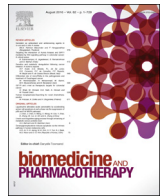




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Original article

Cordycepin inhibits airway remodeling in a rat model of chronic asthma



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ABSTRACT

The potential suppression role of cordycepin (Cor) on airway remodeling in a rat model of chronic asthma was investigated in this paper. We evaluated the anti-remodeling of Cor (50 mg/kg) combined with or without budesonide (BUD) and investigated the possible underlying molecular mechanisms. We found that Cor attenuated immunoglobulin (Ig) E, alleviated the airway wall thickness, and decreased eosinophils and neutrophils in the bronchoalveolar lavage fluid (BALF). Notably, Cor reduced the up-regulation of IL-5, IL-13 and TNF- α in the BALF. Cor also regulated the increase of A_{2A}ARmRNA and the decrease of TGF- β ₁ expression. Furthermore, Cor markedly blocked p38MAPK signaling pathway activation in the OVA-driven asthmatic mice. The combination treatment of Cor and BUD showed profound efficacy in regulating the levels of inflammatory cells and the expression of IL-13, TGF- β ₁ and A_{2A}ARmRNA. Collectively, this study demonstrated that Cor combined with glucocorticoids treatment shows synergistically profound efficacy in inhibiting airway remodeling, and some benefits of Cor may result from the increased A_{2A}ARmRNA expression, the reduced TGF- β ₁ levels and the inhibition of Th2-cytokines through the suppression of the p38MAPK signaling pathways.

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1. Introduction

Cumulative evidence has revealed that allergic asthma is a major global epidemic and a public health problem due to its rapidly rising prevalence, morbidity, and mortality, and the number of individuals with allergic asthma is rapidly increasing worldwide which currently affects approximately 20% of the world's population [1–3]. Although asthma is mostly well controlled by conventional therapies including inhaled corticosteroids, about 5–10% of asthma patients have a severe phenotype described as “steroid-insensitive asthma” or “difficult to control asthma” [4–6], that is to say, glucocorticoids insensitivity, and a number of chronic asthmatic patients develop irreversible alterations of pulmonary function. These alterations result from

structural changes of the airways, known as airway remodeling [7,8], which is a prominent pathologic feature of asthma. The extent of airway wall thickening is associated with disease progression, and this thickening is the most important cause in the reduction of lung function [9]. The severity of asthma is related to the degree of airway remodeling, which is mostly marked in cases of fatal asthma. However, despite advances in understanding the inflammatory features of asthma, the cellular and molecular mechanisms underlying the remodeling process in asthma remain poorly understood.

TGF- β , with important immunomodulatory and fibrogenic characteristics, has been implicated in inflammation and remodeling processes. Gene polymorphisms in the TGF- β ₁ promoter have been associated with asthma development [10]. TGF- β ₁ has direct effects on remodeling in mouse models; instillation into mouse lungs and TGF- β ₁ transgenic overexpression or adenoviral expression in the airway epithelium can induce airway collagen mRNA and protein deposition [11]. Consequently, anti-TGF- β ₁ antibody was effective in inhibiting pulmonary fibrosis and significantly reduced collagen deposition, smooth muscle cell proliferation, and goblet cell mucus production in an asthma model [12]. In OVA-sensitized asthmatic mice, significantly reduced TGF- β ₁ mRNA levels in pulmonary tissue and protein

Abbreviations: Cor, cordycepin; OVA, ovalbumin; AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; PBS, phosphate-buffered saline; Ig, immunoglobulin; IL, interleukin; mRNA, messenger ribonucleic acid; RT-PCR, reverse transcription–polymerase chain reaction; TNF- α , tumour necrosis factor- α ; MAPK, mitogen-activated protein kinase; ERK, extracellular regulating kinase.

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levels in BALF inhibited airway remodeling [13]. These studies suggest that the expression of TGF- β_1 is an important contributor to airway remodeling in asthma.

