Effect of Glucocorticoid in mice of asthma induced by ovalbumin sensitisation and RSV infection

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Summary
Objective: **To investigate the inflammatory changes and the airway hyper-responsiveness in the asthma mouse model infected by respiratory syncytial virus and elucidate the relationship between the infection and the effect of glucocorticoid.**
Methods: **60 BALB/c mice were randomly divided into 6 groups. One of these is the control group; the others are the OVA/sham group, the OVA/sham +Dex group, the PBS/RSV group, the OVA/RSV group and the OVA/RSV+Dex group. The airway resistance was measured using a sealed body plethysmograph. Pathological slides were stained with hematoxylin-eosin, and the peribronchial inflammation was observed microscopically. The concentrations of IL-4、IFN-γ、TGF-β1 in lung tissues were detected by ELISA.**
Results: **Compared with the control group, the degree of the airway inflammation and hyper-responsiveness and the concentrations of IL-4/IFN-γ、TGF-β$_1$ in all four OVA groups increased significantly. And there was a statistically significant difference between the OVA/sham group and the OVA/sham+Dex group, and between the OVA/RSV group and the OVA/RSV+Dex group respectively. Compared with the OVA/RSV group，there was an obvious aggravation of airway inflammation and hyper-responsiveness in the OVA/RSV+Dex group.**
Conclusions: **Glucocorticoid significantly reduces airway inflammation and hyper-responsiveness induced by repetitive OVA challenge in the mouse model of asthma. However, the significant decrease in Th$_1$ and increase in Th$_2$ inflammation and aggravation of airway hyper-responsiveness in the mice in OVA/RSV group show that they are not sensitive to glucocorticoid. The effects of infection with RSV on the mouse model of asthma could be the cause of the glucocorticoid resistance during the therapy.** *(Asian Pac J Allergy Immunol 2011;29:176-80)*

Introduction

Chronic inflammation and airway hyper-responsiveness (AHR) in asthmatics are usually managed effectively by treatment with glucocorticoids. Viral respiratory tract infections can cause increased bronchial reactivity in normal subjects and exacerbate pre-existing asthma. Respiratory viruses are the most important trigger for acute asthma symptoms. Epidemiological evidence suggests that viral respiratory infections may contribute to allergic sensitization[1]. Among the respiratory viruses implicated in asthma, respiratory syncytial virus (RSV) is the most common one[2]. To investigate the effect of glucocorticoid on viral respiratory infections animal models are required, as such studies cannot be performed in humans. In present study, we described the effect of glucocorticoid on airway inflammation and airway hyper-responsiveness in a mouse model of chronic asthma induced by ovalbumin sensitisation and infected by RSV.

Methods
Animals

Female BALB/c mice, 8–10 wk of age, free of specific pathogens, were purchased from Experimental Animal Center of Shandong University. The mice were maintained on ovalbumin (OVA) free diets. All animal experimental protocols were approved by Animal Subjects Committee, Shandong University.

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Viruses

The viruses were cultured on Hep 2 cells. The A2 strain of RSV was originally provided by the Department of Microorganism, Shandong Academy of Medical Sciences.

Mouse Model of OVA-Induced Airway Inflammation

60 BALB/c mice were randomly divided into six groups (10 mice/group): Control group, OVA/sham group, OVA/sham+Dex (dexamethasone) group, PBS/RSV group, OVA/RSV group and OVA/RSV+Dex group. Mice were sensitized intraperitoneally with 1mg of OVA (Grade V, Sigma, UK) and Al (OH)$_3$ in 0.5ml of sterile saline as previously described[3]. Intranasal OVA challenges (20 μg/50 μl in PBS) were administered in 1% (w/v) aerosol for 50 to 60 minutes daily for 20 consecutive days. PBS was administered as placebo.

Mouse infection

On day 17 of OVA or PBS challenge, mice were infected by an intranasal (i.n.) instillation with a dose of 1×10^6 pfu of RSV A$_2$ strain in the PBS/RSV group , the OVA/RSV group and the OVA/RSV+Dex group. Sham-infected mice were inoculated with a working stock by the same procedure as RSV infected mice on day 17 of OVA or PBS challenge. Four days later, the mice were sacrificed and lung specimens were collected after the airway resistance had been measured.

Intervention with Glucocorticoid

To determine whether glucocorticoid could prevent the development of airway inflammation and AHR, dexamethasone (0.2 mg/kg in 100 μl of sterile, endotoxin-free PBS) was administered by intraperitoneal (i.p.) injection in mice of the OVA/sham+Dex group and the OVA/RSV+Dex group. The first dose was administered 2h after the first intranasal OVA challenge, and the therapeutic intervention was continued daily in 20 consecutive days.

