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Effects of fastigial nucleus electrostimulation on airway inflammation and remodeling in an experimental rat model of asthma

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Abstract

Background: Asthma is a chronic disease involving an immune response, which is characterized by non-specific inflammation and airway remodeling. Glucocorticoids are clinically beneficial in controlling asthma, but further options are needed. In our study, fastigial nucleus electrostimulation (FNS) was applied in a rat asthma model for the first time to investigate the effects of pre-intervention.

Objective: To observe the effects of FNS on airway inflammation and remodeling in asthmatic rats.

Methods: Forty rats were assigned randomly to the normal control (CON), model (MDL), FNS, or budesonide (BUD) groups. Asthma was induced with chicken egg (OVA). The animals in the CON and MDL groups were treated with normal saline. The animals in the other two groups received FNS or budesonide, respectively.

Results: The results indicated that IgE in the serum and airway fiber areas were higher in the MDL group than in other groups. After treatment for 3 weeks, collagen fibers in the bronchial wall in the FNS group were significantly lower compared with the MDL group.

Conclusion: FNS significantly reduced IL-4, IL-13, TNF-α, OVA-IgE and TGF-β1 in serum and BALF, and increased IFN-γ. Our results suggest that FNS may ameliorate asthma symptoms and induce changes of cytokines in the serum and lung milieu.

Keywords: asthma, rat model, fastigial nucleus electrostimulation, cytokine, airway remodeling

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List of abbreviations:
BUD, budesonide;
CON, control;
FNS, fastigial nucleus electrostimulation;
MDL, model;
SD, Sprague Dawley;
SJTU, Shanghai Jiaotong University;
TGF, Transforming growth factor.

Introduction

Asthma is a chronic airway inflammatory disease which is characterized by airway inflammation, airway remodeling, reversible airway obstruction, and airway hyper-responsiveness. About 300 million patients are currently suffering from asthma worldwide, and the morbidity and mortality of asthma has shown an increasing trend in recent years, which seriously threatens people's health and quality of life.[1,2] Currently systemic or topical corticosteroids remain the preferred treatments for asthma, but the long-term use of such therapies is often associated with side effects. Therefore, a new strategy is urgently needed for the treatment of asthma.[3]

The pathogenesis of asthma is still unclear due to its complex disease process. Recent studies have shown that asthma is an allergic immune response related to Th1/Th2 lymphocyte imbalance, which results in a significant increase or decrease of cytokines in the blood.[4] Asthmatic lungs contain both Th1 cytokines (IFN-γ) and Th2 cytokines (IL-4 and IL-13).[5]

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Studies using animal models have provided abundant evidence that Th2 cytokines (mainly IL-4 and IL-13), mediating many important features of allergic asthma, are higher in the bronchoalveolar lavage fluid (BALF) of asthmatic mice.[6,7] Some scholars believe that Th1 cells could restrain the asthmatic airway inflammation by inhibiting the responses of Th2 cytokines.[8] The IFN-γ level is upregulated as Th1 response plays a protective role in asthma.[9] Tumor necrosis factor (TNF)-α is a proinflammatory cytokine which might play an important role in severe refractory disease. Preliminary studies have demonstrated that asthmatic patients treated with anti-TNF-α have an improved quality of life, lung function, and airway hyper-responsiveness as well as a reduction in exacerbation frequency.[10-12] Several therapies targeting the IL-13/IL-4/signal transduction pathway have shown promise for asthma. Anti-IL-13 mAbs and IL-4 receptor antagonists are used to reduce the risk of asthma by decreasing the levels of IL-4 and IL-13.[13] Transforming growth factor (TGF)-β1 secreted by airway epithelium and eosinophils is thought to play an important role in airway remodeling in asthmatic subjects. TGF-β1 in BALF is strongly associated with severe airflow limitation and the lack of asthma control.[14-16] The severity of asthma can be reflected by the levels of the cytokines mentioned above.