Cordyceps militaris, is a caterpillar-grown traditional medicinal mushroom. Cordycepin (Cor) (3'-deoxyadenosine), a major bioactive component isolated from cordyceps militaris, has been reported to possess multiple pharmacological activities, including anti-anoxia, anti-inflammatory immunological modulation and anti-hepatic fibrosis, but the molecular mechanisms of Cor on airway remodeling of asthma have not been clearly elucidated yet.

From previous literature, we know adenosine is a key endogenous molecule that regulates tissue function by activating four G-protein coupled adenosine receptors: A₁, A_{2A}, A_{2B} and A₃. Of the four receptors, the adenosine A_{2A} receptor has been strongly linked to controlling inflammation, so we hypothesize that the target of Cor regulating the airway remodeling might be on the A_{2A} receptor. However, there have been no data from respiratory disease models in animals that would support the concept. In addition, in regard to the high risk of adverse effects of glucocorticoids including diabetes, osteoporosis, muscle wasting, skin thinning and weight gain [14,15], and the corticosteroid insensitivity of asthma therapy, more effective therapeutic approaches are needed to explore. So we report here the effects of Cor, a potent and selective adenosine A_{2A} receptor agonist, on the airway remodeling of asthma models of the ovalbumin (OVA)-sensitized Wistar rats, explore its underlying mechanism, observe the synergistic effects of combination therapy of Cor and glucocorticoids, and provide evidence of the potential therapeutic values of Cor.

2. Materials and methods

2.1. Experimental animals

8-week-old female Wistar rats, weighing 200 ± 30 g, were purchased from Shanghai Laboratory Animal Co. Ltd., (Shanghai, China). The rats were divided randomly into each experimental groups and housed five rats in each laminar flow cabinet under specific pathogen free conditions and maintained on 12-h light-dark cycle, with food and water ad libitum. All the animal experiments were strictly conducted in accordance with the protocols approved by the Ethics Committee for Animal Studies at Shanghai General Hospital, China. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.2. Antigen sensitization, challenge and treatment protocol

50 rats were randomly divided into five groups: control group (sensitized and challenged with saline), OVA-sensitized asthma group (sensitized and challenged with OVA), Cor treatment group (50 mg/kg), budesonide (BUD) (10 mg/L) treatment group, and combination treatment group (BUD+Cor). Rats in the last four groups were sensitized on days 0 and 8 by intraperitoneal injection of 1 mg OVA (Grade V; Sigma, St. Louis, MO, USA) together with 200 mg of Al(OH)₃ in 1 mL of saline. Simultaneously, 1 mL of Bordetella pertussis vaccine, containing 6×10^9 heat killed organisms, was administered intraperitoneally as an adjuvant. Then they were challenged to OVA (1% in saline) aerosolized for 30 min via airways thrice weekly since day 15. The aerosol was generated with a nebulizer (PARIBOY N037; PARI, Starnberg, Germany) and was drawn into the transparent plastic challenged chamber (approximate volume of 4L) containing the awake animals. Rats in Cor treatment group were administered intragastrically with Cor at a dose of 50 mg/kg daily since day 58 for 8 weeks. Rats in BUD treatment group were treated with aerosolized

budesonide (AstraZeneca AB) 10 mg/L for 30 min and intragastrically with saline at the same time before challenged, rats in combination treatment group received the same schedule for aerosolized drug as BUD treatment group and intragastrically with Cor 50 mg/kg instead of saline, the duration of treatment is 8 weeks. Animals were killed 24 h after the last intragastric medicine or intranasal inhalation.

2.3. BALF collection and determination of cell numbers

After the rats were killed with sodium pentobarbital, the lungs were lavaged through the tracheal cannula with PBS, airway lumina were washed three times with 0.4 mL sterile saline, then BALF were collected. The cell suspension was concentrated by centrifugation (1000 rpm, 10 min, and 4 °C). The supernants were stored at -80 °C until use. The cell pellet was resuspended in 1 mL of saline. To perform the differential leukocyte cell count, 0.1 mL of the cell suspension was smeared on a glass slide and stained with Wright-Giemsa. Two hundred nucleated cells were then counted under $\times 400$ magnification by the same inspector in a blinded manner. Cells were identified by standard morphology and classified as eosinophils, lymphocytes, neutrophils, and macrophages.

