Pulmonary Pharmacology & Therapeutics 36 (2016) 37–45

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

Long-term azithromycin ameliorates not only airway inflammation but also remodeling in a murine model of chronic asthma

Ji Young Kang, Mi Ran Jo, Hyeon Hui Kang, Sung Kyoung Kim, Myoung Sook Kim, Yong Hyun Kim, Seok Chan Kim, Soon Seog Kwon, Sook Young Lee, Jin Woo Kim*

Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 11 April 2015
Received in revised form
3 November 2015
Accepted 21 December 2015
Available online 9 January 2016

Keywords:
Asthma
Airway remodeling
Azithromycin
Macrolide

ABSTRACT

Objectives: We investigated the effect of long-term treatment with azithromycin on the pathogenesis of chronic asthma with airway remodeling.
Methods: Six-week-old-BALB/c mice were sensitized with ovalbumin (OVA) combined with lipopolysaccharide (LPS) for 1 month, then challenged with OVA for 3 months. Azithromycin at 75 mg/kg was administered via oral gavage five times a week during the challenge period. Inflammatory cells, T helper 2 cytokines in bronchoalveolar lavage fluid (BAL) fluid, and airway hyperresponsiveness (AHR) were measured. Parameters related to airway remodeling were evaluated. The levels of neutrophil elastase, Interleukin (IL)-8, and BRP-39 (human homologue YKL-40) were assessed. The expression of MAPK and NF-κB signaling were investigated.
Results: Long-term treatment with azithromycin improved AHR and airway inflammation compared with the OVA and the OVA/LPS groups. The concentrations of IL-5 and IL-13 in the OVA/LPS group decreased significantly after azithromycin administration. The levels of neutrophil elastase and IL-8, as surrogate markers of neutrophil activation, were reduced in the azithromycin group compared with the OVA/LPS group. Goblet cell hyperplasia and the smooth muscle thickening of airway remodeling were attenuated after azithromycin treatment. The expression of MAPK/NF-kappaB signal and the level of BRP-39 in the lung decreased remarkably in the OVA/LPS with azithromycin-treated group.
Conclusions: This study suggests that in a murine model of chronic asthma, long-term azithromycin treatment ameliorates not only airway inflammation but also airway remodeling by influencing on neutrophilc-related mediators, BRP-39 and MAPK/NF-κB signal pathways. Macrolide therapy might be an effective adjuvant therapy in a chronic, severe asthma with remodeling airway.

1. Introduction

Asthma is a chronic airway inflammatory disease that typically has findings of episodic cough, dyspnea, and airway hyperresponsiveness. However, some phenotypes of asthma exist that have only partially reversible or irreversible airway obstruction. They are not well controlled by standard treatment with inhaled corticosteroids and sometimes have a severe clinical course. Frequently, these conditions can be associated with airway remodeling, including epithelial injury, goblet cell metaplasia, subepithelial fibrosis, smooth muscle hypertrophy/hyperplasia, increased angiogenesis, and alterations to extracellular matrix components [1]. This pathologic change of asthmatic airway is induced by the repeated injury and repair process [2]. It has been thought that airway remodeling occurs in case of chronic asthma in association with persistent inflammation. However, it has been reported that these changes can occur during the first 4–5 years of childhood asthma [3] and that they can be caused by bronchoconstriction without inflammation [4]. Moreover, inhaled corticosteroid treatment to target airway inflammation in asthma cannot prevent airway remodeling [5]. These data suggest that airway remodeling could be a distinct process that occurs parallel to

Abbreviations: OVA, ovalbumin; LPS, lipopolysaccharide; BAL, bronchoalveolar lavage; AHR, airway hyperresponsiveness; IL, Interleukin; MAPK, mitogen-activated protein kinases; NF, nuclear factor; BRP, breast regression protein.

* Corresponding author. Division of Respiratory medicine, Department of Internal Medicine, Uijeongbu St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 65-1, Geumo-dong, Uijeongbu-si, Gyeonggi-do, 480-717, Republic of Korea.
E-mail address: medkjw@catholic.ac.kr (J.W. Kim).

http://dx.doi.org/10.1016/j.pupt.2015.12.002
1094-5539/

inflammation in the airway, and that an appropriate therapeutic intervention is required for the better control of asthma. Some drugs including anti-Th2 cytokines therapies, tiotropium, omalizumab, and imatinib, ameliorate remodeling in asthma [2], there have been few reports about the effect of macrolides on it.

In the past, the beneficial effects of macrolide antibiotics in asthma have been thought to be result of their inhibition of the metabolism of exogenous steroid or their antimicrobial activity against commonly atypical pathogens that cause the exacerbation of asthma [6]. However, it is known that macrolides have not only antibacterial properties, but also immunomodulatory and further antiviral properties [7]. Recent animal and human studies [6,8–16] demonstrated that macrolide treatment improved airway inflammation, hyperresponsiveness, further clinical symptoms, or quality of life in asthma. Most of the animal studies [6,14–16] were conducted in an acute asthma model with a short treatment duration of less than 1 month or an in vitro design. Among macrolides, azithromycin has 15- membered ring by the addition of a nitrogen atom into the lactone ring and resultantly has characteristics of good bioavailability, acid stability, and a long half-life [17]. Azithromycin showed effects on chronic obstructive pulmonary disease and asthma [8,9,18–20] in human studies. In particular, Brusselle et al. [9] confirmed the beneficial effect of the drug in patients with severe neutrophilic asthma and in acute exacerbation, suggesting that some specific groups exist whose asthma can be effectively controlled by macrolides. However, previous animal studies focused on the role of macrolides on airway inflammation in asthma, the effects of the drugs, especially azithromycin, on airway remodeling has not been clearly explored.