Fastigial nucleus electrostimulation (FNS) has been widely used as a physical method to stimulate neural pathways in the rehabilitation treatment of stroke and migraine.[17] Literature suggests that FNS can significantly improve the kinetic parameters of cerebral circulation, increase cerebral blood flow, and mitigate cerebral edema, and probably affect many parts of the body, playing a protective role in patients with cardiovascular and cerebrovascular diseases. FNS may increase IkappaB-α expression by overproducing IkappaB-α mRNA and rendering brain microvessels refractory to IL-1β.[18] The mechanism for its therapeutic effect involves stimulation of the fastigial nucleus, which increases blood supply to the brain and collateral circulation, reduces cerebrovascular inflammatory response after ischemia, promotes astrocyte proliferation, and improves axonal growth following focal cerebral ischemia. Alterations of microvascular inflammation may contribute to the central neurogenic neuroprotection elicited by FNS.[19]

In clinical practice, we found an interesting phenomenon where after FNS treatment, asthma patients can reduce or even stop their drug gradually. We hypothesized that FNS may reduce inflammation and remodeling of the airway caused by allergic response, thereby having a therapeutic effect on asthma. As a result, we designed this study to observe the effects of FNS in an ovalbumin (OVA)-induced rat asthma model by analyzing asthma-related inflammatory factors and histopathological changes.

Methods

Experimental animals

Forty 6-8 week old male Sprague Dawley (SD) rats weighing 180 ±10 g were purchased from Shanghai Hayes Lake Experimental Animal LLC, China (license number: 0032423). All of the rats were fed in a climate-controlled room (temperature: 24°C; relative humidity: 50 ± 5%) on a 12 h/12 h light/dark cycle at the Experimental Animal Center of Shanghai First People's Hospital affiliated to Shanghai Jiaotong University (SJTU). The animals had free access to food and water. Animal handling and experimental procedures were approved by the Animal Investigational Committee of the SJTU and were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the Chinese Health and Family Planning Commission.

Ovalbumin sensitization and challenge

Forty animals were randomly divided into four groups consisting of ten animals each: normal control group (CON), model control group (MDL), fastigial nucleus electrostimulation treated model group (FNS) and budesonide treated model group (BUD). Random numbers were generated using a computer software (spss17.0, SPSS Inc., Illinois, Chicago). The rat model of asthma used in this study was developed by induction using ovalbumin (OVA, Grade V; Sigma, St. Louis, MO) sensitization followed by OVA challenge (based on Labonte´and Kucharewicz et al.[20,21]). On days 0 and 7, rats were sensitized by the subcutaneous injection of either sterile normal saline (NS; control animals) or 1 mg ovalbumin diluted in sterile NS containing 100 mg $Al(OH)_3$ as an adjuvant and with an intraperitoneal injection of a mixture of ovalbumin and $Al(OH)_3$ (Sigma) (1 mg ovalbumin and 100 mg $Al(OH)_3$ diluted in 1 ml sterile NS). All of the rats were injected at the same position: forefoot plantars and groin at both sides, as well as the abdomen, with 0.2 ml injected at each position. The sensitized rats were challenged with aerosolized NS or 1% OVA (Grade II) in sterile NS for 30 min once every two days, beginning on the 14th day of the experiment for 3 weeks, which was accomplished by placing rats in sealed plastic chambers sized 20 cm × 30 cm × 40 cm connected to a nebulizer generating aerosol mist.

Intervention

Rats were treated with different interventions for 30 min before each OVA atomization. The animals in the CON and MDL groups were aerosolized with NS and the rats in the BUD group with 1 mg budesonide (Astra Zeneca, London, UK; 2 ml/ampule: 1 mg). Rats in the FNS group were fixed to fully expose the region of the posterior cerebellar vermis and received FNS treatment at a current intensity of 50 μA (frequency: 50 Hz) using square wave pulse rectangular continuous stimulation for 30 min. The area of the fastigial nucleus is located 11.4–11.8 mm posterior, 0.8–1.0 mm lateral, and 5.7 mm high to the bregma, and 11.4–11.8 mm deep to the pial surface.