2.4. Analysis of tumour necrosis factor (TNF)- α and Th2 associated cytokines in BALF and total IgE in serum

TNF- α , IL-5 and IL-13 of BALF and the level of total IgE in serum was examined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's recommendations (R&D Systems, Minneapolis, MN, USA, and BioSource International, Camarillo, CA, USA). The limit of detection was 0.2 ng/mL. Data were expressed as ELISA units, which was the product of the dilution and the optical density.

2.5. Histological analysis of lung tissue and measurement of bronchial wall thickness

After BALF, the right lobe of the lung from each animal was removed for histological examination. The lungs were fully inflated by intratracheal perfusion with 4% paraformaldehyde. The remains were micro-dissected and placed into liquid nitrogen and kept in -80 °C refreeze till further process for Western blotting. Lungs were then dissected and placed in fresh paraformaldehyde for 48 h. Routine histological techniques were used to paraffin-embed the tissue, and 3- μ m sections of lung tissue were stained with hematoxylin-eosin (HE). Digital image analysis was performed on histological sections, using Image-Pro Plus software version 6.0 (Media/cybernetics, Silver Springs, MD, USA), the bronchial wall thickness was analyzed by applying the Axiovision Rel 4.8 software (Carl Zeiss). In general, 3 bronchi with a diameter of 150–200 μ m while the lumen shortest diameter/longest diameter ≥ 0.6 from each lung tissue sections stained with HE were selected under the Axioplan microscope (Carl Zeiss) under the magnification of 400 $\times$ and quantitative analysis was performed. The thickness of bronchial wall was calculated according to the formula: $Wat/Pi = (The\ outer\ area\ of\ the\ airway\ wall\ (Ao) - The\ inner\ area\ of\ the\ airway\ wall\ (Ai)) / The\ perimeter\ of\ the\ inner\ airway\ wall\ (Pi)$.

2.6. Immunohistochemistry of TGF- β_1

Sections (3 μ m thickness) cut from right lung tissues embedded in paraffin blocks were deparaffinized with xylene and hydrated in ethanol. The presence of TGF- β_1 were confirmed by immunohistochemistry. For immunohistochemical detection of TGF- β_1 , the lung section were incubated overnight at 4 °C with either primary

monoclonal antibody directed against TGF- β_1 (Sigma-Aldrich), or as a negative control rat serum instead of the primary antibody. Immunoreactivity was detected by sequential incubations of the lung sections with biotinylated secondary antibody, followed by incubation with peroxidase reagent and AEC (3-amino-9-ethyl-carbazole) chromogen.

2.7. Real-time PCR experiments

Total RNA was prepared using the TRIzol[®] Reagent (Invitrogen) according to the manufacturer's instructions. A₂₄AR RNA (2 μ g per sample) was used to synthesize single-stranded complimentary DNA (cDNA) using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) in a PTC-200 Peltier Thermal Cycler (MJ Research, Watertown, Mass., USA). The generated cDNA was used as a template in subsequent real-time PCR analyses. Transcript levels were determined by Applied Biosystems 7500 Real Time PCR System (Applied Biosystems, Foster City, CA, USA) using SYBR Green PCR Master Mix Reagent (Qiagen). Relative gene expression was normalized to β -actin. RT-PCR results were analyzed with the $\Delta\Delta$ CT method [16].

2.8. Western blot analysis for p38MAPK and ERK

Lung tissues were homogenized and qualified by bicinchoninic acid assay analysis. Equal amounts of protein were separated by SDS-PAGE and electrophoretically transferred to nitrocellulose membrane and then incubated with primary antibodies against phospho-p38 MAPK, total p38MAPK, ERK, phosphor-ERK (Cell Signaling Technology, Beverly, CA) for blot detection.

