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Inhaled budesonide protects against chronic asthma-induced neuroinflammation in mouse brain

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ABSTRACT

Chronic asthma is one of the most common respiratory diseases, characterized by airway inflammation. However, little is known whether asthma-induced airway inflammation might influence the brain. We found that chronic asthma not only resulted in peripheral inflammation, but also induced neuroinflammation which was characterized by microglial activations and increased levels of TNFα and IL-1β in the hippocampus and prefrontal cortex. Simultaneously, we found that there was significant neuronal loss in the asthmatic mouse brain. Inhaled budesonide, the classic therapeutic drug for chronic asthma, could inhibit asthma-induced microglial activation, down-regulate TNFα and IL-1β but up-regulate TGFβ and IL-10 of mouse brain, and thereby attenuate neuronal loss. Further study showed that chronic asthma increased the expressions of TLR4 and p65/NFκB in the brain, which could be reversed by budesonide treatment. Therefore, the present study reveals that inhaled budesonide protects against asthma-induced neuroinflammation in mouse brain, which might be contributed to attenuate neuronal loss.

1. Introduction

Asthma is one of the most common chronic diseases in the world, affecting over 300 million people(O'Laughlen and Rance, 2012). It is characterized by bronchoconstriction and airway hyper-responsiveness, followed by inflammatory manifestations in the respiratory system (Wanner and Mendes, 2010; Schuijs et al., 2013). Inflammation of the airway wall is a central characteristic of asthma. The release of mediators from the inflammatory cells including eosinophils, lymphocytes, mast cells and macrophages, has been proposed to contribute directly or indirectly to changes in airway structure and function (Vig et al., 2006; Krishnamoorthy et al., 2012; Mikalsen et al., 2014). Therefore, anti-inflammatory medications are the most important treatment options for people with asthma. Anti-inflammatory therapy prevents asthma attacks and works by reducing swelling and mucus production in the airways (Levy et al., 2012). Inhaled corticosteroids are the most effective medications to reduce airway inflammation and mucus production (Braga et al., 2005; Walsh, 2006; Anderson-James et al., 2013). Glucocorticosteroids prevent airway inflammation via inhibiting expressions of pro-inflammatory cytokines and chemokines (Hahn, 2012; Shahid, 2013).

Notably, in individuals with asthma, potential changes in the brain could occur as a consequence of hypoxia or inflammation that resulted from asthma (Rosenkranz and Davidson, 2009). It was found that 13 of 21 people with asthma had brain magnetic resonance imaging abnormalities (Parker et al., 2011). The high rate of incidence suggests that brain abnormalities are relatively common in patients with asthma. Moreover, increasing evidence has revealed that stress and mood disorders adversely affect people with inflammatory diseases such as asthma (Rosenkranz and Davidson, 2009; Busse, 2012). Another study led by researchers from the University of Wisconsin found that certain brain areas could cause worsening of asthma symptoms when subjected to stress (Busse, 2012). So, the brain–body link between asthma and anxiety is being studied (Rosenkranz et al., 2005; Galluccio et al., 2008). But until now, little is known exactly on what or how asthma-induced airway inflammation might influence the brain. Therefore, the present study was to establish a mouse model of chronic asthma and to investigate the impacts of asthma in the brain.

2. Experimental procedures

2.1. Animals

Two-month-old female BALB/c mice, weighing 18 g to 22 g, were obtained from Experimental Animal Center of Jiangsu. All animal experiments were carried out in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice were randomly assigned to three groups: control groups with saline treatment (n = 12); asthma groups with saline treatment (n = 14); and

Abbreviations: (BALF), bronchial alveolar lavage fluid; (PFC), prefrontal cortex; (TLR4), Toll-like receptor 4; (HRP), horseradish peroxidase; (TNFα), tumor necrosis factor α; (IL-1β), interleukin-1 beta; (TGFβ), transforming growth factor beta; (IL-10), interleukin-10.

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asthma mice treated with budesonide (n = 14). Mice were treated as described in detail below. All animals were treated according to protocols approved by Nanjing Medical University.

2.2. Sensitization and inhalational exposure

Allergic mouse models of asthma are generated as we previously reported (Guo et al., 2013). In general, the total schedule time is 12 weeks. After sensitized via 2 intraperitoneal injections of ovalbumin (10 μg) on days 0 and 14 of the experiment, starting on day 21, the mice were exposed to 1% aerosolized ovalbumin for 30 min a day, 3 days a week, for 9 weeks. Importantly, mice were exposed to 10% aerosolized ovalbumin for 30 min at the 9th, 18th and 24th times in order to induce aggravation.

2.3. Budesonide treatment

On day 21 after being sensitized, mice were treated with budesonide 1 h before OVA challenge or aggravation by intranasal administration (25 μl each time, 350 μg/kg), 3 days a week, for 9 weeks. Mice subjected to allergen challenge without budesonide treatment were treated with saline.

