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Short Communication Open Access

Budesonide Suppresses Pulmonary Antibacterial Host Defense by Down-Regulating Cathelicidin-Related Antimicrobial Peptide

Xi Dai[1,2] **and Guoping Li**[2*]

[1]*State Key Laboratory of Quality Research in Chinese Medicine, Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Avenida Wai Long, Taipa, Macao, China*

[2]*Respiratory Department, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan, China*

[*]**Corresponding author:** Guoping Li, Respiratory Department, The Affiliated Hospital of Southwest Medical University, Luzhou, Sichuan, China; Tel: 18982791605; E-mail: lzlgp@163.com

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Abstract

Some previous evidences have proven that Inhaled Corticosteroids (ICS) increased a risk factor for pneumonia in asthma. However, it remains unclear that ICS impacts the antibacterial host defence of lung in asthma. We aim to explore the direct impact of glucocorticoids on host defence against bacterial during asthma. We examined the effect of budesonide in host defence against *P. aeruginosa* and Cathelicidin-Related Antimicrobial Peptide (CRAMP) in HDM-challenged mice and lung epithelial cell lines. We found that inhaled budesonide increased *P. aeruginosa*-induced lung inflammation in OVA-challenged mice. Inhaled budesonide reduced the production of CRAMP in lung in OVA-challenged mice. Total bacterial CFUs were significantly higher in MLE-12 cells exposed to budesonide. The effect of budesonide on bacterial CFUs in MLE-12 cells was dose-dependent. Collectively, these findings demonstrated that inhaled budesonide suppressed pulmonary antibacterial host defence depending on the down-regulation of CRAMP in asthma.

Introduction

As one of the most common chronic conditions affecting both children and adults, Asthma is a global health problem affecting around 300 million individuals [1]. Asthma is a chronic inflammatory disorder characterized by airway inflammation and airway hyper responsiveness, which leads to recurrent episodes of cough, wheezing and breathlessness. Asthma is frequently distinguished by abnormal immune responses to environmental antigens and microbes. However, the etiology of asthma yet remains unclear. Previous studies shown that pneumonia incidence increased in asthma. Respiratory infections are a common precipitant of asthma exacerbation and recurrent wheeze. Up to 92.2% of exacerbations of asthma, pathogens are implicated in the inception and exacerbations of asthma. Virus was one of the main triggering factors [2]. In addition, in the steadies of host microbiome has confirmed that bacterial was an independent or cofactor with viruses by relating the inception and exacerbations of asthma [3]. Bacterial pathogens participated in acute wheezing episodes of preschool children, with a frequency similar to that seen with viruses [4]. It have been demonstrates that the neonatal hypo pharyngeal colonization of *Haemophilus influenzae*, *Streptococcus pneumonia*, and *Moraxella catarrhalis* increased risk of developing recurrent wheezing and childhood asthma [5]. Epidemiological investigations indicated that allergic asthma is also a risk factor of pulmonary infection. Patients with atopic asthma are prone to respiratory infections. Up to 30% of asthma patients suffer from the phenotype of neutrophil, which is characterized by substantial neutrophils in airway. Chronic colonization of bacteria is evident in the phenotype of neutrophil in asthma [6]. Glucocorticoids are the most effective drug for asthma, and widely used in asthma.

Glucocorticoids inhibited airway inflammation with decreasing the production of most inflammatory mediators and the activities of transcription factors controlling the synthesis of inflammatory mediators. Inhaled glucocorticoids are highly effective for most asthmatic subjects. Inhaled glucocorticoids have been used to first-line treatment in adults and children with persistent asthma [7]. Many studies shown that budesonide is efficacious and safe in the treatment of mild to severe asthma [8-10]. Glucocorticoids have a direct impact on the innate immune system involving glucocorticoid receptor-mediated gene transcription [11]. However, Inhaled Corticosteroids (ICS) increase the risk of pneumonia in chronic obstructive pulmonary disease. Although glucocorticoids have a direct impact on the host defence against pathogen, their effect on asthma remains unclear. In our present studies [12], we explore the impact of glucocorticoids on host defence against bacterial during asthma. Interestingly, our results had demonstrated that inhaled budesonide increase the degree of *P. aeruginosa*-induced pulmonary infection in OVA-induced model of mice. After treatment with budesonide, HDM-induced model of asthma mice and a murine lung epithelial cell line (MLE-12) were infected with *P. aeruginosa*. Inhaled budesonide also increased the extent of lung inflammation in asthmatic patients exposed to bacteria. Those results confirmed that inhaled budesonide inhibited host defence against *P. aeruginosa* and increase the severity of *P. aeruginosa*-induced lung infection in OVA-challenged mice (Figure 1A and 1B).

Secondly, 24 h after infection of mice with *P. aeruginosa*, higher numbers of bacterial Colony Forming Units (CFU) were observed in lung tissue from budesonide-treated mice. Inhaled budesonide reduced the clearance of *P. aeruginosa* in OVA-challenged asthma mice (Figure 1C).