Specimen collection

Twenty-four hours after the last OVA challenge, the rats were anesthetized by IP injection with 1 ml/kg urethane and blood was collected from the heart. Briefly, citrate anticoagulated blood samples were immediately centrifuged at 3000 rpm for 10 min at 4°C. Serum samples were aliquoted and frozen at -80°C to prepare for future assays. The right lung was ligated and a tracheal catheter was inserted into the trachea. The left lung was immediately lavaged with NS by gently instilling NS solution into the left lung via the tracheal catheter.

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This process was repeated twice with a total of 6 mL NS. BALF samples were centrifuged, and the supernatants were stored at -80°C until further assay of cytokines. Then, the right lung was fixed with 4% paraformaldehyde in phosphate buffered saline (PBS) for the Masson staining analysis.

Measurement of cytokines and OVA-specific IgE

The levels of the four inflammatory cytokines, including IL-4, IL-13, TNF-α, and IFN-γ, were measured in the BALF and serum samples of all the rats. This was conducted using Bio-plex Pro™ magnetic bead-based assays (Bio-Rad Laboratories, Hercules, CA) on the Bio-plex® platform (Bio-Rad), according to the manufacturer's instructions. Following previous optimization, undiluted samples were evaluated in a blinded manner. Bio-Plex Manager™ software, version 6.0, was used for bead acquisition and analysis.

TGF-β1 and overall OVA-IgE levels in serum and BALF were measured using commercially available ELISA kits (R&D systems, Minneapolis, MN) according to the test protocols. Absorbance was measured at 450 nm using a multifunctional microplate reader (Thermo Scientific Varioskan Flash, Waltham, MA).

Histological evaluation

The right lung tissue was fixed with 4% paraformaldehyde in PBS, embedded in paraffin. The fixed tissue was sectioned at 5-μm thicknesses, placed on glass slides, and deparaffinized. Sections were stained with Masson staining kit (Ruisheng Biotechnology Company, Pudong New District, Shanghai). Histological evaluation was performed by an experienced histologist who was blinded to treatment information. A section of each rat was photographed at 400 magnification microscope for measurements after Masson staining. Three different bronchioles were selected with diameters of ~100 μm, and the area ratio of the collagen fibers was measured using Image-Pro Plus 6.0 software (Media Cybernetics, Bethesda, MD).

Statistical analysis

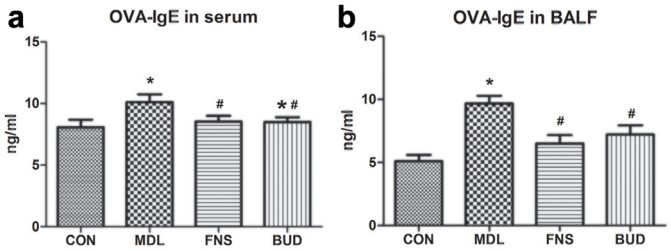
SPSS17.0 statistical software was used for statistical analysis. Data were presented as mean ± SEM (standard error of mean). One-way analysis of variance (ANOVA) was used for evaluation of the data and followed by Tukey-Kramer tests when ANOVA was statistically significant. Differences were considered significant at P <0.05.

Results

Comparison of OVA-specific IgE levels in BALF and serum

We found that when challenged with OVA, the rats with allergic asthma developed mouth breathing, shortness of breath, lip cyanosis, restlessness, nodding, nose scratching, and other symptoms. OVA-specific IgE in serum and BALF in the rat asthma model was significantly higher than in the normal group (n = 8, $P = 0.012$, $P < 0.001$, respectively), which indicated an obvious allergic response in the asthmatic rats. However, after treatment with FNS or BUD, the levels of OVA-IgE in serum and BALF were significantly lower than those in the MDL group (n = 8, $P = 0.048$, $P = 0.001$, and n = 8, $P = 0.043$, $P = 0.011$, respectively). When compared with each other, a statistically significant difference was only found in serum between the FNS and BUD groups ($P < 0.05$) (Figure 1).