2.4. Preparation of bronchial alveolar lavage fluid (BALF)

The mice were anesthetized and placed in a supine position with the head tilted back, and then the trachea was cannulated. The lungs were lavaged three times with 0.3 mL of sterile PBS. The BALF was immediately centrifuged (5 min, 4 °C, 3000 rpm) to separate the cells from the supernatant. The noncellular supernatants of BALF were stored and analyzed with ELISA kit.

2.5. Western blotting

The prefrontal cortex (PFC) and hippocampus tissues of mice were homogenized and solubilized in lysis buffer (Bio-Rad, Hercules, CA, USA). Proteins were separated on Tris–HCl polyacrylamide gels (Bio-Rad) and transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA). After blocking, the blots were incubated with anti-Toll-like receptor 4 (TLR4) (1:800, CST), anti-p65 (1:400, Santa Cruz) and anti-cd11b(1:1000, Abcam) antibodies in TBST overnight at 4 °C, and then with horseradish peroxidase (HRP) conjugated secondary antibodies (Santa Cruz, CA, USA). Immunoreactive bands were detected by enhanced chemiluminescence plus detection reagent (Pierce, Rockford, IL, USA), and analyzed using an Omega 16ic Chemiluminescence Imaging System (Ultra-Lum, CA, USA).

2.6. Immunocytochemistry

After animals were perfused with 4% paraformaldehyde, brains were dissected out and maintained in 4% paraformaldehyde overnight. Brains were cryopreserved in 30% sucrose in phosphate buffered solution and then stored at −70 °C until used. Parallel series of 30-μm-thick coronal sections were obtained in a freezing microtome. Sections were rinsed in phosphate buffer. Every sixth section was kept for immunohistochemistry. Tissue peroxidase was inactivated by incubating in 3% hydrogen peroxide in PBS for 30 min. After three washes in PBS sections were incubated for 2 h in blocking solution (bovine serum albumin in 0.3% Triton X-100 in PBS), the sections were incubated overnight with mouse monoclonal anti-cd11b (1:400, Abcam) and rabbit polyconal anti-NeuN (1:200, Millipore). Then, the sections were incubated with HRP-conjugated secondary antibody (1:800, Chemicon) for 1 h. Positive cells were visualized by incubation in DAB. Control staining was performed without the primary antibodies. The number of NeuN-positive cells was assessed using Stereo Investigator 7, an unbiased stereological procedure with an optical fractionator (MicroBrightField Inc, Williston, VT). The sampling scheme was designed to have coefficient of error (CE) less than 10% in order to get reliable results. All stereological analyses were performed under the 200× objective of an Olympus microscope.

2.7. Measurements of TNFα, IL-1β, TGFβ and IL-10 levels

The levels of tumor necrosis factor α (TNFα), interleukin-1 beta (IL-1β), transforming growth factor beta (TGFβ) and interleukin-10 (IL-10) were determined by ELISA according to the protocol of the manufacturer (Mouse Immunoassay Kits, R&D, USA). Absolute cytokine concentrations were determined by comparison to a standard curve.

2.8. Statistical analysis

All values were expressed as means ± SEM. The significance of the difference between control and samples treated with various drugs was determined by one-way ANOVA, followed by the post hoc least significant difference test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Chronic asthma induces inflammatory response in mouse airway

TNFα and IL-1β levels were determined from the BALF samples by ELISA. In asthmatic mice, the mean concentration of TNFα and IL-1β was significantly increased to 634 ± 114 pg/mg protein, and 184 ± 48 pg/mg protein, respectively. Intranasal administration of budesonide

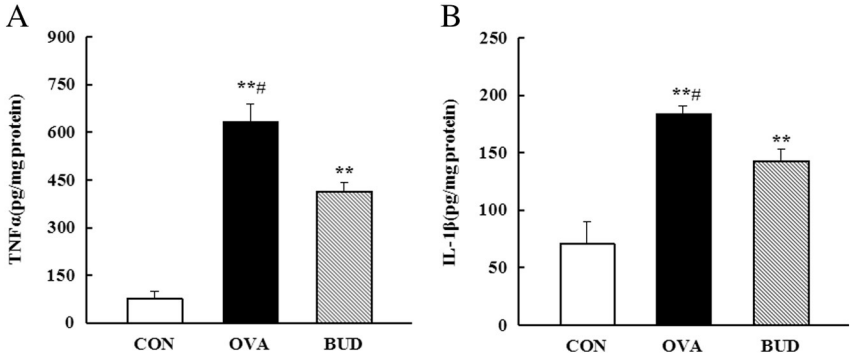

Fig. 1. Effects of budesonide on chronic asthma-induced increases of pro-inflammatory factors in BALF. A. The levels of TNFα in BALF of three groups; B. The levels of IL-1β in BALF of three groups. n = 12–14. Data represent the mean ± SEM. **p < 0.01 vs. CON group; #p < 0.05 vs. BUD group. CON: control group; OVA: asthma model group; BUD: budesonide-treated group.