

Effect of Tongqiao-tang on OVA Induced Allergic Rhinitis Mouse Model

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Objectives : Tongqiao-tang(TQT) has been commonly used for the treatment of common cold, rhinitis etc. Nowadays, TQT becomes one of the most frequently used medicines for allergic rhinitis, but the mechanism of TQT *in vivo* isn't investigated yet. This study was performed to investigate the effect of TQT on OVA-induced allergic rhinitis mouse model by calculating serum cytokines and IgE.

Methods : 8 weeks aged male BALB/c mice were divided into three groups: the normal group, the control group and the medicated group (the TQT group). Each group was consisted of 15 mice. The TQT group was administered TQT extract orally one time a day (1g/kg) from the 1st day of experiment till the 26th day. The control group and the normal group were administered normal saline by the same method of the TQT group. To induce the allergic rhinitis in the control group and the TQT group, mice of each group were sensitized intraperitoneally with ovalbumin (OVA) solution at the 1st, the 7th and the 14th day. After then, intranasal sensitization was performed by dropping 0.1% OVA solution in nasal cavity at the 22th, the 24th and the 26th day. At the 27th day, the mice were killed and the changes of interferon- γ , interleukin-4, interleukin-5, total IgE and OVA-specific IgE were checked.

Results : IFN- γ was increased 36% more in the TQT group than that in the control group. IL-4, IL-5, the total IgE and OVA-specific IgE were decreased in the TQT group as compared with the control group and these results were statistically significant.

Conclusions : Considering the above experimental results, this study showed that TQT could reduce the allergic reaction in allergic rhinitis. Advanced studies are required to investigate the further mechanisms of TQT.

Key Words : Tongqiao-tang, OVA-induced allergy model, cytokines, IgE

Introduction

Tongqiao-tang (TQT) has been used for various nose diseases in Oriental Medicine for a long time. Recently, this herbal medicine is frequently used for patients who suffer from running nose, sneezing and nasal obstruction, especially for the patients who are diagnosed as allergic rhinitis.

There have been some studies on TQT's inhibitory effect on allergic reaction.¹⁻⁵⁾ However, it is still

unclear how TQT regulates the allergic reaction.

The cytokines produced by activated CD4 T helper cells determine their differentiation into Th1 and Th2 cell.^{6,7)} Allergic inflammation is associated with a shift in the balance between cytokines produced by Th1 and Th2 cells toward a Th2 predominance. There are many types of cytokines such as IL-4, IL-5, IL-6, IL-10, INF- γ etc., and these cytokines play an important role in allergic reaction.^{8,9)}

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So, in this study, we made TQT extract and we investigated how TQT extract affected the levels of cytokine and immuno-globulin with using an ovalbumin-induced allergic rhinitis mouse model.

Materials and Methods

1. Animals

8 weeks aged male BALB/c mice (Orient Company, Korea) were used for the experiment. The animals were individually kept in animal cages in the laboratory (temperature: $23\pm 2^\circ\text{C}$, relative humidity: $55\pm 5\%$, and light/dark cycle: 12/12h), and they had access to food and water *ad libitum* throughout the experimental period. After 7 days later, the mice were divided into three groups: the normal group, the control group and the medicated group (the TQT group). Each group was consisted of 15 mice.

2. TQT extract preparation

Extracted powder of TQT was made and used.

To make the extracted powder of TQT, all the herbs were obtained from Kyung Hee Oriental Medical Center. This hospital has been authorized by Ministry of Health and Welfare of Republic of Korea to use herbs as medicine for clinical research and treatment. All the herbs used this experiment conformed to The Korean Pharmacopoeia¹⁰⁾ and The Korean Herbal Pharmacopoeia¹¹⁾ and they met the standard requirements. The composition of the medicinal herb is listed in Table 1. All the herbs were dried and weighed, and the herbs were put into glass bottle with enough water for 1 day. For promoting dissolution, physical stimulus was given to the glass bottle for 1h at 50°C via an ultrasonic washer: this solution was then filtered with filter paper and decompressed for concentration by using a rotary vacuum evaporator (EYELA, Japan). This concentrated solution was transferred to a 1,000ml round flask and lyophilized with using freezing drier (EYELA, Japan) for 24h (yield 11.72%).

