed under the room temperature for 2 h. It shall be centrifuged for 15 minutes under 4˚C 3000 r/min, and the liquid supernatant was taken and stored under the −80˚C refrigerator for testing. ELISA shall detect the IL-4 and IFN-γ contents in serum in strict accordance with the kit instructions.

2.4. Observation for Morphology of Nasal Mucosa

After collecting the blood, the mice were killed immediately to collect the nasal mucosa tissue. It was fixed by paraformaldehyde and dehydrated for multiple layers to prepare the paraffin section. The HE dyeing was carried out to observe the pathologic change of the tissues.

2.5. NF-κB p65 Protein Expression Level Was Detected in Accordance with the Western Blot Method

The nasal mucosa tissue of mice was lysed with the lysate liquid, and the sample treatment liquid was taken for SDS-PAGE electrophoresis and rotary die. After sealing, the primary anti-body was blocked overnight. Then, the secondary anti-body was carried out with NF-κB (concentration 1:1000) and internal refer-

ence protein added. They were incubated under the room temperature for 30 min and developed with the ECL chemiluminescence immunoassay, to determine the relative expression quantity of the target protein.

2.6. The NF-κB p65 Gene Expression of Nasal Mucosa Was Detected in Accordance with RT-PCR

Trizol one-step method was adopted to withdraw the total RNA. NF-κB primer sequence: F5-AGG AGC AGG ACA TGG GAT TTC-3, R5-CCA AGT GCG AGG TGT CTG ATA-3. Internal reference GAPDH sequence: F5-AGG AGA GTG TTT CCT CGTCC-3, R5-GAT GGG CTT CCC GTT GATGA-3; The reaction was carried out on fluorescence quantitative PCR instrument, and the two-step PCR amplification standard procedure was adopted to confirm its amplification curve and solubility curve, and measure the relative expression quantity of mRNA of the target gene.

2.7. Statistical Treatment

It adopts the SPSS 21.0 software for statistics, and the results are shown as $\bar{x} \pm s$. The comparison among groups was analyzed with one-way variance and $P < 0.05$ indicated that the difference was statistically significant.

3. Results

3.1. Comparison of the Mice's Symptoms in Each Group

The result showed that except the normal group, the mice in the model group, Yupinfengsan Oral Liquid group and loratadine group suffered from AR symptoms with the symptom score over 5 points. Compared with the normal group, the symptom of mice in each group was significant alleviated ($P < 0.01$). See Table 1.

3.2. Result of Nasal Mucosa HE Dyeing of Mice in All Groups

For the normal group, the epithelial cells of nasal mucosa were columnar epithelium with clear outline and regular and orderly arrangement. No cellular damage or inflammatory infiltration was found in the tissue, and the integral structure was basically normal. For the model group, the local epithelial cells of nasal mucosa were denatured, and edema was found on local mucosa with connective

Table 1. Comparison of nasal symptom score of mice in four groups before and after treatment ($\bar{x} \pm s$, Point, N = 10).

Group	Before treatment	After treatment
Normal group	1.26 ± 0.18	1.25 ± 0.22
Model group	6.45 ± 0.93$^{\#}$	6.58 ± 0.96$^{\#}$
Yupinfengsan Oral Liquid group	6.61 ± 0.66$^{\#}$	3.57 ± 0.68$^{\#*}$
Loratadine group	6.95 ± 0.86$^{\#*}$	3.73 ± 0.60$^{\#*}$

Compared with the normal group, $^{\#}P < 0.01$; compared with that in the model group, $^{*}P < 0.01$.

tissue hyperplasia and loose tissue arrangement. The diffuse infiltration of inflammatory cells was observed and there is the edema of some mucosal epithelial cells with cell enlargement and pale cytoplasm. For the Yupinfengsan Oral Liquid group and Loratadine group, few inflammatory cell infiltration was seen on local nasal mucosa, and it was significantly reduced when compared with the model group. See Figure 1.

3.3. Comparison on IL-4 and IFN-γ Levels in Serum of the Mice in All Groups

The result showed that the IL-4 level of the mice in the model group was increased, while the IFN-γ level of serum was decreased when compared with that in the normal group ($P < 0.01$). The IL-4 level was decreased and the IFN-γ level was increased in the serum of Yupinfengsan Oral Liquid group and Loratadine group when compared with that in the model group ($P < 0.01$), see Table 2.