After pre-treated with budesonide, the levels of internalized GFP-labeled *P. aeruginosa* and total bacterial CFUs in MLE-12 cells were increased, and budesonide showed a dose-dependent effect in bacterial CFUs in cells (Figure 1D).

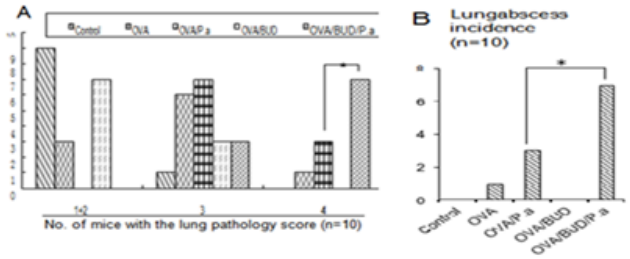

Figure 1 : The extent of lung inflammation score and lungabscess incidence were analyzed by microscopical histopathologic 24 h after intrartracheal challenge with *P. aeruginosa*. The cellular infiltration scoring of OVA/Bud/P. a mice was higher than that in OVA/P. a mice(*p<0.01).

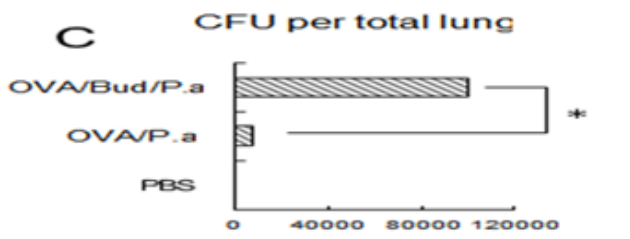

Figure 1C: Represents the bacterial colony forming units(CFUs) of OVA/P.a mice and OVA/Bud/P.a mice on blood agar plates. Compare with OVA/P.a mice, the number of *P. aeruginosa* CFUs was significantly higher, while control mice(PBS group) was zero.

Allergen processing and presentation to allergen specific T cells through antigen presenting cells is a key initiation step for IgE in asthma [13]. Th2 cells cytokines such as IL-4, IL-5, IL-6, IL-9, and IL-13 are important to host defence against parasites. The imbalance in Th1/Th2 immunity plays an important role in the pathogenesis of allergic asthma [14]. Cytokines also have direct effects in the airway inflammation through the recruitment of inflammatory cells, particularly eosinophil [15]. In our results, inhaled budesonide decreased IL-4 levels in OVA-challenged mice, demonstrated the therapeutically capacity of budesonide in asthma (Figure 1E). However, Th2 cytokines such as IL-4, IL-5, and IL-13 and serum IgE obviously increase during lung infection. IL-4 and interferon (IFN)-γ reflect antibacterial host defense. Sriram [16] reported that IL-4 suppressed the gene expression of IFN-β and IFN-responsive genes and inhibited IFN-dependent MHC Class I expression and amplification of IFN signalling pathways triggered upon TLR stimulation. Moreover, IL-4 suppressed TLR7- and TLR9-induced cDC production of pro-inflammatory cytokines such as TNFα, IL-12p70 and IL-6 by inhibiting IFN-dependent and NFκB-dependent responses. IL-4 also decreased the IFN response and increased permissiveness to viral infection of cDCs exposed to HIV-based lentivirus. IL-4 played a negatively role in viruses and intracellular parasites. Some studies showed that lung infectious inhibited Th2 cell response [17]. In our studies, after the OVA-challenged mice were infected with *P. aeruginosa*, IL-4 levels showed an increase in OVA-challenged mice treated with budesonide (Figure 1E).

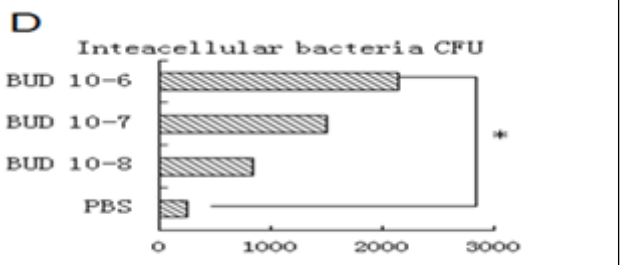

Figure 1D: MLE-12 cells, pretreated with budesonide (concentration were 10-8, 10-7 and 10-6 respectively), were infected with GFP-labeled *P. aeruginosa*. The presence of intracellular bacteria CFUs was significantly obvious in high concentration budesonide group than low concentration groups and control group(*p<0.001).

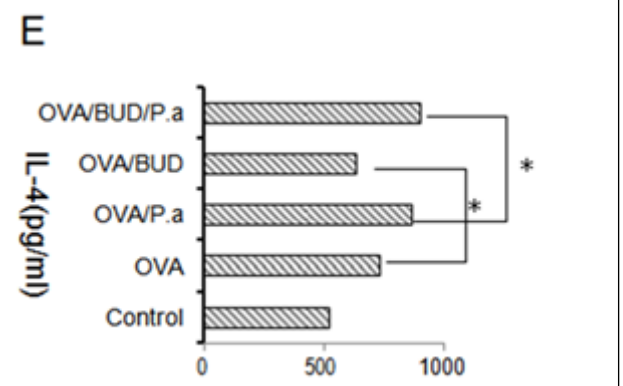

Figure 1E: IL-4 concentration in serum were detected with ELISA method. IL-4 in OVA/Bud/P.a mice was higher than in OVA/P.a mice.