2.9. Statistical analysis

Data were expressed as mean $\pm$ SEM and n presented the number of animals. Statistical analyses were performed using GraphPad Prism 5.0 software (San Diego, CA, USA). Analysis of variance (ANOVA) was used to compare mean value for the different groups, followed by Student-Newman-Keul test for multiple comparisons. Statistical analysis was performed using GraphPad Prism 5.0 software (San Diego, CA, USA). A p value <0.05 (two-sided) was considered significant.

3. Results

3.1. Cor treatment attenuates inflammatory cells in BALF

Firstly, we examined the effects of Cor on the numbers of inflammatory cells in BALF. In the saline-treated rats, OVA challenge resulted in a marked increase of total cells, eosinophils and neutrophils when compared with the control rats ($P < 0.01$,

$P < 0.01$, and $P < 0.01$, respectively [Fig. 1A–C]). Compared with the OVA-sensitized rats, there were significant reductions in eosinophil counts in all the treatment groups, but no obvious changes were seen among each treatment group (Fig. 1B). Only in the combination treatment group, the numbers of total cells and neutrophils were significantly reduced, but were not seen in the monotherapy of Cor or BUD group.

3.2. Cor decreases the level of cytokines in BALF and total levels of IgE in serum

In order to determine whether Cor treatment affects cytokine secretion in the BALF, the levels of IL-5, IL-13, and TNF- α were determined via ELISA. The BALF were obtained 4 h after the final administration. The levels of IL-4, IL-5 and TNF- α in the BALF were increased significantly by airway challenge with OVA when compared with that of the control. The treatment of Cor reduced the concentrations of IL-5, IL-13, and TNF- α ($P < 0.01$, $P < 0.001$, and $P < 0.05$, respectively [Fig. 2A–C]). Moreover, as depicted in Fig. 2B, the combination treatment also resulted in a significant reduction in the secretion of IL-13, to a significant degree, than the monotherapy of Cor.

we also evaluated the effect of Cor on total levels of IgE in serum. Compared with the control group, the OVA group had a significantly higher concentration of total IgE, while concentrations in all the treatment groups were significantly lower than the OVA group ($P < 0.01$, $P < 0.001$, and $P < 0.001$, respectively [Fig. 2D]), no obvious changes were seen among all the treatment group.

3.3. Morphometric analysis and the thickness of bronchial wall

Representative photomicrographs in Fig. 3A showed significant peribronchial myofibroblast distribution, which reflected the bronchial wall thickness occurring in the animals. The bronchial wall thickness in rats repetitively challenged with OVA was significantly greater than that in the control animals. Cor treatment group significantly reduced the area of staining, as compared with the corresponding area in OVA group. The combination treatment significantly reduced the thickness of the airway wall than the BUD treatment group (Fig. 3).

3.4. Effects of Cor on TGF- β_1 expression in lung tissue

To further determine the effects of Cor, we examined the expression of TGF- β_1 in lung tissues. Expression of TGF- β_1 was identified in lung tissue by immunohistochemical analysis (Fig. 4A). Compared with the OVA challenged group, TGF- β_1 expression was low in all treatment groups, including Cor treatment group. When compared with monotherapy of BUD,