Figure 1. Effect of fastigial nucleus stimulation (FNS) on OVA-specific IgE in serum and bronchoalveolar lavage fluid (BALF) of OVA-induced rat model of asthma. The BALF and serum samples were harvested during the 24 h after the last OVA challenge. ELISA was used to measure OVA-IgE in BALF (a) and serum (b). CON: rats sensitized and challenged with normal saline. MDL: rats sensitized and challenged with OVA. FNS: OVA-sensitized and challenged rats treated with FNS (30 min/day). BUD: OVA-sensitized and challenged rats treated with BUD (1 mg/day). The data are presented as mean ± SEM, n = 8 rats per treatment group. * P < 0.05 versus CON; # P <0.05 versus MDL.



Comparison of bronchioles collagen fiber area and TGF-β1

The area ratio of bronchiole fiber in the asthma group was significantly higher than that in the normal group (n = 8, $P = 0.008$). The results showed that fibers in the wall around the bronchioles of asthmatic rats increased, which is an important reason for airway remodeling. After treatment with FNS or BUD, the area ratio of bronchiole fibers reduced, but the difference was not statistically significant (n = 8, $P > 0.05$, $P > 0.05$, respectively). Upon further testing of TGF-β1 levels in serum and BALF, we found that the levels of TGF-β1 in the MDL group were significantly higher than those in the CON group (n = 8, $P = 0.006$, $P = 0.018$, respectively). After treatment with FNS or BUD, the levels of TGF-β1 in serum and BALF were lower than those in the MDL group (n = 8, $P = 0.032$, $P = 0.001$, and n = 8, $P = 0.046$, $P = 0.030$, respectively). However, upon comparison with each other, there was no statistically significant difference between the FNS and BUD groups (n = 8, $P > 0.05$) (Figure 2).

Comparison of cytokine levels in BALF and serum

Allergic asthmatic inflammation is known to be caused by secretion of a series of Th1 cytokines (IFN-γ) and Th2 cytokines (TNF-α, IL-4, and IL-13).[22,23] To assess the effect of FNS on airway inflammation in asthmatic rats, the levels of these cytokines in sera as well as BALF were measured. We observed significant increases in the levels of the inflammatory cytokines IL-4, IL-13, and TNF-α in the serum samples of the MDL group compared with the CON group (n = 8, $P = 0.010$, $P = 0.034$, $P = 0.013$, respectively). Significantly lower levels of IL-4, IL-13, and TNF-α in the serum samples were observed in the FNS- or BUD-treated rats compared with the MDL group (n = 8, $P = 0.011$, $P = 0.031$, $P = 0.040$, and n = 8, $P = 0.008$, $P = 0.038$, $P = 0.026$, respectively). A significant decrease was observed in the levels of the protective cytokine IFN-γ in the serum samples in the MDL group compared with the CON group (n = 8, $P < 0.001$). IFN-γ in the FNS-treated rats was significantly higher compared with the MDL group (n = 8, $P = 0.005$). IFN-γ in