TQT extract was suspended in 1g/100cc of normal saline and this had been administered orally to the

Table 1. Composition of Tongqiao-tang

Medicinal plants	Species	Dose(g)
Astragali Radix	<i>Astragalus membranaceus</i> Bunge	15.00
Angelicae Radix	<i>Angelica dahurica</i> Bentham et Hooker	15.00
Atractylodis Rhizoma	<i>Atractylodes lancea</i> D. C, <i>Atractylodes chinensis</i> Koidzumi	12.00
Glycyrrhizae Radix	<i>Glycyrrhiza uralensis</i> Fischer	8.00
Xanthii Fructus	<i>Xanthium strumarium</i> Linne	8.00
Sileris Radix	<i>Saposhnikovia divaricata</i> Schiskin	8.00
Angelicae Koreanae Radix	<i>Ostericum Koreanum</i> Maxim.	8.00
Angelicae Tennissimae Radix	<i>Angelica tenuissima</i> Nakai.	8.00
Cimicifugae Rhizoma	<i>Cimicifuga heracleifolia</i> Komarov	8.00
Puerariae Radix	<i>Pueraria lobata</i> Ohwil	8.00
Cnidii Rhizoma	<i>Cnidium officinale</i> Makino	6.00
Ephedrae Herba	<i>Ephedra sinica</i> Stapf	4.00
Zanthoxyli Fructus	<i>Zanthoxylum piperitum</i> De Candolle	4.00
Magnoliae Flos	<i>Magnolia denudata</i> Desrousseaux	4.00
Asiasari Radix	<i>Asiasarum heterotropoides</i> F. Maekawa var. <i>mandshuricum</i> F. Maekawa	3.00
Menthae Herba	<i>Mentha arvensis</i> var. <i>piperascens</i> Malinvaud	3.00
	Total	122.00g

TQT group once a day during the experimental period (1g/kg). The control group and the normal group had been administered normal saline orally once a day during the experimental period (1g/kg).

3. Sensitization of mice

The ovalbumin (OVA chicken egg albumin, grade V, Sigma, USA) was used as an antigen by adapting Levin&Vaz's method.¹²⁾ 10 μ g OVA was dissolved in a mixture of Al(OH)₃ gel (Sigma, U.S.A.) and phosphate buffered saline (PBS) (1:1) to make 1ml of OVA solution. Both the control group and the TQT group were injected OVA solution intraperitoneally at the 1st, the 7th, and the 14th day. The normal group was injected PBS at the same way as negative control. After 7 days intermission, 0.1% OVA solution was dropped in the nasal cavity of both the control group and the TQT group at the 22st, the 24th, and the 26th day for inducing intranasal sensitization.

4. Serum separation

Three groups of mice were anesthetized by an IM injection of zoletil (0.1cc/100g) at the 27th day: blood samples were immediately collected by cardiac puncture, and the sera were stored at -20°C.

5. Splenocyte preparation

After the blood was collected, the spleens were removed and prepared for cell culture. The spleens were washed with RPMI-1640 supplemented with 10% fetal bovin serum (FBS). The splenocytes were prepared by disrupting the spleen between glass slides in complete medium and then the cell mixture was filtered with a 0.40 μ m nylon cell strainer. After 10min coagulation at 1,000rpm to separate cells from debris, the cells were washed in medium: this was followed by lysis of the erythrocytes using ammonium chloride reagent (Pharmingen, CA, USA). After the 2nd centrifugation, the splenocytes were washed again.

6. Cell culture

The splenocytes were cultivated with OVA (1mg/ml) in a 37°C, 5% CO₂ incubator for 48h after they were seeded at 1 \times 10⁶ cells/ml on 24 well plates. After cell harvest, the supernatants were collected and stored at -20°C for cytokine analysis.

