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Original Research Article

Effects of caffeoylxanthiazonoside on airway inflammation in an allergic asthma mice model

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Abstract

Purpose: *To investigate the inhibitory effects of caffeoylxanthiazonoside (CYT) on airway inflammation in mice and its mechanism of action.*
Methods: *An allergic asthma mice model was established by intraperitoneal injection and aerosol nebulization with ovalbumin (OVA). After treatment with CYT, the blood and bronchoalveolar lavage fluid (BALF) were collected from the mice. The leukocytes were classified and counted with Giemsa solution. Enzyme-linked immunosorbent assay (ELISA) was used to determine the serum levels of IgE, and IL-4, IL-5, IL-13 and IFN-γ in the BALF of mice. Lung tissues were obtained from the mice and MUC5AC protein expression was measured by western blot.*
Results: *CYT significantly decreased the serum level of IgE in asthmatic mice. Inflammatory cells in BALF of mice were markedly reduced ($p < 0.05$) by CYT treatment at varying doses (10, 20, and 40 mg/kg). Treatment with CYT also significantly suppressed the cytokines of IL-4, IL-5 and IL-13 and increased the IFN-γ in the BLAF of OVA-induced allergic asthma mice ($p < 0.05$). Western blot results indicate that CYT treatment significantly decreased the expression of MUC5AC protein in the lung tissues of asthmatic mice. In addition, no significant effects on the body weight of the mice were found after CYT treatment.*
Conclusion: *Caffeoylxanthiazonoside inhibits airway inflammation in allergic asthma mice by altering Th1/Th2 via re-balancing of related cytokines and downregulation of lung MUC5AC protein expression. Therefore, this compound can potentially be developed for the therapeutic management of inflammation in allergic asthma.*

Keywords: *Caffeoylxanthiazonoside, Ovalbumin, Airway inflammation, Allergic asthma*

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INTRODUCTION

Asthma is a chronic airway inflammatory disease with the characterizations of reversible airflow obstruction, goblet cell and smooth muscle hyperplasia, increased mucus production, hyperactivity and ultrastructural remodeling of the airways [1,2]. The prevalence of asthma in the developed countries is approximately 10 % of the population [3]. Inhaled glucocorticoids and β_2-agonists are common pharmacological treatments for asthma [4]. However, the effectiveness of these agents is not satisfactory for numerous patients [5]. Therefore, it is imperative to discover new agents which are

well-tolerated and effectively mitigate the symptoms of asthma.

Xanthium strumarium L. (XSL), belonging to the Asteraceae family, is a widely used medicinal plant in China [6]. Its fruits, called “Cangerzi” in Chinese, have been used for treating inflammatory diseases including allergic rhinitis, tympanitis, urticaria, arthritis and ozena [7]. Various compounds have been found in the the fruits of XSL, includings flavonoids, saponins, caffeyolquinic acids, caffeic acids, and sesquiterpene lactones [8]. Caffeoylxanthia-zonoside (CYT, Figure 1) is an active thiazinedione heterocyclic compound isolated from the fruits of XSL, and investigations have shown that CYT possesses favorable therapeutic effects on allergic rhinitis, sepsis, and chronic heart failure [9,10]. The aim of this study was ti determine the effects of CYT on airway inflammation in an allergic asthma mice model.

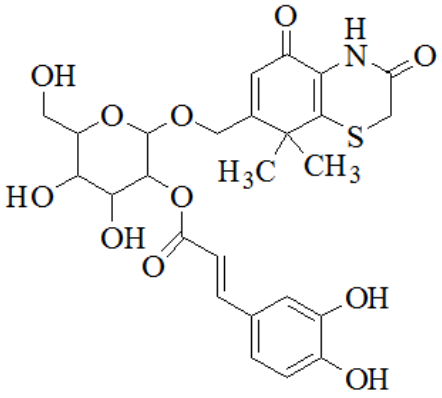

Figure 1: Structure of CYT

EXPERIMENTAL

Chemicals and reagents

Caffeoylxanthiazonoside (purity ≥ 98 %) was purchased from Shanghai PureOne Biotechnology (Shanghai, China). Ovalbumin (OVA, ≥ 90 %) was obtained from Sigma Aldrich (St Louis, MO, USA). Interleukin (IL-4), IL-5, IL-13, interferon (INF)-γ and Immunoglobulin E (IgE) ELISA kits were obtained from R & D Systems (Minneapolis, MO,USA). Dexamethasone was purchased from Cisen Pharmaceutical Co, Ltd (Jinan, China). The primary antibody against musin 5AC (MUC5AC) and secondary antibodies were obtained from Abcam (Cambridge, MA).