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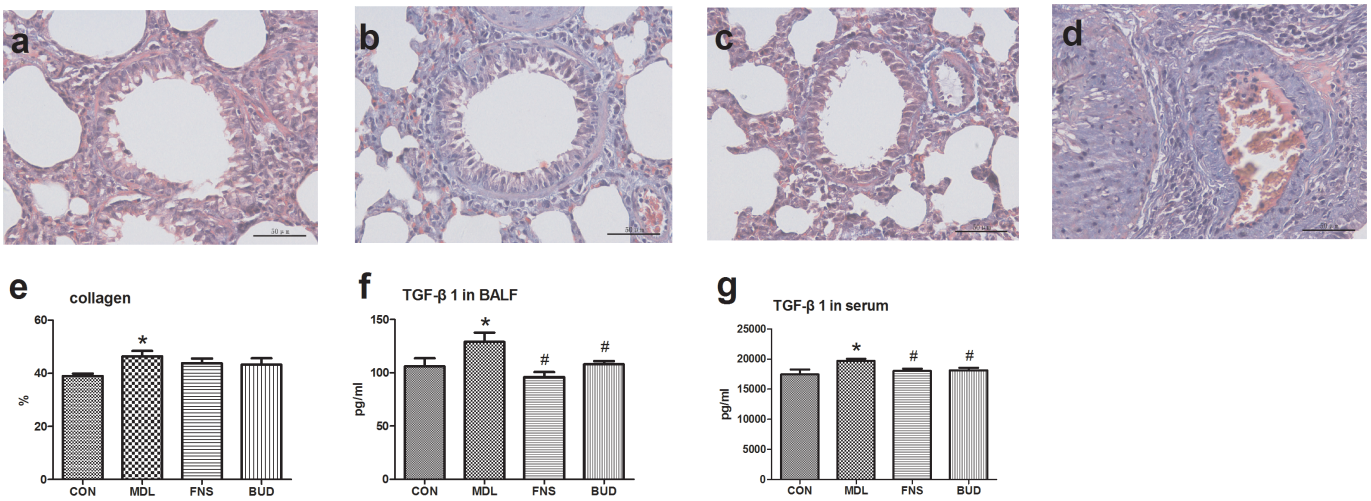
Figure 2. Effect of fastigial nucleus stimulation (FNS) on airway remodeling in OVA-induced rat model of asthma. Lung tissues and serum as well as BALF were obtained on the day after the last OVA challenge. Tissues were stained with Masson (400x) (a-d). The area ratio of the collagen fibers was measured with Image-Pro Plus 6.0 software (e). ELISA was used to measure TGF-β1 in BALF (f) and serum (g). CON: rats sensitized and challenged with normal saline. MDL: rats sensitized and challenged with OVA. FNS: OVA-sensitized and challenged rats treated with FNS (30 min/day). BUD: OVA-sensitized and challenged rats treated with BUD (1 mg/day). Data are expressed as mean ± SEM, n = 8 rats per treatment group. *P < 0.05 versus CON; #P <0.05 versus MDL.



the BUD-treated rats was not significantly higher than that in he MDL group (n = 8, $P > 0.050$). However, on comparison with each other, a statistically significant difference was found for IFN-γ between the FNS and BUD groups (n = 8, $P = 0.013$) (Figure 3a).

In BALF, compared with the CON group, there was a significant increase in the levels of inflammatory cytokines IL-4 and IL-13 as well as the levels of TNF-α in the MDL group (n = 8, $P < 0.001$, $P < 0.001$, $P = 0.001$, respectively). However, the levels of IL-4 and IL-13 as well as TNF-α in the FNS-treated rats were significantly reduced compared with the MDL group (n = 8, $P < 0.001$, $P = 0.035$, $P = 0.037$, respectively). The levels of IL-4 and IL-13 as well as TNF-α in the BUD-treated rats were not significantly lower than those in the MDL group (n = 8, $P > 0.050$, $P > 0.050$, $P > 0.050$, respectively). A significant decrease was observed in the levels of protective cytokine IFN-γ in the BALF in the MDL group compared with the CON group (n = 8, $P = 0.004$). IFN-γ in the FNS-treated rats was significantly higher compared with the MDL group (n = 8, $P = 0.021$). IFN-γ in the BUD-treated rats was not significantly higher than that in the MDL group (n = 8, $P > 0.050$). However, in comparison with each other, a statistically significant difference was only found for IL-4 between the FNS and BUD groups (n = 8, $P < 0.001$) (Figure 3b).