Regarding the signaling pathways involved in lung disease, Maneechotesuwan et al. [21] showed that p38 mitogen-activated protein kinase (MAPK) mediates the phosphorylation of GATA-3, which plays a critical role in allergy by regulating production of cytokines from Th2 lymphocytes. In addition, Morinaga et al. [22] showed that extracellular signal-regulated kinase (ERK) and nuclear factor-kappa B (NF-κB) pathway were involved in *Chlamydophila pneumoniae*-induced MUC5AC production in bronchial epithelial cells and that it is was reduced by macrolides, especially clarithromycin and telithromycin. Some previous studies [16,23,24] provided evidence that NF-κB, a ubiquitous transcription factor complex, could play a central role in asthma through increasing expression of many inflammatory genes, thereby perpetuating eosinophil inflammation. Kanoh et al. [25] proposed that major three pathways were involved in macrolide immunomodulation as follows: i> receptor tyrosine kinase − MAPK cascade − activator protein-1, ii> toll-like receptors − transforming growth factor-activated protein kinase 1 − NF-κB, iii> G-protein-coupled receptor-phospholipase C- protein kinase C and calcium/calmodulin signaling. They described that macrolide could inhibit MAPK activation, NF-κB translocation, and intracellular calcium increase and modulate immune response.

Mammals including human do not synthesize chitin, but do synthesize chitinases [26]. Chitinase-like proteins contain evolutionarily conserved 18-glycosly-hydrolases similar with chitinases, except the lack of enzymatic activity [27]. In human diseases, it has been known that the proteins has a role on the inflammation and tissue remodeling. In particular, Chupp et al. [28] showed that the expression of human chinitase-like protein YKL-40 increased in the serum and lung in patients with asthma.

In the present study, we used a murine model of chronic asthma to investigate whether azithromycin has an effect not only on airway inflammation but also remodeling. To explore further the underlying mechanisms, we measured the activities of signal pathways such as MAPK and NK-κB, and the expression of mouse breast regression protein 39 (BRP-39, human homologue YKL-40) in lung tissue.

2. Materials and methods

2.1. Sensitization and antigen challenge protocol

Female BALB/c mice (Dae-Han Experimental Animal Center, Daejon, Korea), 6 weeks of age, were used in the experiments. They were sensitized and challenged with ovalbumin (OVA, grade V; Sigma−Aldrich, St. Louis, MO, USA). Lipopolysaccharide (0.1 μg) (LPS; Sigma−Aldrich) was added during OVA sensitization to induce LPS-enhanced inflammation, as described in the previous studies [29,30]. Mice were divided into five groups: control, azithromycin (Pfizer Pharmaceuticals, Dublin, Ireland), OVA, OVA/LPS, and OVA/LPS with azithromycin treatment ($n = 5$ for the first four groups, and $n = 8$ for the OVA/LPS with azithromycin-treated group). To verify the result more clearly, we used 8 mice in the last treated group. The mice were immunized subcutaneously with mixture of 25 μg OVA and 1 mg of aluminum hydroxide (Sigma−Aldrich, Milwaukee, WI, USA) in 200 μl of normal saline once a week for 1 month. 20 ng OVA in 50 μl of phosphate-buffered saline [PBS] were challenged via intranasal inhalation from day 31 and repeatedly twice a week for 3 months, based on the protocol for inducing a model of chronic asthma described previously [31,32]. LPS (0.1 ug) was administered intraperitoneally during the sensitization period. Mice were sacrificed 24 h after the final OVA challenge. Airway hyperresponsiveness, bronchoalveolar lavage (BAL) fluid, and lung tissues were analyzed. http://www.jimmunol.org/cgi/content/full/173/12/7556 − F1#F1All animal experimental protocols were approved by the Animal Subjects Committees of the Catholic University of Korea.

2.2. Administration of azithromycin

Referring to the experiment by Beigelman et al. [15] who used 50 mg/kg of azithromycin, we conducted the preliminary study of a murine model of acute model with three different doses (25, 50, 75 mg/kg). We observed that the best effective dose was 75 mg/kg of azithromycin. Therefore, we used the dose given via oral gavage in a chronic asthma model. From day 38, and 0.5 h before the challenges, 75 mg/kg azithromycin was administered for 5 days a week for 3 months. Control groups and azithromycin groups were treated in the same way with control group receiving PBS. The azithromycin was generously donated by Pfizer.

2.3. Measurement of airway hyper-responsiveness (AHR)

AHR was assessed 24 h after the final OVA inhalation using the flexiVent system (SCIREQ, Montreal, QC, Canada). After the administration of aerosolized methacholine (Sigma) to the mice, we measured the changes in the airway resistance (Rrs; cmH_2O/ml/s) as described elsewhere [33]. In brief, mice were anesthetized, tracheostomized, and then mechanically ventilated (150 breaths/min, tidal volume of 10 ml/kg, and positive end-expiratory pressure of 3 cmH_2O). Mice were challenged with saline aerosol at baseline and then sequentially increasing concentrations of methacholine (6.25, 12.5, 25, and 50 mg/ml). Methacholine aerosol was administered via nebulizer for about 4 min at each concentration, after which Rrs was continuously monitored and recorded. Data were calculated as peak Rrs value for each methacholine concentration.

2.4. Bronchoalveolar lavage (BAL)

Mice underwent anesthesia via intraperitoneal injection of ketamine and xylocaine and were subjected to thoracotomy, after