Animals

Female BALB/c mice (Six-week-old, 19 ± 1 g) were purchased from SLAC Laboratory Animal Co. Ltd. (Shanghai, China). Animals were housed in a condition-controlled room with 12:12-h light-dark cycle (21 ± 2°C) and were free access to food and water.. The animal experiments in the present study were in accordance with the Guide for the Care and Use of Laboratory Animals [11] and were approved by the institutional Ethics Committee on Animal Use (no. 2017KW124) in The Second Hospital of Shandong University (Ji’nan, China).

Model and groups

The allergic asthma model was induced by OVA using a previously described method with some modifications [12]. After one week’s acclimatization, mice were randomly divided into 6 groups (n = 12): Normal control group; negative control group; DEX group (5 mg/kg); and varying doses of CYT groups (10, 20, and 40 mg/kg). The mice were sensitized by intraperitoneal injection (i.p.) with OVA (20 μg) on day 7 and 14. At the day 15 to 21, mice were challenged with aerosol nebulization with 1 % OVA (dissolved in PBS) for 30 min each day. Meanwhile, mice in CYT groups were administered with CYT (i.p.) once a day during this period. Normal control group received normal saline at each step. At day 22, blood were collected by extirpating eyeball and the serum were prepared by centrifugation (3500 rpm, 10 min) at 4 °C.

Preparation of bronchoalveolar lavage fluid (BALF)

The left lungs of the mice were lavaged with 0.5 mL normal saline to obtain the BALF. After centrifugation (1000 rpm) for 10 min at 4 °C, the cell culture supernatants were collected for the determination of inflammatory cytokines. The precipitated cells were stained with Giemsa solution, and the number of total cells, neutrophils, eosinophils (EOS) and lymphocytes in BALF were classified and counted based on the morphology and staining profile.

Enzyme-linked immunosorbent assay (ELISA)

The serum levels of IgE, and inflammatory cytokines including IL-4, IL-5, IL-13 and IFN-γ in the BALF of mice were measured by commercial ELISA kits according to the manufacturer’s instructions.

Western blot analysis

Lung tissues of mice were surgically collected and the total proteins were extracted. The total protein (50 μg) was separated by 8 % sodium

dodecyl sulfate - polyacrylamide gel electropheresis (SDS-PAGE), and then transferred onto a polyvinylidene fluoride (PVDF) membrane. After blocking with 5 % skimmed milk for 1 h, and the PVDF was incubated with different primary antibodies overnight at 4 °C, respectively. Subsequently, the membrane was incubated with horseradish peroxidase labelled secondary antibodies for 1 h at room temperature. The protein bands were visualized by using Electro-Chemi-Luminescence (ECL) reagents.

Statistical analysis

SPSS 17.0 software (IBM Corp., Armonk, NY) was used to analyze the data. The data are expressed as mean ± standard deviation. Statistical signifcance between different groups was compared using student's *t*-test or variance analysis. $P < 0.05$ was regarded as statistically signifcant.

RESULTS

Concentration of IgE in mouse serum

As shown in Figure 2, the IgE level in the serum of negative control group was significantly increased ($p < 0.01$), when compared with those of normal control group. After the treatment of DEX, the serum level of IgE in the mice significantly decreased compared with negative control group. More importantly, the serum IgE level of the CYT-treated groups was also decreased ($p < 0.01$) compared with negative control group at doses of 10, 20 and 40 mg/kg.

