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Nebulized lidocaine ameliorates allergic airway inflammation via downregulation of TLR2

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ABSTRACT

Nebulized lidocaine has been suggested to be beneficial in asthma therapy, but the underlying mechanisms are little known. We aimed to investigate whether Toll-like receptor (TLR) 2 was involved in the protective effect of lidocaine on allergic airway inflammation. Female C57BL/6 mice were sensitized and challenged with ovalbumin (OVA). Meanwhile, some of the mice were treated with TLR2 agonist (Pam3CSK4, 100 μg) intraperitoneally in combination with OVA on day 0. Just after allergen provocation, mice were treated with inhaled lidocaine or vehicle for 30 min. In this model, we found that lidocaine markedly attenuated OVA-evoked airway inflammation, leukocyte recruitment and mucus production. Moreover, lidocaine abrogated the increased concentrations of T cytokines and TNF-α in bronchoalveolar lavage fluid (BALF) of allergic mice, as well as reducing the expression of phosphorylated nuclear factor (P-NF)-κBp65 and the NOD-like receptor pyridine containing 3 (NLRP3), which are important for the production of pro-inflammatory cytokines. In addition, our study showed that lidocaine dramatically decreased OVA-induced increased expression of TLR2 in the lung tissues. Furthermore, activation of TLR2 aggravated OVA-challenged airway inflammation, meanwhile, it also elevated OVA-induced expression of P-NF-κBp65 and NLRP3 in the lungs. However, lidocaine effectively inhibited airway inflammation and counteracted the expression of P-NF-κBp65 and NLRP3 in allergic mice pretreated with Pam3CSK4. Taken together, the present study demonstrated that lidocaine prevented allergic airway inflammation via TLR2 in an OVA-induced murine allergic airway inflammation model. TLR2/NF-κB/NLRP3 pathway may serve as a promising therapeutic strategy for allergic airway inflammation.

1. Introduction

Allergic airway inflammation is a chronic inflammatory disease, in which both innate and adaptive immunity play a critical role (Kim et al., 2010). Despite significant progresses in its treatment, the control of allergic airway inflammation remains poor (Beeh et al., 2013). Nowadays, glucocorticoids are the most widely used antiinflammatory drugs for allergic airway inflammation. However, 1 or 2% of asthma patients are entirely corticosteroid-insensitive (Barnes, 2010). Therefore, exploring the mechanisms and seeking alternative antiinflammatory therapy are urgently needed.

Emerging evidence shows that Toll-like receptors (TLRs) are key mediators that are crucial to the pathogenesis of allergic airway diseases (Phipps et al., 2007). TLR2 has been extensively investigated in allergic airway inflammation such as asthma (Buckland et al., 2008; Bjornvold et al., 2009). TLR2 recognizes common allergens and initiates downstream signaling pathways (Zuo et al., 2015), particularly the myeloid differentiation factor 88 (MyD88)-dependent pathway (Liew et al., 2005). Subsequently, MyD88 activates nuclear factor (NF)-κB signaling pathways, which triggers the production of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6 (Piras and Selvarajoo, 2014). Moreover, recent studies have demonstrated that in murine macrophages TLR-induced NF-κB activation leads to the upregulation of the NOD-like receptor pyridine containing (NLRP) 3 (Qiao et al., 2012), which in turn results in cleavage of the pro-form of IL-1β into their mature forms (Jin and Flavell, 2010). Therefore, targeting TLR2 may be potentially utilized in the treatment of allergic airway inflammation.

Lidocaine, a widely used local anaesthetic and blocker of voltage-sensitive sodium channels, has been used in medicine for > 100 years. Treatment of asthmatic with lidocaine is a new concept that has been mainly motivated by findings on its anti-inflammatory effects (Okada

Abbreviations: TLR2, toll-like receptor 2; P-NF-κBp65, phosphorylated nuclear factor -κBp65; NLRP3, NOD-like receptor pyridine containing 3; OVA, ovalbumin

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et al., 1998; Tanaka et al., 2002; Olsen et al., 2011). Several studies have revealed that lidocaine exerts its anti-inflammatory effects by suppressing cytokine production and T cell proliferation and the function (Tanaka et al., 2002; Serra et al., 2012; Serra et al., 2016). Moreover, recent studies further demonstrated that lidocaine protects against inflammation by abolishing the expression of TLR4 (Lee et al., 2008; Liu et al., 2014). However, whether lidocaine inhibits TLR2-mediated signaling to ameliorate allergic airway inflammation has not been elucidated.

In the present study, we aimed to evaluate the protective effect of nebulized lidocaine on allergic airway inflammation and to assess whether TLR2 plays a role in this process.

2. Materials and methods

2.1. Animal

Female C57BL/6 mice used in this study were obtained from the Shanghai Laboratory Animal Center (Shanghai, China). The mice were 6 to 7 weeks old, weighing 20–25 g. All mice were kept in a controlled-temperature and -light animal facility under specific pathogen-free conditions. All protocols and experimental procedures involving animals had received prior approval from the Committee on the Ethics of Animal Care and Use of Anhui Medical University.