3.4. Effect of NF-κB P65 Proteins and Genes of the Nasal Mucosa Tissue of the Mice in All Groups

The result showed that compared with that in the normal group, the NF-κB p65 protein and gene expression of the mice's nasal mucosa in the model group were increased ($p < 0.01$). Compared with that in the model group, the NF-κB p65 protein and gene expression were decreased in the Yupinfengsan Oral Liquid group and Loratadine group ($p < 0.01$). See Figures 2-4.

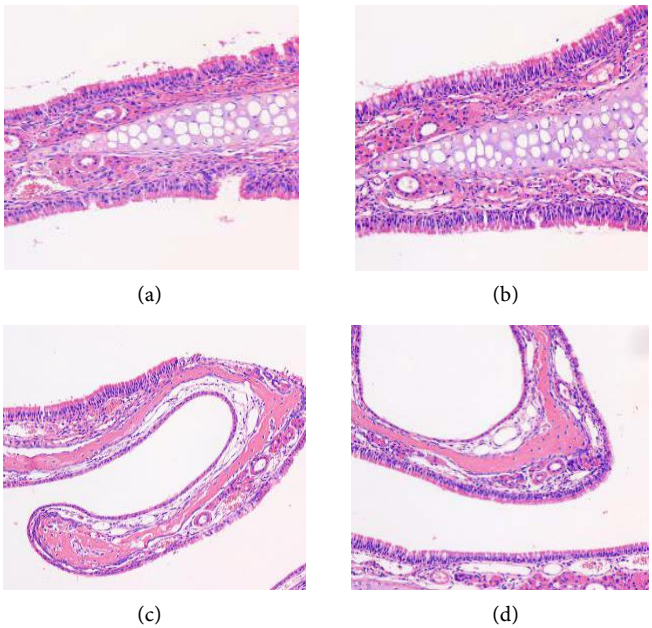

Figure 1. Hematoxylin-eosin dyeing for nasal mucosa of the mice in all groups (×200). Notes: (a) is the normal group. No inflammatory infiltration was observed and the integral structure was basically normal; (b) is the model group, and the significant inflammatory cell infiltration was observed under the nasal mucosa; (c) and (d) refer to Yupinfengsan Oral Liquid group and Loratadine group, and few inflammatory cell infiltration was observed in local nasal mucosa.

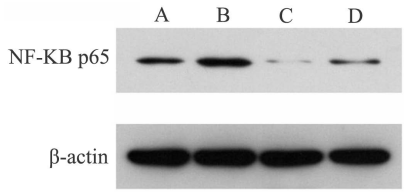

Figure 2. NF-κB P65 Protein Expression Electrophoresis of the Mice's Nasal Mucosa in-All Groups. Notes: A: Normal group; B: Model group; C: Yupinfengsan Oral Liquid group; D: Loratadine group.

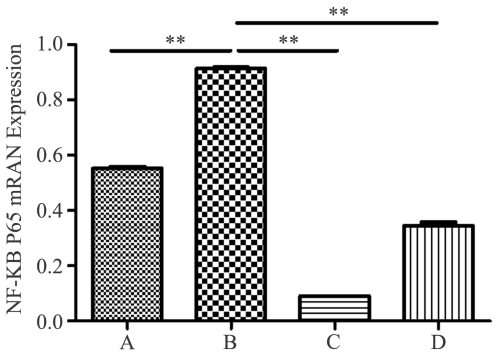

Figure 3. NF-κB P65 protein expression level of the mice's nasal mucosa in all groups. Notes: A: Normal group; B: Model group; C: Yupinfengsan Oral Liquid group; D: Loratadine group. Compared with the normal group, **P < 0.01; Compared with that in the model group, **P < 0.01.