Our results indicated that inhaled budesonide reduced the clearance of *P. aeruginosa*, and increased the severity of pulmonary infection in OVA challenged mice exposed to *P. aeruginosa*. Infection with *P. aeruginosa* was associated with increased levels of IL-4 in OVA-challenged mice treated with budesonide. Thus, our study indicated that inhaled budesonide increased pneumonia incidence in the medol of OVA-challenged mice. Antimicrobial peptides (AMPs) are important components of host defence against pathogen and secreted by airway epithelial cells, macrophages, neutrophils, and other classical host defence cells. AMPs kill pathogens through, disturbing cytoplasmic membrane septum formation and the synthesis of nucleic-acid synthesis and protein or inhibiting enzymatic activity [18]. Glucocorticoid inhibits the transcription of antibacterial peptides encoding genes by of NFκB alpha [19]. In our studies, inhaled budesonide significantly inhibited the expression of CRAMP [12]. Budesonide also inhibited the expression of CRAMP in lung epithelial cells. Total bacterial CFUs were significantly increased in MLE-12 cells

in present of budesonide. The effect of budesonide on bacterial CFUs in MLE-12 cells showed a dose-dependent effect. Meantime, the number of internalized GFP-labelled *P. aeruginosa* and bacterial CFUs increased in MLE-12 cells treated with CRAMP-neutralizing antibody (Figure 1F).

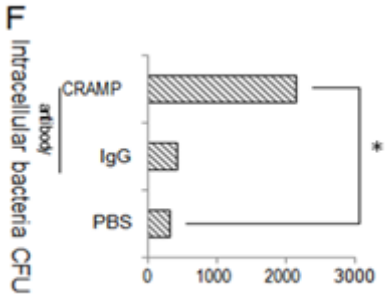

Figure 1F: After treated with CRAMP neutralizing antibody, the intracellular bacteria CFU was obviously higher than control group.

Taken together, these findings indicate that budesonide attenuate the antibacterial host defence associated with down-regulating CRAMP. The diseases of the respiratory infections are the most common diseases because of our lungs always exposed to the environment and its microbial components [20]. The innate immune system is the first line of host defence against pathogens. Being responsible for the immediate recognition and regulation of microbial invasion, host defence system consists of neutrophils, macrophages, epithelial cells, mast cells, eosinophil, and natural killer cells. Airway epithelium is part of the host's first line of defence against microbial invasion by secreting numerous antimicrobial molecules. The innate pulmonary immune system recognizes microorganisms and secretes numerous host defence cytokines, including antimicrobial and antiviral proteins. The airway epithelium is a necessary physical and chemical barrier to inhaled opportunistic pathogens. Immune effectors for pathogens are located in airway epithelial surface. Airway epithelial cells contribute markedly to their local antimicrobial peptides and complement [21]. Glucocorticosteroids are the true immunomodulators and recommended as a first line treatment for asthma. Glucocorticosteroids depress or enhance immune system depending on hormone-activated GC receptors (GCR) and other immunioactive. These selective effects of Glucocorticoid are partially mediated through activation of the transcription factor C/EBP [22]. High concentration of hydrocortisone suppressed innate immunity by decrease the expression and function of Toll-like receptors (TLR) in human corneal epithelial cell [23]. Glucocorticoid could impair innate pulmonary defences through modulate epithelial antimicrobial peptide expression [24]. The early application of glucocorticoids was a risk factor for human enterovirus 71 (HEV71) infections [25]. GCR change the transcription of pro-inflammatory cytokine gene and modify immune responses. Dexamethasone modulated the pro-inflammatory cytokine produced of human peripheral lymphocytes such as IL-1β, IL-6 and TNF-α [26]. Treatment with glucocorticoids or pyrazolones increased the risk factor for life-threatening HEV71 infection in Fever patients [27]. Meanwhile, the level of IFN-α showed approximately 25-fold decrease in the blood in patients treated with glucocorticosteroids [28]. Glucocorticoids suppress CX3CL1, CX3CR1, and CD22 expression in the hippocampus [29]. Previous studies showed that 5mg prednisolone increased about 30%, 46% or 100% risk of serious infection when continuously using for the last 3 months, 6 months or 3 years [30]. These findings indicate that glucocorticosteroids impact host defence against pathogens. Above all, we showed that inhaled budesonide could increase the severity of *P. aeruginosa* infection in OVA-challenged mice and attenuate antibacterial host defence in airway epithelial cells by down-regulating CRAMP. These novel findings may have implications for glucocorticoid treatment of asthma patients.

Acknowledgements

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