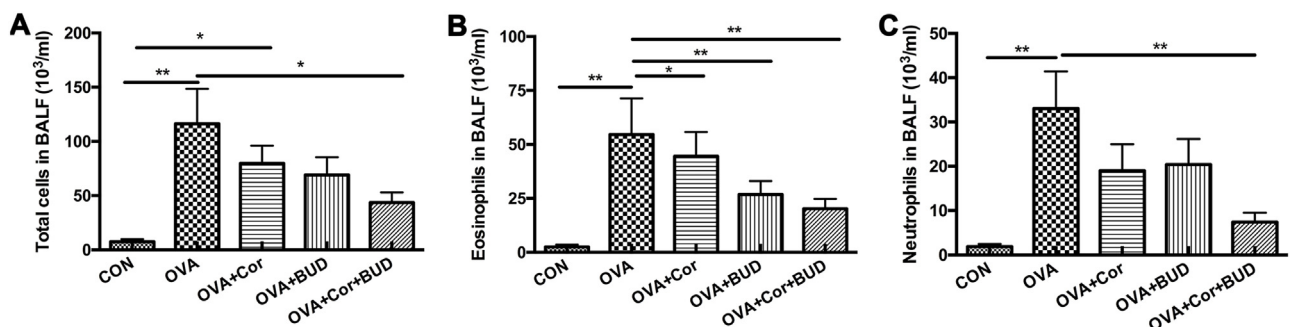


Fig. 1. Effects of Cor on inflammatory cells in BALF. Mean numbers of total cells (A), eosinophils (B) and neutrophils (C) recovered from bronchoalveolar lavage fluid (BALF). Data are expressed as means $\pm$ SEM for n = 10 for each group. * $P < 0.05$, and ** $P < 0.01$.

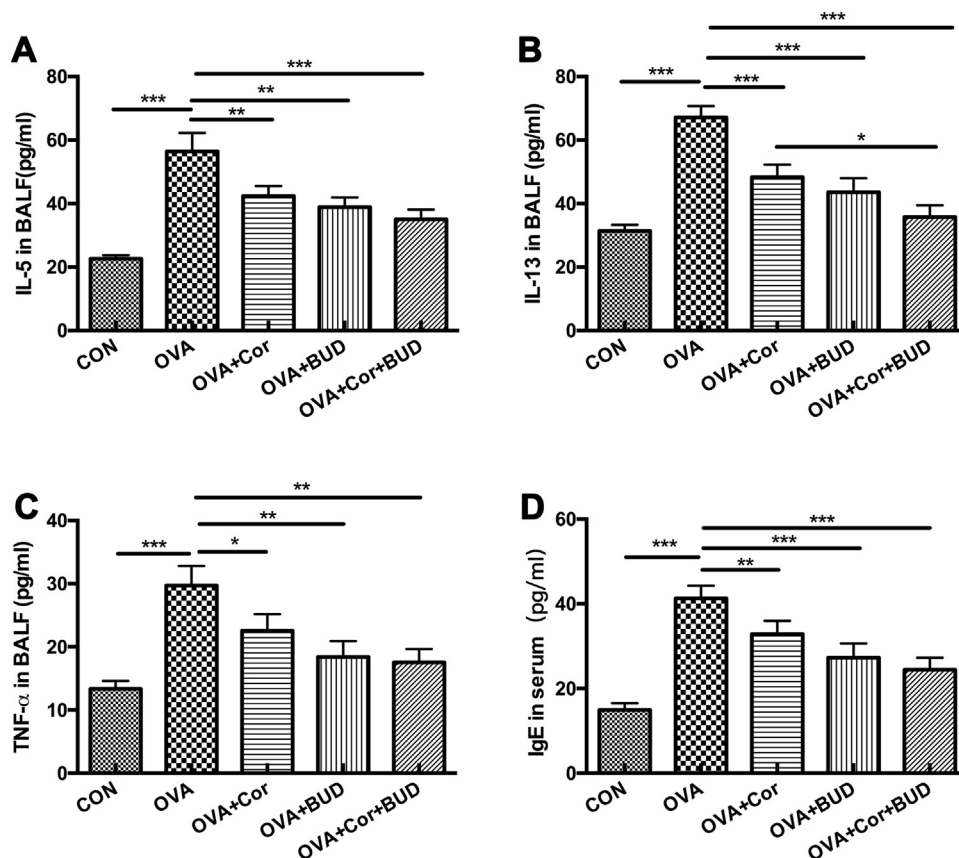


Fig. 2. Effects of Cor on cytokines in BALF and serum of each group. Mean levels of IL-5 (A), IL-13 (B), and TNF-α (C) in BALF and IgE in serum (D) were detected by ELISA. Data are expressed as means $\pm$ SEM for $n = 10$ for each group. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