Measurement of AHR

AHR was assessed by whole-body plethysmography (Buxco Electronics, Troy, NY) and aerosolized methacholine (Mch, Sigma) in a dose-dependent manner (3.1 mg, 6.25 mg, 12.5 mg, 25.0 mg and 50.0 mg in 1 ml PBS). Penh (enhanced pause) was used as the indicator of AHR in this study. The Penh values were expressed for each dose of MCh as the percentage of baseline Penh values after PBS exposure (% baseline Penh).

Baseline Penh values did not differ significantly between any of the 6 groups.

Lung histology

Mice were killed 24h after the final OVA challenge, and the lungs were analyzed. Left lungs from the mice were fixed with 1ml of formalin solution (10% neutral buffered formaldehyde). Subsequently, the lungs were embedded in paraffin, sectioned at a thickness of 4 μm, and stained with hematoxylin-eosin. The quantitative histological and image analysis of all coded slides was performed by researchers who were unaware of the coding of the slides. The degree of peribronchial inflammation was assessed from 1 to 6 based on the histological grading system as previously described[4].

Measurement of cytokines in lung tissues

After the mice were sacrificed, the right lungs were collected and cut into 1 mm^3, homogenated in ice-cold Hanks' buffer (pH 7.5). Thereafter, the homogenates were centrifuged at 3000×g at 4℃ for 10 min. The supernatant was collected and stored at -70℃ for assaying of the IL-4, IFN-γ and TGF-β1 levels. The concentrations of Cytokine IL-4, IFN-γ and TGF-β1 in lung tissues of the mice were measured by commercially available ELISA kits (R&D Co.) in accordance with the manufacturer's instructions .

Statistical Analysis

The Kruskal-Wallis test was applied for statistical analysis of categorical variable data. The statistical significance of inter-group differences was further assessed with the Mann-Whitney test for independent samples with Bonferroni's correction. Numerical variable data were expressed as mean ± S.D. Differences among the groups were analyzed by one-way analysis of variance (ANOVA), Student-Newman-Keuls Test (SNK) was used to compare data between groups. Statistical analyses were performed using SPSS 13.0 software (version 13.0; SPSS Inc, Chicago, IL) .P values less than 0.05 were considered statistically significant.

Results

Effect of Glucocorticoid on the Physical Signs

There were no asthma-related physical signs in mice in the control group and the PBS/RSV group. However, the mice in OVA/sham group, OVA/RSV group and OVA/RSV+Dex group showed significant signs of asthma such as dysphoria, tachypnoea,

Table 1. The degree of peribronchial inflammation in mice

GROUPS	The indices of peribronchial inflammation						Total
	1	2	3	4	5	6	
Control	9	1	0	0	0	0	10
OVA/sham[*]	0	1	8	0	1	0	10
OVA/sham+Dex[#]	6	1	2	0	1	0	10
PBS/RSV[*]	0	1	7	2	0	0	10
OVA/RSV[*#]	0	0	1	6	3	0	10
OVA/RSV+Dex[*#Δ]	0	0	1	1	4	4	10

OVA : ovalbumin, DEX : dexamethasone, RSV : respiratory syncytial virus ; * vs Control group p <0.05 ; # vs OVA/sham p <0.05 ; Δ vs OVA/RSV p <0.05

wheezing, incontinence, etc. However, the physical signs of asthma in OVA/sham+Dex group were milder than those in OVA group.

Effects of Glucocorticoid on the airway inflammation

Effects of Glucocorticoid on airway inflammation are shown in Table 1.

Compared with OVA/sham group, the degree of airway inflammation in OVA/sham+Dex group was significantly reduced ($p < 0.05$). But the degree of inplammation in OVA/RSV+Dex group was greater than that in OVA/RSV group.

Effect of Glucocorticoid on the AHR

Effects of Glucocorticoid on the AHR are shown in figure 1.

The differences of Penh values in PBS/RSV group, OVA/sham group and OVA/RSV group were significant compared with those in the control group when stimulated by Ach (12.5, 25.0 and 50.0 mg/ml) ($p < 0.05$). The degree of AHR was further increased in mice previously exposed to repeated OVA inhalations and subsequently infected with RSV. Compared with OVA/sham group, the Penh values in OVA/sham+Dex group were significantly reduced ($p < 0.05$). Dex significantly prevented the AHR in mice subjected to repetitive OVA challenge. However, the AHR in OVA/RSV+Dex group was significantly greater than that in OVA/RSV group ($p < 0.05$).