Discussion

In the present study, OVA-sensitized asthmatic rats were treated with FNS or BUD to explore the effects of FNS on asthma. The main outcome of the study is that FNS elicits an anti-inflammatory response in OVA-sensitized asthmatic

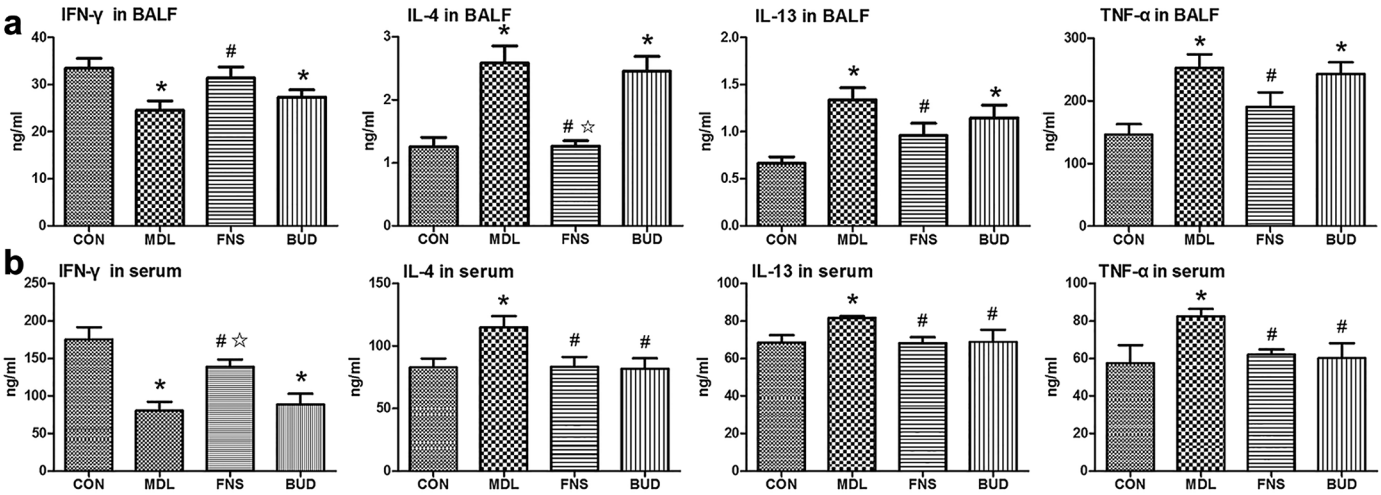


Figure 3. Effect of Fastigial nucleus stimulation (FNS) on IFN-γ, TNF-α, IL-4, and IL-13 in bronchoalveolar lavage fluid (BALF) and serum of OVA-induced rat model of asthma. The BALF and serum samples were harvested in 24 h after the last OVA challenge. The Bio-plex Pro™ magnetic bead-based assays was used to measure IFN-γ, IL-4, IL-13, and TNF-α in BALF (a) and serum (b). CON: rats sensitized and challenged with normal saline. MDL: rats sensitized and challenged with OVA. FNS: OVA-sensitized and challenged rats treated with FNS (30 min/day). BUD: OVA-sensitized and challenged rats treated with BUD (1 mg/day). Data are expressed as mean ± SEM, n = 8 rats per treatment group. *P < 0.05 versus CON; #P < 0.05 versus MDL;☆P < 0.05 versus Budesonide.

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rats and inhibits airway remodeling by reducing the formation of collagen fibers. This is the first study to focus on the effect of FNS in an asthmatic rat model. The data presented in this study indicate that the positive effect of FNS is accompanied by an increased release of Th1 cytokine (IFN-γ) and a decrease in Th2 cytokines (IL-4 and IL-13), as well as proinflammatory cytokines (TNF-α and TGF-β1) and IgE in the serum and BALF (particularly in the BALF).

