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Ligustrazine attenuates inflammation and the associated chemokines and receptors in ovalbumine-induced mouse asthma model

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ABSTRACT

Ligustrazine which is isolated from Chinese herb ligusticum chuanxiong hort, has been widely used in traditional Chinese medicine (TCM) for asthma treatment. In this study, we aim to observe the effect of ligustrazine on inflammation and the associated chemokines and receptors in ovalbumin (OVA)-induced mouse asthma model. Our data demonstrates that ligustrazine suppresses airway hyperresponsiveness to methacholine and lung inflammation in OVA-induced mouse asthma model. Ligustrazine also induces inhibition of inflammatory cells including neutrophils, lymphocytes and eosinophils. In addition, ligustrazine significantly reduces IL-4, IL-5, IL-17A, CCL3, CCL19 and CCL21 level in BALF of asthma mice. Furthermore, ligustrazine induces down-regulation of CCL19 receptor CCR7, STAT3 and p38 MAPK protein expression. Collectively, these results suggest that ligustrazine is effective in attenuation of allergic airway inflammatory changes and related chemokines and receptors in OVA-induced asthma model, and this action might be associated with inhibition of STAT3 and p38 MAPK pathway, which indicates that ligustrazine may be used as a potential therapeutic method to treat asthma.

1. Introduction

Asthma is a complex and chronic inflammatory disorder affecting approximately 300 million individuals worldwide (GINA Report 2014 update). With inflammatory changes throughout the airways, asthma has been traditionally ascribed to be driven by T helper type 2 (Th2) cytokine responses characterized by the production of the cytokines IL-4, IL-5 and IL-13 (Robinson, 2010). According to the latest results, asthma is considered to be highly heterogeneous due to the diverse immune response and the associated inflammatory mediators including cytokines and chemokines (Murdoch and Lloyd, 2010). In addition to the Th2 phenotype, asthma has been reported to be associated with Th17 cells dysfunction with hypersecretion of IL-17 (Dias and Banerjee, 2013; Herbert et al., 2013; Morishima et al., 2013; Weaver et al., 2013).

Chemokines, a family of small proteins secreted by a variety of cell types, play significant roles in inducing directed chemotaxis in nearby responsive cells (Juan et al., 2013). The contribution of chemokines and their receptors to the pathogenesis of allergic asthma has been well established (Shimizu and Dobashi, 2012; Yamashita et al., 2006). Dendritic cells (DCs) which are professional antigen presenting cells play significant roles in initiating immune responses (Wang et al., 2013). Early studies identify that DCs migrate from circulation in peripheral blood during the allergen-induced asthmatic responses (Burchell et al., 2010; Maazi et al., 2013). It has been postulated that migration of DCs is regulated by their differential expression of chemokine receptors and the response to chemokine gradients. The chemokine CCL3 (also called MIP-1α) is considered one of the most abundantly expressed chemokines on immune cells. CCR1, receptor of CCL3, is considered to be associated with the chemoattraction of immature DCs, which had CCR1 highly expressed on their surface. Previous studies demonstrate that administration of CCL3 could rapidly recruit DC precursors into peripheral blood (Shi et al., 2011), and blockade of CCR1 results in their reduced mobilization into the peripheral blood (Li et al., 2011a). CCR7, which has CCL19 (also called MIP-3-β and ELC) and CCL21 (also called 6Ckine and SLC) as its ligands, is one of the most prominent chemokine receptors in the adaptive immune system (Comerford et al., 2013). Researches indicate that CCL19/CCL21/CCR7 has been involved in establishing a microenvironment in lymphoid tissues, which contributes to the encounter

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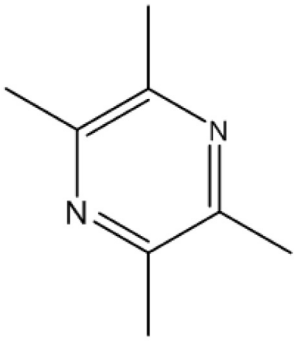

Chemical Formula: $C_8H_{12}N_2$
Exact Mass: 136.10
Molecular Weight: 136.20

Fig. 1. Chemical structure of ligustrazine.

of naïve T cells and mature DCs (Choi et al., 2013; Li et al., 2011b; Ricart et al., 2011). CCL21 is reported to promote DCs migration and maturation (Britschgi et al., 2010; Jalili et al., 2010). CCL19 and CCL21 gene-deleted mice fail to induce DC localization (Gunn et al., 1999), therefore, has slight immune responses.