Effect of Glucocorticoid on the concentrations of IL-4、IFN-γ、TGF-β1 in lung tissue

The levels of IL-4、IFN-γ and TGF-β1 are shown in Table 2.

Table 2. The concentrations of Cytokine proteins IL-4, IFN-γ and TGF-β1 in lung tissues （ng/L, x ±SD）

GROUPS	n	IL-4	IFN-γ	TGF-β$_1$
Control	10	23.5±1.6	210.7±21.6	12.3±3.2
OVA/sham	10	42.3±3.7[*]	130.4±16.4[*]	41.8±10.5[*]
OVA/sham+Dex	10	32.4±1.7[*#]	196.5±26.7[*#]	27.2±8.1[*#]
PBS/RSV	10	40.7±3.2[*]	126.1±14.0[*]	43.2±11.5[*]
OVA/RSV	10	51.6±4.3[*#]	99.4±15.3[*#]	53.6±13.2[*#]
OVA/RSV+Dex	10	57.3±3.9[*#Δ]	81.5±14.7[*#Δ]	64.9±12.5[*#Δ]

OVA : ovalbumin, Dex : dexamethasone, RSV : respiratory syncytial virus ; * vs Control group P<0.05 ; # vs OVA/sham p <0.05 ; Δ vs OVA/RSV p <0.05

The levels of IL-4 and TGF-β1 in the lung tissue of OVA/sham+Dex group were significantly lower and IFN-γ was higher than those of OVA/sham group respectively ($p < 0.05$). Dex significantly reduced levels of lung IL-4 and TGF-β1 and increased the level of IFN-γ in mice subjected to repetitive OVA challenge. However, the levels of IL-4 and TGF-β1 in the OVA/RSV+Dex group were higher and IFN-γ was lower than those in OVA/RSV group ($p < 0.05$).

Discussion

In this study we demonstrated that glucocorticoid significantly reduces airway inflammation and AHR induced by repetitive allergen challenge in a mouse model of the asthma. When glucocorticoid was applied, we found the significant decrease in Th$_1$ and increase in Th$_2$ inflammation and aggravation of the AHR, which could result from RSV infection. Thus RSV infection in the mouse model of the asthma could be the cause of glucocorticoid resistance during the therapy.

Asthma is a common respiratory disease characterized by chronic airway inflammation leading to AHR and reversible airway obstruction. Chronic inflammation and AHR in asthmatics are usually managed effectively by treatment with glucocorticoid. Although current therapies targeting both airway inflammation and AHR effectively relieve and prevent symptoms in the majority of patients, a subset of patients experience persistent symptoms and a progressive decline in lung function and remains refractory to therapy, described as irreversible or refractory asthma. The underlying mechanisms are poorly known. RSV may play a role in the induction and maintenance of airway remodeling. The effects of glucocorticoid in virus-induced asthma are controversial[5].
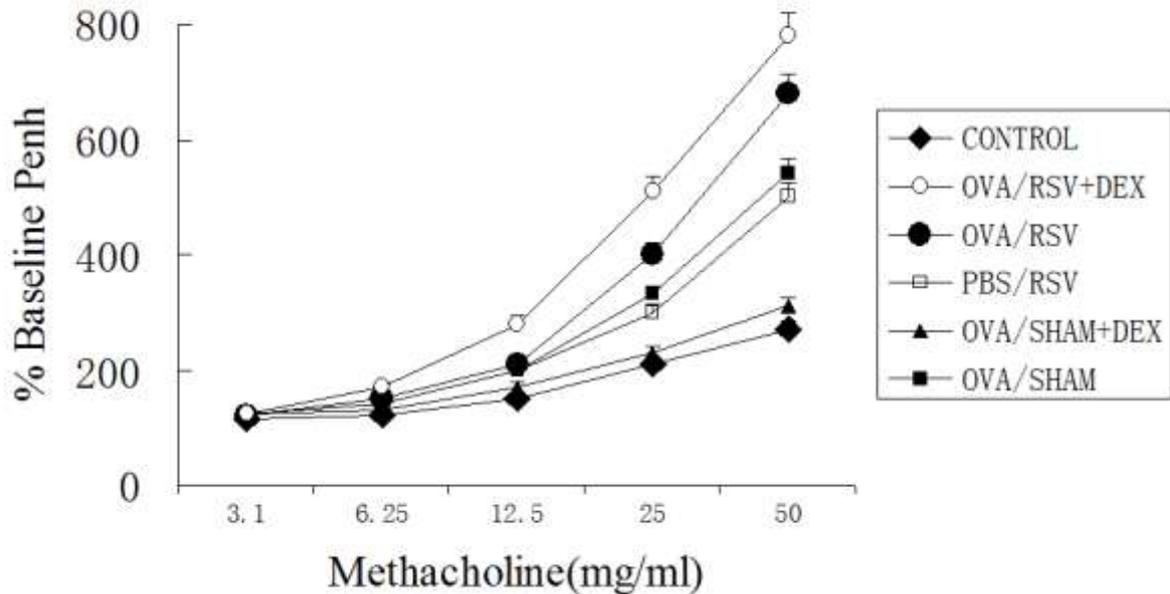
Figure 1. AHR to MCh in mice. Penh was measured at baseline and after increasing doses (3.1 to 50 mg/ml) of inhaled MCh.