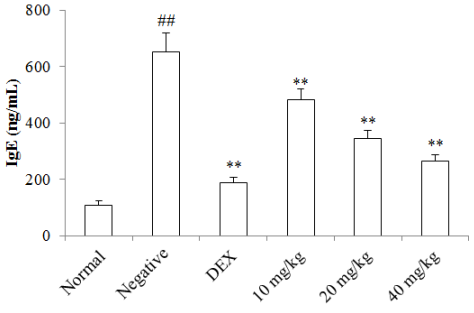

Figure 2: Serum level of IgE in mice; ##$p < 0.01$, compared with the normal control group; **$p < 0.01$, compared with negative control group

Inflammatory cells in BALF

The number of total cells, neutrophils, EOS and lymphocytes were counted to evaluate the effects of CYT on the influx of inflammatory cells. The number of inflammatory cells in the BALF of mice was markedly increased after the model was established (Figure 3). DEX treatment significantly reduced the number of inflammatory cells in the BALF ($p < 0.01$). Interestingly, CYT at different doses also had significant inhibitory effect on inflammatory cells in mice BALF (Figure 3).

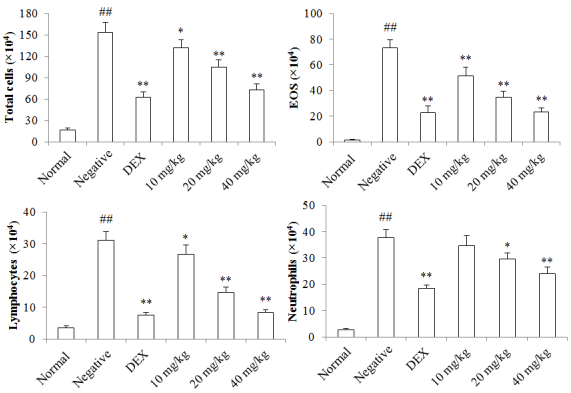

Figure 3: Effect of CYT on inflammatory cell number in BALF; ##$p < 0.01$, compared with the normal control group; *$p < 0.05$ and **$p < 0.01$, compared with the negative control group

Inflammatory cytokines production in BALF

Compared with the normal control group, IL-4, IL-5 and IL-13 levels of BALF increased, whereas IFN-γ level decreased in negative control group (Figure 4). Treatment with DEX and different doses of CYT (10, 20 and 40 mg/kg) significantly suppressed cytokines (IL-4, IL-5 and IL-13) compared with negative control group ($p < 0.01$). Furthermore, IFN-γ level in BALF of DEX- and CYT-treated groups (20 and 40 mg/kg) was higher than that of negative control group.

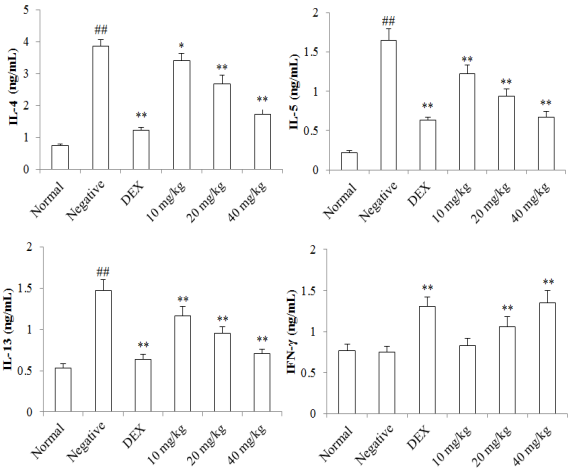

Figure 4: Levels of cytokines (IL-4, IL-5, IL-13 and IFN-γ) in BALF; ##$p < 0.01$, compared with normal control group; *$p < 0.05$ and **$p < 0.01$, compared with negative control group

Effect of CYT on expression of MUC5AC protein

To further explore the mechanism of action of CYT in allergic asthma, the expression of MUC5AC protein was determined by western blot. As shown in Figure 5, the MUC5AC protein was markedly upregulated in negative control group when compared with normal control group. Both DEX and different doses of CYT significantly downregulated the protein expression of MUC5AC in lung tissues of mice, compared with negative control group ($p < 0.01$).