7. Measurement of the cytokines and total IgE

ELISA was used with OPT EIA set (Pharmingen) for the analysis of the total IgE and the cytokines. The 96-well plates were coated with capture antibodies in PBS: overnight at 4°C. The primary antibodies were discarded and the plates were blocked with Assay Diluent (Pharmingen, San Diego, CA, USA) for 1h at room temperature. The plates were washed 3 times with wash buffer (0.05% Tween 20 in PBS). The standard IgE and standard cytokines were added in triplicate and then the plates were incubated for 2h at room temperature. The supernatant was discarded and the wells were washed 5 times with wash buffer. Biotinylated IL-4, IL-5, IFN- γ and IgE detection antibodies plus Avidin-HRP were added and then the plates were incubated for 1h at room temperature. After washing, 100 μ l tetramethylbenzidine substrate solution (Pharmingen, San Diego, USA) was added. The color was allowed to develop for 30 min in the dark before the reaction was quenched with stop solution (0.2M H₂SO₄ 50 μ l). The plates were read at 450-570nm by using a Microplate reader (Molecular devices, USA) and the sample concentrations were determined with the help of a standard curve.

8. Measurement of OVA-specific IgE

ELISA was also used as described above for the OVA-specific IgE. 96-well plates were coated overnight at 4°C with OVA (100 μ g/ml). After washing, the plates were blocked with 10% FBS in PBS for 1h at room temperature. Diluted samples were incubated for 2h at room temperature. After washing, biotinylated OVA was added and the plates incubated for 2h at room temperature. The OVA was

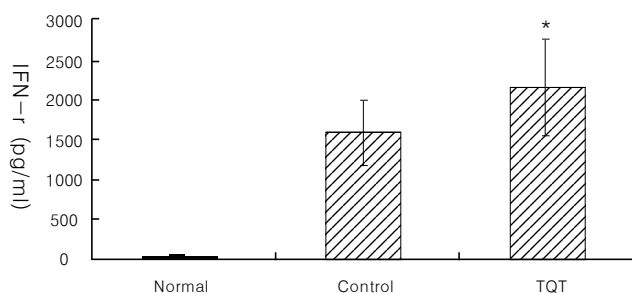


Fig. 1. Effect of TQT on the production of IFN- γ at OVA-induced allergic rhinitis mice. Normal is intact group. Control is immunized by OVA and medicated with normal saline group. TQT is immunized by OVA and medicated with TQT extract one time a day from the 1st day till the 26th day group. Each value represents the mean \pm SD. The statistical analysis was performed by independent T-test and * means $p < 0.05$.

biotinylated using a Biotin N hydroxysuccinimide ester-water soluble (Vector Laboratories, CA, USA). After washing, Avidin-Horseradish Peroxidase was added and the plates incubated for 30min at room temperature. After washing, the plates were incubated with 3,3'-5,5'-Tetramethylbenzidine and then stop solution was added. The optical densities were measured at 450-570nm.

9. Statistical analysis

All the data was expressed as means \pm standard deviations (Mean \pm SD) for all the measurements of each group. The statistical analysis was performed by independent T-test. The statistical significance was set at $p < 0.05$. The data of the normal group

was used as reference data.

Results

1. The effect of TQT on the production of IFN- γ , IL-4 and IL-5

IFN- γ was increased 36% in the TQT group (2182.212 \pm 596.595pg/ml) compared with the control group (1599.986 \pm 415.018pg/ml). In the normal group, IFN- γ was 33.657 \pm 28.569pg/ml (Fig. 1). IL-4 and IL-5 were significantly decreased in the TQT group compared with the control group. IL-4 was 4.984 \pm 0.626pg/ml in the normal group, 17.391 \pm 4.304pg/ml in the control group and 12.241 \pm 4.855pg/ml in the TQT group (Fig. 2). IL-5 was not found in the normal