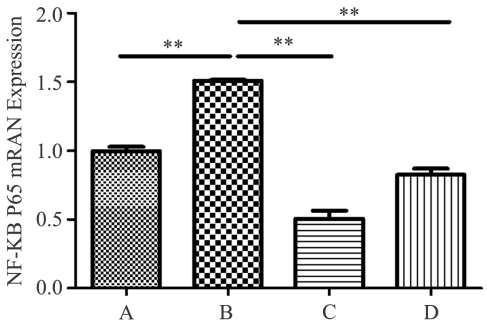

Figure 4. NF-κB P65 gene expression level of the mice's nasal mucosa in all groups. Notes: A: Normal group; B: Model group; C: Yupinfengsan Oral Liquid group; D: Loratadine group. Compared with the normal group, **P < 0.01; Compared with that in the model group, **P < 0.01.

Table 2. Comparison on IL-4 and IFN-γ contents of the mice's serum in four groups ($\bar{x} \pm s$, n = 10, pg/mL).

Group	IL-4	IFN-γ
Normal group	5.98 ± 1.06*	24.43 ± 2.59*
Model group	34.71 ± 3.68#	7.10 ± 1.59#
Yupinfengsan Oral Liquid group	22.91 ± 2.38#*	22.40 ± 2.61*
Loratadine group	13.39 ± 3.22#*	19.56 ± 2.23#*

Compared with the normal group, #P < 0.01; Compared with that in the model group, *P < 0.01.

4. Discussion

Yupingfengsan is an important category in the Traditional Chinese Medicine. The recent researches has shown that Yupingfengsan can give the role of immune regulation, anti-inflammatory, antibacterial and other effects via different mechanisms, and it can prevent and treat the allergic rhinitis, upper respiratory tract infection and other diseases. Clinically, there are numerous kinds of drugs in treatment of AR, and it mainly includes glucocorticoids, antihistamines and other drugs. There are little research on the relevant mechanism for AR treatment by Yupingfengsan, so it has no available support for Ypingfengsan on AR treatment [7].

AR is a type-I allergic disease resealed by the IgE-mediated mediators, and involved with multiple immune cells and cytokines after the body is exposed to the allergen. Now, it is generally accepted that the balance disorder of the T helper cells (Th1/Th2) is the key to cause AR. IFN-γ is the key effect factor of Th1 secretion, and it can inhibit the synthesis of IgE by B cells and the differentiation of Th0 cells into Th2 cells. IL-4 is mainly generated from Th2 cells, and it promotes the synthesis of IgE. Besides, it can induce the isomorphic transformation of immune globulin, which is secreted by B cells, from IgM to IgE, and enhance the degranulation of mast cells [8]. The abnormal expression of IL-4 and IFN-γ suggests the differentiation deviation of Th cells. NF-κB is a transcription factor with the nucleated cells that are located in the TLR downstream signaling hub, which plays a pivotal role in the inflammation and immune response through regulating the cascade amplification waterfall effect among relevant factors of immunity, inflammation and inflammatory transmitter [9]. The numerous studies showed that TLR-NF-κB signal pathway plays an important role in the balance disorder of Th1/Th2 cytokines.

In this experiment, OVA and aluminum hydroxide adjuvants stimulated the mice repeatedly to generate the allergic reaction, to successfully establish the AR mice model according to the score of the mice's symptoms. The result showed that Yupinfengsan Oral Liquid can improve the clinical symptoms and histopathological manifestations of AR mice. Compared with the normal group, the studies observed that the IL-4 level in the serum of AR model group was significantly increased and the IFN-γ level was significantly decreased. After the treatment of Yupinfengsan Oral Liquid, the IL-4 level in serum was decreased and IFN-γ level was increased when compared with the model group. It indicated that the Yupinfengsan Oral Liquid can effectively regulate the AR mice's cytokines, and play the immunomodulatory effect on the allergic rhinitis, which was consistent with the studies of immunologic equilibrium mechanism of Th1/Th2 cytokines of the most allergic rhinitis. Through the further research, it was found that Yupinfengsan Oral Liquid can inhibit the NF-κB expression, and it was speculated that it treated the allergic rhinitis by inhibiting the NF-κB activation and maintaining Th1/Th2 cytokines balance. This study carries out the preliminary discussion for the mechanism of action of Yupinfengsan Oral Liquid on the

treatment of AR. However, it remained to be studied for the method to inactivate NF-κB, so as to block inflammation and immune response.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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