

Effectiveness of thymoquinone in the treatment of experimental asthma

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Abstract

Background. Thymoquinone (TQ), the main active constituent of the volatile oil extracted from *Nigella sativa*'s seeds, is used for the treatment of inflammatory diseases and exhibits a variety of pharmacological effects.

Methods. Twenty-eight BALB/c female mice were divided into four groups: I (sham-operated control group), II, III, and IV. All groups except for the sham-operated group were sensitized and challenged with ovalbumin. The sham-operated group received nebulized saline in challenge period. Mice in groups III and IV were administered TQ at a dose of 3 mg/kg and dexamethasone 1 mg/kg, respectively, intraperitoneally once a day for the final 5 days of the challenge period. Animals were sacrificed 24 h after the last drug administration and the airway samples were evaluated histologically by light microscopy.

Results. All histological parameters in Group III, similar to Group IV, were improved when compared to Group II. All variables except numbers of goblet cells were found to be significantly better in Group III and Group IV compared to Group II.

Conclusions. In our study, we demonstrated that TQ administration alleviates the pathological changes of chronic asthma. TQ might be a promising therapy for asthma in the future. *Clin Ter* 2013; 164(3):e155-158. doi: 10.7417/CT.2013.1559

Key words: anti-inflammatory, chronic asthma, thymoquinone, mouse, treatment

Introduction

Asthma is a chronic airway disease which is characterized by reversible airway obstruction, chronic inflammation and remodeling (1). It is the most common pulmonary disease in industrial countries, which has significant economic costs (2). Remodeling is a potential consequence of asthma (3). Airway remodelling, a potentially important consequence of asthma, comprises progressive structural changes in the composition, content, and organisation of the cellular and molecular constituents of the airway wall, as well as enhanced turn over of extracellular matrix components. It includes

goblet cell hyperplasia, basement membrane thickening, subepithelial fibrosis, airway smooth muscle hypertrophy/hyperplasia, and angiogenesis. Airway remodelling is poorly responsive to current anti-inflammatory asthma therapies, including inhaled corticosteroids and antileukotrienes (4). Thus, new therapeutic options are required.

Thymoquinone (TQ) is an active constituent of the volatile oil extracted from *Nigella sativa*'s seeds (5). It shows its effects by suppressing the production of leukotriene B₄, thromboxane B₂, and inflammatory mediators via 5-lipoxygenase and cyclooxygenase pathway of arachidonic acid metabolism (6). Antioxidant and immunomodulatory activities of thymoquinone have been demonstrated. Animal experiments proved effectiveness of thymoquinone on the cancer (7, 8). Its effectiveness on allergic diseases was also shown (allergic rhinitis, atopic eczema, asthma) (9, 10).

Consequently, TQ may have potential efficacy in controlling inflammatory diseases. The possible benefits of TQ in asthma treatment have yet to be thoroughly investigated. Therefore, in this study, our aim is to investigate the efficacy of TQ on lung histopathology in a murine model of chronic asthma.

Materials and Methods

Experimental animals

Specific pathogen-free, 6- to 8-week-old, female BALB/c mice, weighing 18-20 g, were maintained in a pathogen-free laboratory of Dokuz Eylul University. They were kept in hygienic macrolene cages in air-conditioned rooms and allowed ad libitum with food and water on a 12-h light/12-h dark cycle. All experimental procedures complied with the requirements of the Animal Care and Ethics Committee of Dokuz Eylul University.

BALB/c mice were divided into four groups (7 mice per group). Group I - sham group. Group II - Chronic asthma. Group III - Thymoquinone Treated. Group IV - Dexamethasone Treated.

