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Comparable Function of γ -Tocopherols in Asthma Remission by Affecting Eotaxin and IL-4*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of article

Abstract

Background. Bronchial asthma is one of the world's most common chronic disorders dangerous to human health. It has been hypothesized that the increased number of asthma sufferers may be due to changing antioxidant intake or vitamin deficiency. However, the influence of vitamins on asthma has rarely been considered.

Objectives. The aim of this study was to explore the effects of γ -tocopherols, a specific form of vitamin E, on asthma remission together with the possible mechanism behind the process.

Material and Methods. Eosinophil counting was applied to detect the total number of cells, eosinophils and lymphocytes. Meanwhile, HE staining was used for morphological detection. In addition, the eotaxin and IL-4 levels in the serum and bronchoalveolar lavage fluid were measured using ELISA technology.

Results. The cell counting results showed that γ -tocopherols possesses the capability to reduce the number of eosinophils. Moreover, the exudation of inflammatory cells together with the hyperplasia of goblet cells was also found to experience significant inhibition when treated with γ -tocopherols. Furthermore, the high levels of eotaxin and IL-4 in the asthma group were evidently reduced under the treatment of γ -tocopherols which was comparable with hexadecadrol.

Conclusions. γ -tocopherols can remit asthma by regulating the level of eotaxin and IL-4. Moreover, γ -tocopherols may be regarded as a potential candidate for asthma treatment after much deeper explorations (*Adv Clin Exp Med* 2016, 25, 4, 643–648).

Key words: asthma, γ -tocopherols, dexamethasone, eotaxin, IL-4.

Bronchial asthma is one of the world's most common chronic disorders, characterized by recurring symptoms, chronic lung airway inflammation, variable airflow obstruction and bronchial hyper-responsiveness [1]. The marked increase in asthma prevalence has made bronchial asthma a public health concern. More and more researchers recently are considering the possibility that this phenomenon may be due to the changing antioxidant intake and maladjusted dietary ratio [2]. Meanwhile, a great deal of attention has been focused on the fat-soluble dietary vitamin E, a protective dietary factor with desirable antioxi-

dant functions. Vitamin E has been proved to have the capabilities of rescuing allergen-induced inhibition of antioxidant enzymes, preventing the detrimental effects of air pollution as well as asthmatic features in asthma [3–5]. Furthermore, it has also been concluded that vitamin E supplements could improve clinical manifestations and pulmonary function tests in children with moderate asthma [6].

Vitamin E consists of two closely related subgroups: tocopherols and tocotrienols, each existing in 4 isomeric forms (α , β , γ and δ) [7]. Mechanistic studies have demonstrated that specific forms

* The study was funded by Xi'an health science project of China (No. 2013054).

of vitamin E such as gamma-tocopherols (γ T) and tocotrienols (especially γ TE) have desirable anti-inflammatory effects by inhibiting eicosanoids or suppressing NF- κ B signaling pathways [8, 9]. Moreover, the investigation of γ T in the airway inflammation of various animal models has attracted significant attention [10]. It is proposed that the supplementation of γ T may be helpful in asthma treatment. However, studies referencing this application to support the hypothesis have been limited.

Eosinophilic granulocyte, the crucial effector cell in the process of bronchial asthma, is closely associated with the severity of asthma [11]. Meanwhile, eotaxin, as the eosinophil activation chemokine, plays an important role in eosinophil adhesion, recruitment and degranulation [12]. There are synergies between eotaxin and Th2 cytokines, which mainly participate in humoral immune responses. The imbalance of Th1/Th2 immune cells has been regarded as the foundation of asthma pathogenesis. It is reported that the IL-4 gene can upregulate the expression level of eotaxin mRNA in pulmonary granuloma, airway smooth muscle cells or bronchial epithelial cells and is associated closely with asthma [13–15]. In order to study the association of γ T with asthma and provide a theoretical foundation for the clinical application of γ T, a test whether γ T supplementation could mitigate airway inflammation in a mouse allergic airway response model was carried out in comparison to the effects of dexamethasone, which is one of the most effective drugs for asthma but with some side effects.

Material and Methods

Material and Agents

Albumin (OVA), γ -T (95% purity) and Aluminum Hydroxide Dried Gel were purchased from Sigma Chemical CO (St. Louis, USA). BUD Pulmicort Respules was bought from AstraZeneca. Antibodies, an Immunostain SP kit, an *in situ* hybridization kit and Hoechst 33342 were purchased from Invitrogen (Carlsbad, USA). Dexamethasone was produced by Tin Yiu Technology Co., LTD of Zhengzhou City.