It has been shown that the main mechanism of asthma is a Th1/Th2 imbalance and increased IgE levels. When exposed to inhaled antigens, Th2 cells are predominantly induced. Then, Th2 cells might recruit eosinophils and induce IgE^+ B cells through the production of IL-4 and IL-13.[12,24] Cytokines like IL-4 generally stimulate IgE^+ B cells to produce IgE, which binds to its high-affinity and low-affinity receptors on the cells of the airway mucosa. High-affinity receptor-bound IgE will be ligated by allergens on mast cells and basophils, which leads to degranulation and synthesis of inflammatory mediators and cytokines. It is a crucial characteristic of asthma pathology and may cause the symptoms of immediate hypersensitivity, such as plasma extravasation, smooth muscle contraction, and itching.[25-27] Th1 cells are thought to inhibit asthmatic airway inflammation. Numerous studies have demonstrated that Th1 cytokines, especially IFN-γ, may inhibit antigen-induced airway hyper-responsiveness in asthma.[28] TNF-α is known as a pro-inflammatory cytokine, the level of which in the serum and BALF of patients is examined to elevate the severity of asthma. It is thought to play a key role in the progression of airway inflammation in asthma.[29] TGF-β1 is a multifunctional cytokine produced by airway epithelial cells, eosinophils, macrophages, and Th2 lymphocytes. It contributes to the pathophysiology of asthma, such as subepithelial fibrosis and smooth muscle cell proliferation in airways. TNF-α can promote the production of TGF-β-induced eosinophilia.[30] In OVA-sensitized rats and mice, the number of eosinophils and the levels of TNF-α, IL-4, IL-5, IL-13, and TGF-β1 increased in the BALF[12,31,32] In this study, when challenged with nebulized OVA, the level of Th1 cytokine (IFN-γ) in the serum and BALF of the asthmatic rats increased and Th2 cytokines (IL-4 and IL-13) and proinflammatory cytokines (TNF-α and TGF-β1), as well as IgE in the serum and BALF decreased. Bronchiole collagen fiber areas in the asthmatic rats also increased, indicating that there was airway remodeling. This is similar to the results reported in previous studies.

Several studies have reported that atomized budesonide and electro-acupuncture (EA) can inhibit increase of eosinophils, TNF-α, IL-4, IL-5, and IL-13 in the BALF and promote the increase of IL-1 and IFN-γ in OVA-sensitized rats or mice.[5,33,34] Symptom relief and gradual dose reduction were found in volunteers with asthma after FNS treatment, the therapeutic effect of which was similar to EA and budesonide. However, the mechanism of FNS in relieving asthma is unclear. In this study, we found that the levels of IL-4, IL-13, TNF-α, and TGF-β1, as well as IgE in the serum and BALF decreased and alleviated in the FNS- or BUD-treated rats compared with the MDL rats. The levels of IFN-γ in the serum and BALF increased in the FNS- or BUD-treated rats compared with the MDL group. Bronchiole collagen fiber areas in rats of group FNS or BUD decreased compared with group MDL, but the difference was not statistically significant. This may be partly due to the short treatment time and small sample size. However, between-group comparison showed no significant difference between the FNS and BUD groups. All of these factors indicate that FNS could reduce increased bronchiole collagen fiber area and OVA-induced IgE in asthmatic rats. This phenomenon may be caused by its effects of increasing the inflammatory cytokines, IFN-γ and decreasing TNF-α, IL-4, IL-5, IL-13, and TGF-β1. The effect was similar to nebulized BUD. One possible explanation of these findings is that FNS may affect the hypothalamic-pituitary-adrenal axis and mediate the production of ACTH and cortisol in the serum, thus promoting the amelioration of asthma.

Conclusion

The present study has shown that FNS has the similar effect as BUD in alleviating airway inflammation and decreasing airway remodeling on the OVA-sensitized asthmatic rats. The mechanism by which FNS increases the inflammatory cytokines, IFN-γ and decreases TNF-α, IL-4, IL-5, IL-13, and TGF-β1 remains unclear and needs to be further explored. Our data demonstrate that FNS is a promising therapeutic strategy for the treatment of asthma and may be helpful in preventing the progression of asthma. Further studies are required to elucidate the mechanism underlying its therapeutic effect and benefits.

Conflict of interests

The authors declare that they have no conflict of interest.

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