Ligustrazine (tetramethylpyrazine) which is isolated from Chinese herb ligusticum chuanxiong hort (chuanxiong), has been widely used in traditional Chinese medicine (TCM) for asthma treatment. Previous studies show that ligustrazine has anti-inflammatory effect on TNF-α-induced endothelial dysfunction (Hu et al., 2010), and has protective effect on pulmonary damage (Deng et al., 2011). Furthermore, ligustrazine could correct Th1/Th2 and Treg/Th17 imbalance in mouse asthma model (Ying et al., 2011). However, effect of ligustrazine on chemokines and their receptors associated with DCs functions in asthma has not been fully elucidated. In this study, we aim to observe the effect of ligustrazine on airway inflammation of ovalbumin (OVA)-induced mouse asthma model and the associated role of ligustrazine on chemokines and their receptors important for DCs chemotaxis.

2. Materials and methods

2.1. Reagents and animals

Ligustrazine (purity > 98%), structure shown in Fig. 1, is purchased from Chengdu Must Biological Technology Co., Ltd. OVA and methacholine (Mch) are purchased from Sigma-Aldrich Co. LLC. IL-4, IL-5 and IL-17A ELISA kits are purchased from R&D systems, and CCL3, CCL19 and CCL21 ELISA kits are purchased from Raybiotech. Anti-mouse CCR1, CCR7, Janus kinase (JAK) 1, signal transducer and activators of transcription (STAT) 3 and p38 MAPK antibodies are purchased from abcam technology. Anti-mouse GAPDH and HRP-conjugated IgG are purchased from KangChen Bio-Tech.

Six-week-old female BALB/c mice (16 ± 3 g) are purchased from Shanghai Sippr BK Laboratory Animal Co. Ltd and housed in a pathogen-free rodent facility with food and water freely available according to standards for animal care of the Committee on the Ethics of Animal Experiments of Fudan University.

2.2. Asthma model preparation and treatment

Mice are randomly divided into normal control (NC), asthma (A), ligustrazine (L 25, L 50, L 100 mg/kg) and dexamethasone (DXM) groups (12 mice/group). As shown in Fig. 2*, the asthma model is induced by multiple OVA sensitization and challenge. Briefly, mice are sensitized by intraperitoneal injection of 20 μg OVA and 2 mg aluminum hydroxide in 0.2 ml saline on days 0, 7, 14 and 21. OVA challenge is administered by nebulization of 3% OVA from day 25 to day 31 using an ultrasonic nebulizer (402AI ultrasonic nebulizer, Yuyue, China). Mice in the NC group are sensitized and challenged with saline. Ligustrazine and DXM (0.085 mg/kg) are intragastric administrated from day 24–31.

2.3. Airway hyperresponsiveness (AHR) measurement

Airway responsiveness to Mch is determined by Buxco pulmonary mechanics with an invasive method within twenty-four hours after last OVA exposure as described previously(Luo et al., 2014). Briefly, tracheal intubation is performed under anesthesia and gradients of Mch (3.125, 6.25 and 12.5 mg/ml) is administered by nebulization to assess airway resistance (R_L) and lung dynamic compliance (Cdyn) of mice in each group. Data is expressed as a percent change from the baseline value.

2.4. Bronchoalveolar lavage fluid (BALF) inflammatory cell analysis

After AHR measurement, BALF is collected by lavaging the left lung with 0.3 ml aliquots of PBS for two times through the tracheal cannula (total volume 0.6 ml) and centrifuges at 800 × g, 4 °C for 10 min. The supernatant is collected and stored at −80 °C for further ELISA assay, and the pellet is resuspended in 0.1 ml PBS for cell count with a hemacytometer (HEMAVET 950FS, DREW, USA).

2.5. Histological analysis

Hematoxylin and eosin (H&E) staining is used to investigate the inflammatory changes in the lung tissues of mice. Briefly, the lung tissue is collected, fixed in 4% paraformaldehyde, embedded in paraffin and cut into 5 μm sections for H&E staining. The histopathological changes are determined by an optical microscope (ECLIPSE 80i, Nikon, Japan).

2.6. Analysis of cytokine and chemokine levels in BALF

Levels of IL-4, IL-5, IL-17A, CCL3, CCL19 and CCL21 in BALF are examined by ELISA assay according to the manufacturer's instructions.

2.7. Protein extraction and western blot assay

Expression of chemokine receptors CCR1, CCR7, JAK2, STAT3 and p38 MAPK are determined by western blot analysis. Briefly, total protein in the lung tissue is extracted and then isolated by 13% SDS-PAGE. The protein samples from each group are then electrophoretically transferred onto PVDF membranes. After electrotransfer, the PVDF membranes are blocked with 5% BSA and subsequently incubated with primary and secondary antibodies. The incubation conditions for the primary antibodies are overnight at 4 °C with CCR1 1:150 dilution, CCR7 1:5000 dilution, JAK1 1:1000 dilution, STAT3 1:500 dilution and p38 MAPK 1:1000 dilution, and for secondary antibodies are 37 °C for 1 h with 1:5000 dilution. In the end, the blots are visualized and analysed with Bio-Rad's Image Lab software.

2.8. Data analysis

Data is expressed as mean ± SD. Statistical significance of the differences is performed by one way analysis of variance (ANOVA)