The Establishment of the Mouse Airway Response Model

The 40 BALB/C6 mice (clean class), provided by the laboratory animal center of Xi'an Jiaotong university, were randomly divided into 4 sub-

groups: normal subgroup (A), asthma subgroup (B), dexamethasone-treated subgroup (C) and γ T-treated subgroup (D). A mixture (0.2 mL) of Ovalbumin (OVA, 40 μ g) and Al(OH)₃ (10 mg) was applied to the mice for sensitization by means of intraperitoneal injection in the first and second week respectively. A combined application of OVA atomization inhalation for 30 min was aimed to establish the asthma model in the mice since the third week and lasted for 8 weeks, 3 times per week. Normal saline (NS), instead of the sensitization liquid, was added to the control subgroup while the dexamethasone-treated subgroup and γ T-treated subgroup were intraperitoneally injected with dexamethasone or γ T, respectively, prior to OVA inspiration.

Specimen Collection

During the period of final inspiration, the mice were anesthetized and dissected following chloral hydrate injection. Inferior vena cava blood was collected and centrifuged. The upper serum was retained at -70° . A bronchoalveolar lavage fluid (BALF) of physiological saline lavage was then gathered after the operation of bilateral lung tissue explosion, tracheal ligation and puncture. The upper serum after centrifugation was cryopreserved at -70° . The cell counting of the BALF can be used as an index to assess the mouse asthma model.

Histopathology Detection

The middle section of the left lung was fixed with 4% paraformaldehyde and then transected at the hilum pulmonis level. HE staining was proceeded after embedding, dehydrating and slicing. Each subgroup was incubated with HE for 15 min and finally, the airway inflammation and structural changes of the airway are made clear at a glance after HE dye.

ELISA

The eotaxin and IL-4 levels in the serum and bronchoalveolar lavage fluid were measured using ELISA (R&D Systems, Minneapolis, USA). In brief, 96-well microtiter plates were coated overnight at room temperature. Triplicate samples of each treatment were applied and incubated overnight at 4° C. Samples were then incubated for 1 h at room temperature with biotinylated goat antibodies, followed by 1 h incubation with HRP-conjugated streptavidin (R&D Systems, Minneapolis, MN). After three washes with PBS containing 0.05% Tween 20 (PBST), the final reaction product was detected and the plate was read at 450 nm.

Statistical Analysis

All values in the experiments were expressed as mean ± SD. Statistical analyses were carried out using SPSS 17.0 software. The significance of inter-subgroup differences was evaluated by ANOVA (one-way analysis of variance) when the variables showed normal distribution. The SNK-q test and Dunnett's c (U) test were used for pairwise comparison and multiple comparisons after each analysis. A p-value less than 0.05 was considered to be significant.

Results

The Establishment of the Asthma Model

The mouse asthma model was established successfully and the results usually occurred within 30 min of OVA inspiration. Obvious features were easy to find in different subgroups. For instance, the asthma model mouse demonstrated restlessness, shortness of breath, nose incitement, incontinence and serious limb collapse while the control mouse behaved as usual, without any abnormal occurrences. The symptoms of the dexamethasone-treated subgroup were distinctly reduced but were found with increasing activity and scratching to the face or nose. The degree of asthma episodes was also mitigated.

Results of Cell Counting

The BALF counting showed us the number of total cells, eosinophils and lymphocytes. As is shown in Table 1, the cell number of the asthma

subgroup was significantly higher than the control subgroup. And that of the dexamethasone-treated subgroup and γT-treated subgroup were decreased in comparison to the asthma subgroup. Obviously there was little difference between the dexamethasone-treated subgroup and the γT-treated subgroup. In addition, the percentage of eosinophils and lymphocytes was noticeably lower following the addition of dexamethasone or γT. The rate of eos decreased by 15% in the dexamethasone-treated subgroup and γT-treated subgroup without a great difference, but showed a large reduction compared to the asthma subgroup. A similar phenomenon was also found in the Lym (%) measurement.

HE Staining

HE staining was used to represent the morphology of airway lumen in the four model subgroups (Fig. 1). The staining results revealed the distinct characteristics between them. Firstly, the normal control subgroup contains the whole airway epithelium with tiny amounts of inflammatory cells around without damage in the bronchial mucosal epithelium and muscularis mucosae structure. In comparison with the staining results of the normal subgroup, the airway lumen of the asthma subgroup was found with evident incrasation and blood vessels infiltrated a large majority of inflammatory cells, which could also be seen in the pulmonary mesenchyme and alveolar space. When referred to the dexamethasone-treated subgroup or γT-treated subgroup, the above-mentioned phenomenon was repeated to a certain degree, with not only the exudation of inflammatory cells but also the inhibited hyperplasia of goblet cells. Moreover, the asthma-catabatic function of γT was comparable to the dexamethasone, there-

Table 1. Comparison of cell count between the four groups (mean ± SD)

Groups	N	Total cells (×10 ⁵ /mL)	Eos (%)	Lym (%)
Control	10	15.321 ± 2.102	0.482 ± 0.027	13.405 ± 1.662
Asthma	10	28.952 ± 5.001*	21.734 ± 3.251*	36.981 ± 3.724*
Dexamethasone-treated	10	20.785 ± 3.710*#	6.923 ± 1.840*#	22.729 ± 4.002*#
γT-treated	10	19.638 ± 1.126*#	6.146 ± 1.006*#	21.764 ± 3.152*#

* p < 0.01 vs. control; # p < 0.01 vs. asthma group.