Sensitization and inhalational exposure

BALB/c mice are high responders to ovalbumin (11). The mice in study groups II, III, IV were sensitized via two intraperitoneal injections, on days 0 and 14 of the experiment, of 10 µg/0.1 mL chicken egg albumin (ovalbumin, grade V, ≥98% pure; Sigma, St. Louis, MO, USA) with alum as an adjuvant. The mice in study groups II, III, IV were then exposed to aerosolized ovalbumin for 30 min per day on 3 days per week for 8 weeks, beginning from the 21st day of the study. The mice in group I (sham) were administered normal saline with alum intraperitoneally on 0 and 14th days of the experiment, and exposed to aerosolized saline for 30 min per day for 3 days per week for 8 weeks, beginning from the 21st day of the study (11,12). Exposures were carried out in a whole body inhalation exposure system. Temperature and relative humidity were maintained between 20–25 °C and 40–60%, respectively. A solution of 2.5% ovalbumin in normal saline was delivered by aerosolization via compressed air to a sidestream jet nebulizer injected into a chamber. The aerosol generated by this nebulizer comprised >80% particles with a diameter of <4 µm. Particle concentration was maintained in the range of 10–20 mg/mm³ (12).

Study drugs

Mice in group I received saline solution. Mice in groups III, IV instead received TQ (Sigma&Aldrich®) at a dose of 3 mg/kg, dexamethasone 1 mg/kg respectively, intraperitoneally once a day in the last 5 days of the challenge period. Intraperitoneal doses of TQ and dexamethasone were chosen from other studies conducted also with BALB/c mice (10,12).

Histological Analysis

After study, the mice were sacrificed by ketamine 24 h after the last drug administration. Mid zone lung tissues were obtained and fixed in buffered 10% formalin and embedded in paraffin wax. Five-micron-thick serial sections were obtained and the first 10 samples stained with haematoxylin and eosin (H&E). In these samples, general tissue features were examined and the thicknesses of epithelium and subepithelial smooth muscle layers of the medium and small airways were measured. In order to evaluate the thicknesses of epithelium and subepithelial smooth muscle layers, measurements were performed from four points of each airway. Considering that each section contained approximately two to three airways, around 20 or more airways were evaluated for each mouse. Photomicrographs were taken by Olympus DP71® camera (Japan), which adapted on Olympus DP70 model microscope (Olympus Optical®, Tokyo, Japan). Measurements were carried out with UTHSCSA Image Tool for Windows Version 3.00 software®.

The consecutive 10 sections were stained with toluidine blue and the other 10 sections with periodic acid-Schiff (PAS). Photomicrographs were taken randomly from five fields of each section which were stained with toluidine blue. For mast cell enumeration, a standard transparent counting frame representing an area of 16,400 µm² was used manually and eight fields in each photograph were examined for each

mouse. Goblet cells stained with PAS were enumerated in 10 sections of each mouse. In each section, three to five randomly selected airways were photographed. Circumferences of all airways were measured and goblet cell numbers in these areas were recorded. For standardization, goblet cell numbers in 100 µm were analyzed by division of total goblet cell number to the total length of airway circumferences and multiplying the result by one hundred.

Statistical analysis

All values were expressed as the mean ± standard deviation (SD). The Kruskal Wallis (between all groups) and Mann–Whitney U-test (for two groups) was used to compare staining intensity values between groups. All statistical procedures were performed by SPSS software for Windows, Version 15.0 SPSS®, Chicago, IL, USA). A value of $p \leq 0.05$ was considered significant.

Results

Lung tissues of the sham group was observed normal histologic features (Figure 1, 1A, 1B, 1C, 1D). In the chronic asthma group, the number of mast cells, goblet cells and the subepithelial smooth muscle and epithelial thicknesses were significantly higher than the Sham group ($p=0.000$, $p=0.000$, $p=0.000$, $p=0.009$ respectively) (Table 1 and Figure 1, 2A, 2B, 2C, 2D).

These results considered that the model was established. Subepithelial smooth muscle and epithelial thickness and the number of mast cells and goblet cells in thymoquinone treated group was significantly lower than asthma group (Thymoquinone $p=0.008$, $p=0.000$, $p=0.001$, respectively) (Table 1 and Figure 1, 3A, 3B, 3C, 3D).

Subepithelial smooth muscle and epithelial thickness and the number of mast cells and goblet cells in dexamethasone treated group was significantly lower than asthma group ($p=0.002$, $p=0.000$, $p=0.000$, respectively) (Table 1 and Figure 1, 4A, 4B, 4C, 4D). There was no statistically significant difference in all histological parameters between thymoquinone and treated groups. Thymoquinone significantly improved subepithelial smooth muscle and epithelial thickness and number of mast and goblet cells similar to dexamethasone, the gold Standard treatment of asthma.