We employed a mouse model with characteristic features of chronic airway inflammation and its AHR similar to those observed in patients with asthma. These results are consistent with previous observations of acute inflammation in mouse models of asthma[6]. The current data support the notion that prior airway exposure to inhaled allergen may predispose to the development of exaggerated airway responsiveness upon subsequent RSV infection. These results are in agreement with earlier studies[7,8].

We observed significantly suppressed airway inflammation when treating with Dex. Similarly, a noticeable reduction in AHR was found in OVA-treated mice that were administered Dex. Consistent with previous observations[9], we found that airway inflammation and AHR were significantly decreased in OVA-sensitized mice treated with Dex compared with those of OVA group. We further examined IL-4 and IFN-γ concentrations in this mouse model. Consistent with these previous findings, our results have shown that concentration of IL-4 in lung tissue increased and IFN-γ decreased after OVA challenge and administration of Dex in OVA-induced mice decreased the IL-4 and increased the IFN-γ. Our studies suggest that glucocorticoid reduce level of lung IL-4 and raise that of IFN-γ. Thus Dex may inhibit mice airway inflammation and AHR through changing the overall production of Th1 and Th2 cytokines (IFN-γ and IL-4, respectively). TGF-β1 represents a profibrotic cytokine that is produced by a number of cells, including macrophages, epithelial cells, fibroblasts and eosinophils. TGF-β1 is known to enhance the fibrotic process by augmenting fibroblast growth and collagen production, as well as promoting differentiation of fibroblasts into myofibroblasts that secrete collagen and other extracellular matrix components[10]. Thus the ability of glucocorticoid to inhibit allergen-induced TGF-β1 expression could play a significant role in the inhibition of airway remodeling by glucocorticoid in asthma. Consistent with these previous findings[11], our results have shown that the level of TGF-β1 in lung tissue increased after OVA challenge, but the administration of Dex in OVA-induced mice could inhibit the increase of TGF-β1.

RSV infection produced a marked recruitment of neutrophils and lymphocytes into the airways and an increase in IL-4, TGF-β1 and a decrease in IFN-γ levels in the lung tissue. By contrast, treatment with Dex altered the Th1 immunoreaction induced by RSV through decreasing the IFN-γ/IL-4. It did not relieve the increased inflammatory response or decrease the production of TGF-β1, but markedly increased the recruitment of inflammatory cells and AHR, resulting in decreased Th1 cytokines (a potentially Th2-prone environment) in this model. Our findings suggest Dex may inhibit the immunity to viral infection in mice so that the damage to

epithelial cells caused by the viruses outweighs Dex inhibition on AHR.

While many of these patients may respond to an extremely high dose of glucocorticoids, such treatment carries the risk of serious side effects. Further research is needed to define the mechanisms of action for ongoing airway immune activation, correlation of response to particular treatment strategies and thereby to identify the most appropriate treatment regimen for a particular pathway of cellular activation. An understanding of the mechanisms by which glucocorticoids fail to resolve inflammation and AHR in asthma may provide important insight into its pathogenesis, especially as it related to progressive pulmonary deterioration.

In summary, this study demonstrates that administration of glucocorticoids can prevent increase in the inflammation and AHR. This ability is likely mediated by several mechanisms, including inhibition of IL-4 and TGF- β1, increase of IFN-γ in lung tissue. Significant decrease in Th_1 and increase in Th_2 inflammation and aggravation of AHR in the mice in OVA/RSV group indicates that they are not sensitive to glucocorticoid. The effect of infection of RSV on the mouse model of the asthma could be the cause of the glucocorticoids resistance during therapy.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

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