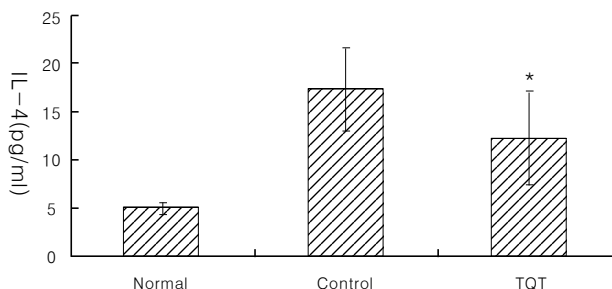


Fig. 2. Effect of TQT on the production of IL-4 OVA-induced allergic rhinitis mice. Normal is intact group. Control is immunized by OVA and medicated with normal saline group. TQT is immunized by OVA and medicated with TQT extract one time a day from the 1st day till the 26th day group. Each value represents the mean \pm SD. The statistical analysis was performed by independent T-test and * means $p < 0.05$.

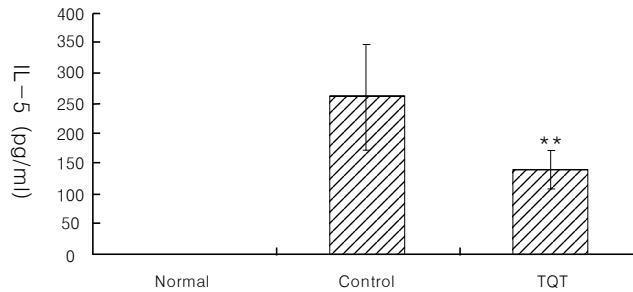


Fig. 3. Effect of TQT on the production of IL-5 at OVA-induced allergic rhinitis mice. Normal is intact group. Control is immunized by OVA and medicated with normal saline group. TQT is immunized by OVA and medicated with TQT extract one time a day from the 1st day till the 26th day group. Each value represents the mean±SD. The statistical analysis was performed by independent T-test and ** means $p < 0.01$.

group. The level of IL-5 was $260.886 \pm 86.23 \text{ pg/ml}$ in the control group and $140.044 \pm 31.169 \text{ pg/ml}$ in the TQT group (Fig. 3).

2. The effect of TQT on the total IgE and the OVA-specific IgE

Both the total IgE level and the OVA-specific IgE level were significantly decreased in the TQT group compared with the control group. The level of total IgE was $804.383 \pm 80.764 \text{ ng/ml}$ in the normal group, $4982.495 \pm 2044.453 \text{ ng/ml}$ in the control group and $2165.868 \pm 976.464 \text{ ng/ml}$ in the TQT group (Fig. 4). In conclusion, compared with the control group, the total IgE was reduced 57% in the TQT group. The level of OVA-specific IgE in the normal group

was low as 0.059 ± 0.008 , it was 0.557 ± 0.220 in the control group and 0.356 ± 0.190 in the TQT group (Fig. 5).

Discussions

Allergic rhinitis is an IgE-mediated inflammatory disease^{13,14} and a number of recent studies have suggested that the level of IL-4 and IL-5 were increased in allergic rhinitis.^{13,15} Allergic inflammation is associated with a shift in the balance between cytokines produced by the Th1 and Th2 cells toward a Th2 predominance.^{9,16} The subtypes of T helper cells are determined by their cytokines secretion patterns.^{17,18} Th1 cells contribute to cell-mediated

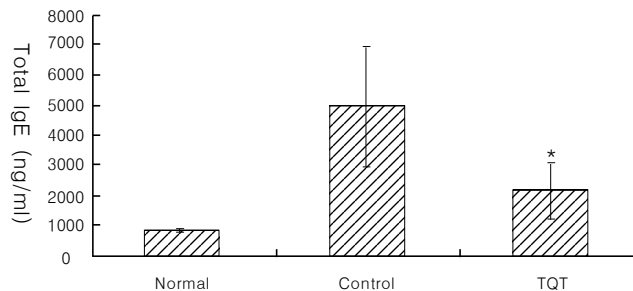


Fig. 4. Effect of TQT on the serum level of total IgE at OVA-induced allergic rhinitis mice. Normal is intact group. Control is immunized by OVA and medicated with normal saline group. TQT is immunized by OVA and medicated with TQT extract one time a day from the 1st day till the 26th day group. Each value represents the mean±SD. The statistical analysis was performed by independent T-test and * means $p < 0.05$.