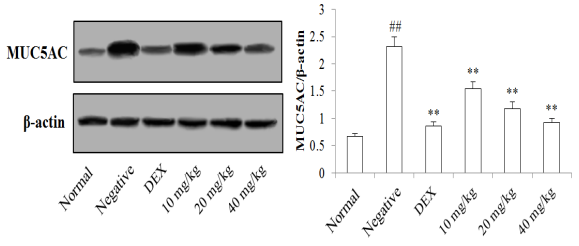

Figure 5: MUC5AC protein expression in lung tissues of mice; ##$p < 0.01$, compared with the normal control group; **$p < 0.01$, compared with the negative control group

Effect of CYT on body weight of mice

The body weight of mice was used to evaluate the toxicity of CYT. As can be seen from Figure 6, the body weight of DEX group was significantly lower than that of normal control group. However, there was no significant difference in body weight for CYT-treated and normal control groups. These results indicate that CYT did not exert significant toxicity in mice.

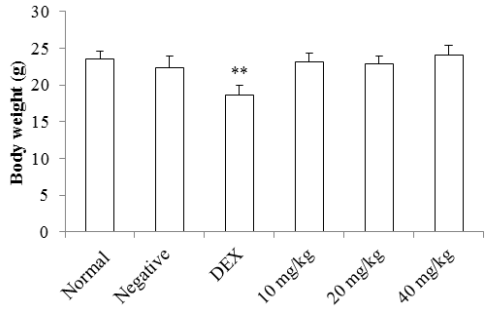

Figure 6: Effect of CYT on mouse body weight; **$p < 0.01$, compared with normal control group

DISCUSSION

In the present study, the protective effects of CYT on airway inflammation were investigated in an OVA-induced allergic asthma mice model. The results indicate that CYT significantly suppressed the airway inflammation in allergic asthma. Specifically, CYT significantly downregulated the expression of MUC5AC protein in mouse lung tissues, but had no significant effect on the body weight of the mice.

Asthma is a Th2 cell-associated lung inflammatory disease which is characterized by eosinophil recruitment, IgE increase, inflammatory cytokines release, mucus hypersecretion and bronchoconstriction [13]. The recruitment of eosinophils into the airways is a characteristic of asthma, and transmigration of leukocytes into the airways can be regulated by cytokines such as IL-4, IL-5 and IL-13 [14]. The Th1 (IFN-γ) and Th2 (IL-4, IL-5 and IL-13) cytokines are reported to be closely associated with the pathogenesis of asthma [15]. The alteration of Th1 and Th2 cytokines induces secretion of allergen-specific IgE, overproduction of airway eosinophilia and mucus secretion [16]. In the present study, CYT treatment reduced the leukocytes number (especially eosinophils) in the BALF, suppressed the IgE production in the serum, decreased the Th1 cytokines (IL-4, IL-5 and IL-13) expression and increased the Th2 cytokine (IFN-γ) expression. The results indicate that CYT may inhibit allergic inflammation in the OVA-induced allergic asthma mice model by altering Th1/Th2 via re-balancing the related cytokines.

It has been reported that mucus contains a lot macromolecules, and mucins are the major components of mucus. As an important gel-forming mucin, MUC5AC is up-regulated when airway inflammation occurs [17]. In asthmatic patients, up-regulation of MUC5AC production contributes to mucous plugs and airflow obstruction [18]. In the present study, CYT inhibited allergic inflammation and this was linked to the downregulation of MUC5AC protein expression.

CONCLUSION

Caffeoylxanthiazonoside significantly inhibits airway inflammation by altering Th1/Th2 via re-balancing of related cytokines and downregulation of lung MUC5AC protein expression in allergic asthma mice. Therefore, this compound may be developed into a suitable drug for inflammation in allergic asthma.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. Wei Wang conceived and designed the study; Hui Wang and Xiaowen Che collected and analyzed the data, Qian Wu wrote the manuscript. All authors have read and approved the manuscript for publication in this journal.

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