Discussion

In asthma, some structural changes called remodeling develop as a result of damage/repair processes in the airways. Today, drugs used in the treatment of asthma have limited effect on the structural changes. Therefore there is need for effective treatment options (13). Although TQ provides a reduction in asthma symptoms and inflammatory markers, the tissue-level activity has not entirely been demonstrated (14). Thus, we aimed to investigate the efficacy of TQ on pulmonary histological changes and especially on remodeling in a murine model of chronic asthma.

In our study, the structural changes observed in the asthmatic group revealed that the model was well established. It

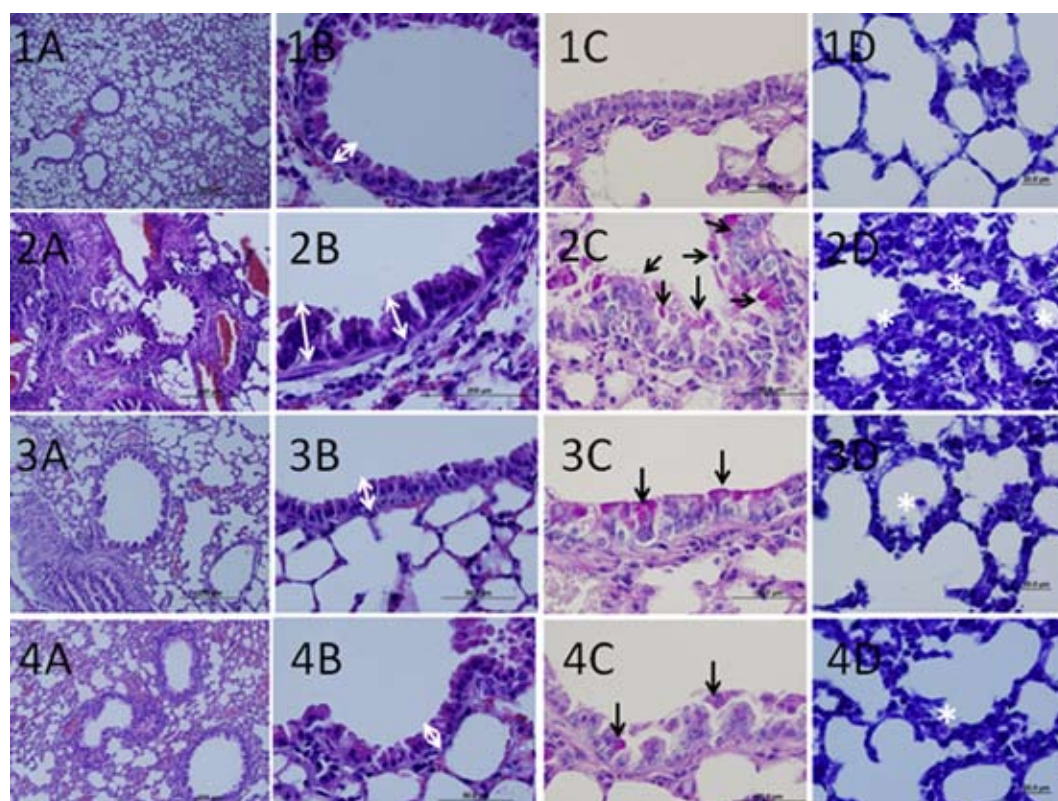


Fig. 1. Groups. I; Sham, II; Asthma, III; Thymoquinone Treated group, IV; Dexamethasone Treated group. 1A, 2A, 3A and 4A (H&E staining) are shown in the histological structure of lung tissue. 1B, 2B, 4B 3Bve (H & E staining). 1C, 2C, 3C and 4C (PAS staining). Light microscopic findings (1a, 1b) revealed a regular respiratory epithelium and airways, a thin, regular subepithelial smooth muscle layer (arrow with two heads), and normal PAS-stained parenchymal structures (1c). Parenchymal structures with toluidine blue staining were regular (1d). In asthma group; H&E staining (2a, 2b) revealed thickened epithelium, thickened subepithelial smooth muscle (arrow with two heads), high numbers of goblet cells (Gc) were seen with PAS-staining (arrow) (2c), and mast cells with toluidine blue staining (2d); * was indicated mast cells. In Thymoquinone treated group (3a, 3b, 3c, 3d) and dexamethasone treated group (4a, 4b, 4c, 4d) group; no difference in light microscopic findings was found compared with sham rats.