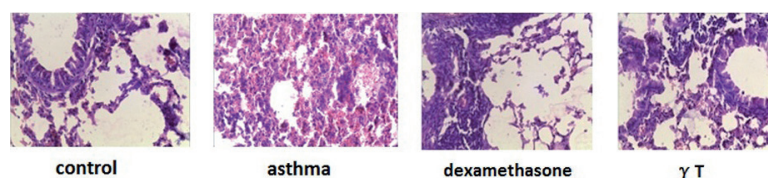


Fig. 1. The comparison of cell count between the four subgroups. **: p < 0.01 vs. control; #: p < 0.05 vs. asthma; ##: p < 0.01 vs. asthma.

fore γ T might be applied instead of dexamethasone for the treatment of asthma.

The Decreased Level of Eotaxin

Unlike the results of the control subgroup, the concentration of eotaxin in the asthma subgroup was obviously enhanced (Table 2). However, the eotaxin in BALF was remarkably reduced when treated with dexamethasone or γ T compared to the asthma subgroup. Meanwhile, eotaxin in the serum decreased in comparison to the asthma subgroup. Furthermore, the effects of γ T were even better than dexamethasone in eotaxin reduction which indicated the potential of γ T in aspects of asthma relief.

The Decreased Level of IL-4

Next, we assess the IL-4 level in serum and BALF of the four distinct subgroups. The IL-4 level of the asthma mice was conspicuously higher than the in normal mice. The addition of dexamethasone or γ T decreased the IL-4 level, especially γ T, which reduced the IL-4 in the serum of nearly half of the asthma subgroup better than dexamethasone as is shown in Table 3. The function of γ T to IL-4 in BALF was comparable to dexamethasone without large differences.

Discussion

Bronchial asthma is a chronic inflammatory disorder of the airways in which many cells, particularly eosinophils, may play important roles

through the release of various mediators [16–18]. There is strong evidence that an imbalance between the reducing and oxidizing systems favoring a more oxidative state is present in the airway inflammation and a deficiency in the amount of antioxidants exists in the asthmatic airway [19, 20]. A plant source of vitamin E has been found to reduce key mitochondrial dysfunctions, alleviate asthmatic features and serve as a marker of clinical status in asthma [5, 21]. In this study, using a mouse model of allergic asthma, we demonstrated the potential function of γ T, one of the vitamin E isoforms, in preventing airway inflammation with a distinct decline of eosinophilic granulocyte, followed by obvious remission in aspects of morphology.

In addition, eotaxin is the most specific and strongest factor which can affect the function of eosinophil [22]. The finding of an eotaxin decrease compared to the asthma subgroup in blood serum and BALF further showed the efficiency of γ T in asthma relief. Furthermore, there were comparable or even better effects of γ T than dexamethasone, which is a good drug for asthma treatment but with some of side effects [23–25].

Eotaxin could regulate eosinophil trafficking into the airways along with other chemotactic factors, such as IL-4 which is a pleiotropic cytokine that elicits a wide spectrum of physiological and pathogenic events including cell proliferation, differentiation, apoptosis and inflammation [26–28]. The enhanced level of IL-4 in the asthma subgroup was attenuated with the addition of γ T, with almost the same function as dexamethasone. It is most likely that a weakening of the level of IL-4 and eotaxin could reverse the unbalanced

Table 2. The detection of eotaxin in serum and BALF (mean \pm SD)

Group	N	Serum (ng · L ⁻¹)	BALF (ng · L ⁻¹)
Control	10	208.67 \pm 19.97	427.13 \pm 49.58
Asthma	10	297.30 \pm 41.34	573.78 \pm 52.82
Dexamethasone-treated	10	289.73 \pm 28.79	492.13 \pm 40.36
γ T-treated	10	255.82 \pm 25.84	445.48 \pm 37.18

Table 3. The IL-4 level in serum and BALF (mean \pm SD)

Group	N	Serum (pg · mL ⁻¹)	BALF (pg · mL ⁻¹)
Control	10	9.98 \pm 2.36	14.44 \pm 1.64
Asthma	10	34.22 \pm 2.40	34.08 \pm 2.62
Dexamethasone-treated	10	21.00 \pm 2.03	22.60 \pm 1.75
γ T-treated	10	19.10 \pm 1.55	24.12 \pm 2.39

condition of immune response and contribute to the prevention or treatment of asthma. However, this hypothesis still needs to be verified in the near future.

In conclusion, the desirable capability of γ T in reducing eotaxin and IL-4 in asthma mice serum or BALF has been discovered. A strategy in asthma therapy might be found utilizing this functional vitamin.

Acknowledgements. We thank Shaanxi College of Traditional Chinese Medicine for assisting us with the related laboratory equipment and venues.

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Conflict of interest: None declared

Received: 14.11.2014

Revised: 24.03.2015

Accepted: 1.04.2015