2.2. Experiment protocols and treatment

A previously established allergic airway inflammation protocol was used (Wu et al., 2015). The flowchart of our experimental design is shown in Fig. 1A. The mice were randomly divided into the following 6 groups (n = 6 each): saline control (Con), OVA-induced asthma group (OVA), OVA + 1% lidocaine (OVA + Lido), control mice treated with Pam3CSK4 (Con + Pa), OVA + Pam3CSK4 (OVA + Pa) and OVA + Pa + 1% lidocaine (OVA + Pa + Lido). The OVA model was sensitized by intraperitoneal (i.p.) injection of 0.5 mL saline mixed with 10 μg OVA (Sigma, St. Louis, MO, USA) and 1 mg alum (potassium aluminum sulfate, Sangon Biotech, Shanghai, China) at day 0. Then, 1% aerosolized OVA challenge was administered on days 14–20 for 30 min per day. For the control mice, OVA was replaced by saline solution. To further confirm our hypothesis, 100 μg Pam3CSK4 (InvivoGen, San Diego, USA) was administered i.p. in combination with OVA on day 0. Just after OVA provocation, the mice from OVA + Lido and OVA + Pa + Lido group were exposed to an aerosol of 1% lidocaine (Chaohui Pharma, Ltd., Shanghai, China) in a chamber for 30 min for seven consecutive days. One day after the last challenge, the mice were sacrificed, and bronchoalveolar lavage fluid (BALF) from the left lung was collected for subsequent experiments. The right lung was dissected for histological examination and western blotting.

2.3. BALF and differential cell counts

Immediately after sacrifice, the trachea via the endotracheal tube was flushed thrice with PBS to collect BALF. After centrifugation (700*g*, 4 °C, 5 min), the supernatant of the BALF was stored at −80 °C, and the cell pellets were resuspended in 200 μL of PBS for further analysis. The cells in BALF were counted by using a hemocytometer. Furthermore, different cell counts were performed after staining with Wright stain solution (Sigma).

2.4. Enzyme-linked immunosorbent assay (ELISA)

The cell-free supernatants of the BALF were quantified using commercial ELISA kits (Cusabio, Wuhan, China). The concentrations of IL-13, IL-6, TNF-α and interferon (IFN)-γ in the BALF were determined according to the manufacturer's instructions. The limitations for IL-6, IL-13, TNF-α, and IFN-γ were 1.56 pg/mL, 31.25 pg/mL, 3.9 pg/mL, and 15.6 pg/mL, respectively.

2.5. Lung histological examination

After bronchoalveolar lavage, the lung tissues of each mouse were fixed in 4% paraformaldehyde and embedded in paraffin. Then 5 μM-thick sections were stained with hematoxylin/eosin (H&E) or periodic acid-Schiff (PAS). A semi-quantitative scoring system was used to assess peribronchial inflammation in the H&E-stained lung sections of six mice, and quantitative analysis of PAS-stained sections was performed by counting the number of goblet cells (PAS^+) in the airway by the perimeter of the basement membrane (Pbm) (Wu et al., 2016).

2.6. Western blotting

Lung tissue of each mouse was homogenized in RIPA buffer with protease inhibitor (Roche, Indianapolis, IN, USA) and phosphatase inhibitor PhosSTOP (Roche). The microbicinchoninic acid assay (BCA, Pierce) was used for determining protein concentrations. Samples of lung (25 μg) were separated by 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis ((SDS-PAGE)) and then transferred onto PVDF membranes (Millipore, Billerica, MA, USA). After blocking with 5% nonfat milk for 1 h, the membranes were incubated with primary antibodies overnight at 4 °C. The primary antibodies we used included: P-NF-κBp65 (Signaling Technology Inc., Beverly, MA, USA), NF-κBp65 (Signaling Technology Inc., Beverly, MA, USA), TLR2 (Millipore, Billerica, MA, USA), NLRP3 (Signaling Technology Inc., Beverly, MA, USA), and GAPDH (KANGCHEN Biotech, Shanghai, China). Protein bands were detected by using enhanced chemiluminescence (Thermo Scientific, Tewksbur, MA, USA), and ImageJ software was used to quantify protein band density.

2.7. Statistical analysis

The Shapiro-Wilk test was utilized to assess normal data distribution. All data showed normal distribution except for the histological scoring of H&E-stained lung sections. The data were expressed as the mean ± SD or median within an interquartile range. Statistical analysis was performed with SPSS19. When the data showed normal distribution, statistical analysis was performed using one-way ANOVA with post hoc Bonferroni tests. A nonparametric Kruskal–Wallis test, with a post hoc Dunn test applied to nonnormally distributed data. Significance was assumed at $P < 0.05$.

3. Results

3.1. Lidocaine alleviates airway inflammation and mucus production in allergic mice

Histological sections showed that a large number of inflammatory cells infiltrated into the peribronchial regions of allergic mice when compared with that in the healthy controls (Fig. 1B and C). Interestingly, lidocaine treatment led to a notable inhibition of inflammatory cell recruitment (Fig. 1B and C). Concomitantly, OVA-challenged mice exhibited PAS^+ hyperplasia and mucus hypersecretion (Fig. 1D and E), which was significantly attenuated by nebulization of lidocaine (Fig. 1D and E).

3.2. Lidocaine prevents the influx of inflammatory cells into BALF of allergic mice

After OVA challenge, the total number of cells (Fig. 2A), neutrophils (Fig. 2B), monocytes (Fig. 2C), lymphocytes (Fig. 2D), and eosinophils (Fig. 2E) in BALF significantly increased in comparison with that in the control mice. However, nebulization with lidocaine after OVA provocation showed a lower degree of increase in the number of cells