Table 1. Histomorphometric analysis of lung tissues.

	Histomorphometric Measurements			
	Sham	Chronic asthma	Thymoquinone Treated	Dexamethasone Treated
Subepithelial smooth muscle thickness (μm)	4.69 $\pm$ 2.16	13.8 $\pm$ 4.02 *	9.84 $\pm$ 2.85**	8.50 $\pm$ 2.67**
Epithelium thickness (μm)	41.4 $\pm$ 15.8	60.7 $\pm$ 19.0*	49.5 $\pm$ 10.0 **	48.3 $\pm$ 9.00**
Numbers of mast cells/16400 (μm^2)	0.67 $\pm$ 0.58	2.64 $\pm$ 0.94*	1.70 $\pm$ 1.33**	1.50 $\pm$ 1.33**
Numbers of goblet cells/100 (μm)	0.88 $\pm$ 1.03	2.35 $\pm$ 2.29*	1.55 $\pm$ 1.77	1.35 $\pm$ 1.15

*: Significantly increased vs. Sham group. **: Significantly decreased vs. chronic asthma group. ($p < 0.05$)

seems very important to prevent airway remodeling in the chronic management of asthma (3), because once formed, remodeling is resistant to asthma therapy (15). In our study, dexamethasone and TQ significantly ameliorated all the structural changes of chronic asthma.

Antileukotrienes, theophylline, inhalation of corticosteroids are insufficiently responsive to these structural changes. Inhaled corticosteroids may be effective in reducing these

structural changes when used for a long period of time (5). In our study, dexamethasone and TQ significantly ameliorated all the structural changes of chronic asthma. This finding is very important because reticular basement membrane thickness is considered a hallmark for airway remodeling in asthma (16).

Thymoquinone (TQ) is the major active compound derived from black seed (*Nigella sativa*). *Nigella sativa* have

long been used in the Middle and Far East as a conventional medicine for a wide range of illnesses (17) TQ is a member of bioflavonoid with antioxidant, anti-inflammatory, neuroprotective, antiallergenic, antiviral, antidiabetic, and anti-carcinogenic properties (18-20). TQ shows its effects by suppressing the production of leukotriene B₄, thromboxane B₂, and inflammatory mediators via 5-lipoxygenase and cyclooxygenase pathway of arachidonic acid metabolism (14). In addition, it has been identified to have inhibitory effects on histamine receptors (21). Airway response in asthma occurs as a result of IgE mediated mast cell degranulation and consequent release of histamine and leukotriene mediators. We think that the mechanism of action on asthma occurs in this way.

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El Gazzar (10) administered TQ in mice before sensitization with ovalbumin in the experimental asthma model of mice and demonstrated that TQ has inhibitory effects on airway inflammation. And they considered that TQ probably suppresses inflammation by increasing the secretion of interferon-gamma. In contrast, we have administered TQ after creating an experimental model of asthma and have proven its effectiveness also after the development of remodeling in asthma.

El-Sayed (22) compared the effectiveness of curcumin and TQ in a similar asthma model. They administered TQ 10 mg/kg/day and curcumin 15 mg/kg/day for 8 days between 30th and 38th days of the experimental model of asthma and demonstrated that TQ is a more potent agent than curcumin. In our study, achieving similar efficacy with TQ administered at lower doses and durations is noteworthy.

Based on our results, it is suggested that measurement of cytokine levels and electron microscopic examinations should be included in such studies in order to make further conclusions about the changes at cellular level in asthma.

In conclusion, TQ injection caused a reduction in chronic inflammatory changes in experimental asthma model created in mice. Several studies are needed for better understanding of its effectiveness.

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