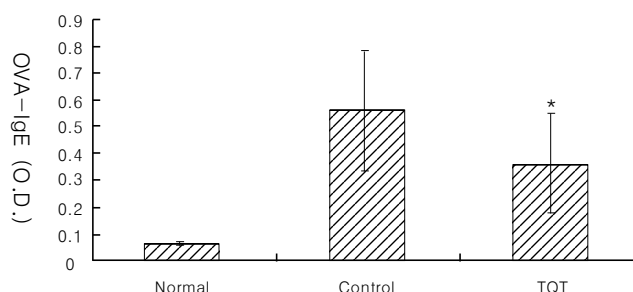


Fig. 5. Effect of TQT on the serum level of OVA-specific IgE at OVA-induced allergic rhinitis mice. Normal is intact group. Control is immunized by OVA and medicated with normal saline group. TQT is immunized by OVA and medicated with TQT extract one time a day from the 1st day till the 26th day group. Each value represents the mean±SD. The statistical analysis was performed by independent T-test and * means $p < 0.05$.

inflammatory immunity, while Th2 cells are responsible for humoral responses.¹⁸⁾ Th1 cells produce IL-2, IFN- γ and tumor necrosis factor- β whereas Th2 cells secrete IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13 and granulocyte-macrophage colony-stimulating factor (GM-CSF).^{15,19-21)} The Th1/Th2 cytokines interact in a reciprocal manner to maintain a balanced immune status.^{15,22)}

Production of IFN- γ has been associated with autoimmune inflammatory conditions.²³⁾ IFN- γ is a potent inhibitor of IgE production by interfering with the IL-4 mediated switch to ϵ -gene expression. IFN- γ mediated-antagonism of the effects of IL-4 and IFN- γ could occasionally be detected in the absence of significant proliferating responses, which suggests that proliferation can be dissociated from cytokine release.^{23,24)}

IL-4 is involved in many aspects of allergic inflammation, including IgE production and cellular influx.²⁵⁾ IL-4 is important as it induces switching of B-cells from IgM/G to the IgE antibody isotype.^{14,15,18,19,26-27)} IL-4 production is critical for the development of Th2 cells.^{15,26)} and IL-4 is essential in promoting the commitment Th1 cell precursors to produce the Th2 subset of cytokines: this is important for the development of humoral-mediated immunity. In addition to promoting Th2 differentiation, IL-4 promotes T-cell proliferation, and IL-4 activates IgE isotype switching of B cells. IL-4 is the

most important factor for induction of Th2-cell development and the resultant production of Th2-cell cytokines.²⁸⁾

IL-5 is involved in the accumulation and activation of eosinophil. This cytokine is expressed to a greater degree in allergic patients compared with non-allergic patients, and its level increase after allergen challenge.¹³⁾ IL-5 promotes eosinophil maturation and endothelial adhesion, activation and survival. It contributes to eosinophil recruitment from bone marrow and to the release of inflammatory mediators.

Production of IgE critically depend on the ratio of the cytokines IL-4 and IFN- γ , which are both elicited after exposure to allergen.²³⁾ The pathogenesis of perennial allergic rhinitis has been reported to correlate with excessive production of allergen-specific IgE and IgG4.

In this study, both IL-4 and IL-5 were significantly decreased in the TQT group as compared with the control group. IFN- γ was also increased in the TQT group as compared with the control group. The total IgE was decreased in the TQT group as compared with the control group, and OVA-specific IgE was more decreased in the TQT group than that in the control group. From these results, we could form a hypothesis that TQT has an inhibitory effect on allergic inflammation by inducing cell-mediated inflammatory immunity of Th1 cells and by inhibiting the humoral responses of Th2 cells.

Conclusions

This study indicates that Tongqiao-tang extract inhibits allergic reaction by decreasing the expression of IL-4, IL-5, the total IgE and OVA-specific IgE and by increasing IFN- γ .

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