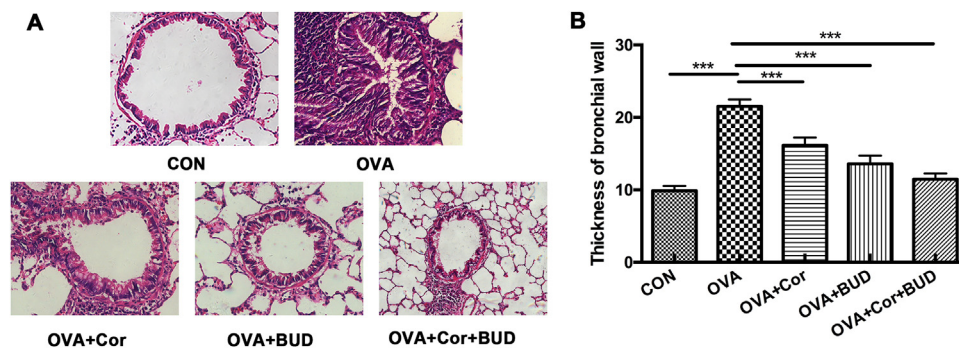


Fig. 3. Effects of Cor on thickness of bronchial wall in each group. Representative histological sections of bronchial wall (A) (400 $\times$) were stained with haematoxylin and eosin. Samples of serum and BALF were collected 24 h after the last administration. Mean values of thickness of bronchial wall in rats (B). Data are expressed as means $\pm$ SEM for $n = 10$ for each group. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

combination treatment rats further reduced the expression of TGF- β_1 to some extent (Fig. 4B). Representative photomicrographs in Fig. 4A also show the difference of mean density of TGF- β_1 occurring in OVA challenged group and other treatment groups.

3.5. The effects of Cor on the levels of A_{2A} ARmRNA and phosphorylation of p38MAPK and ERK in lung tissues

As shown in Fig. 5A, OVA challenged induced the decrease in the expression of A_{2A} ARmRNA. Rats in monotherapy of Cor and combination treatment demonstrated significantly increases in the expression of A_{2A} ARmRNA compared with OVA challenged group and BUD treatment group (Fig. 5A). In contrast, there was no

difference between OVA challenged group and BUD treatment group (Fig. 5A).

The level of phosphorylation of p38 MAPK is calculated by the phosphor-p38 MAPK/total p38 MAPK. The phosphorylation of p38 MAPK in all the treatment groups were significantly higher than control group, but lower than OVA challenged group, and the phosphorylation of p38MAPK in combination group was significantly suppressed than monotherapy of BUD group (Fig. 5B), while there was no difference between monotherapy of Cor group and monotherapy of BUD group on the phosphorylation of p38MAPK (Fig. 5B).

The level of phosphorylation of ERK is presented by phosphor-ERK/actin in Fig. 5C. The levels of phosphorylation of ERK in all

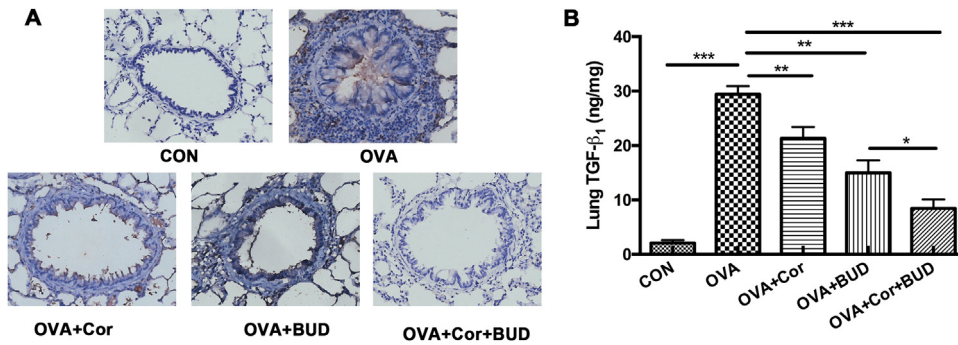


Fig. 4. Immunohistochemical staining. Paraffin embedded section of lung tissues of every group. Representative immunohistochemical staining of mouse lung sections (A) (original magnification $\times 400$). Mean density of TGF- β_1 in lung tissue (B) from different groups. Data are expressed as means $\pm$ SEM for $n = 10$ for each group. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

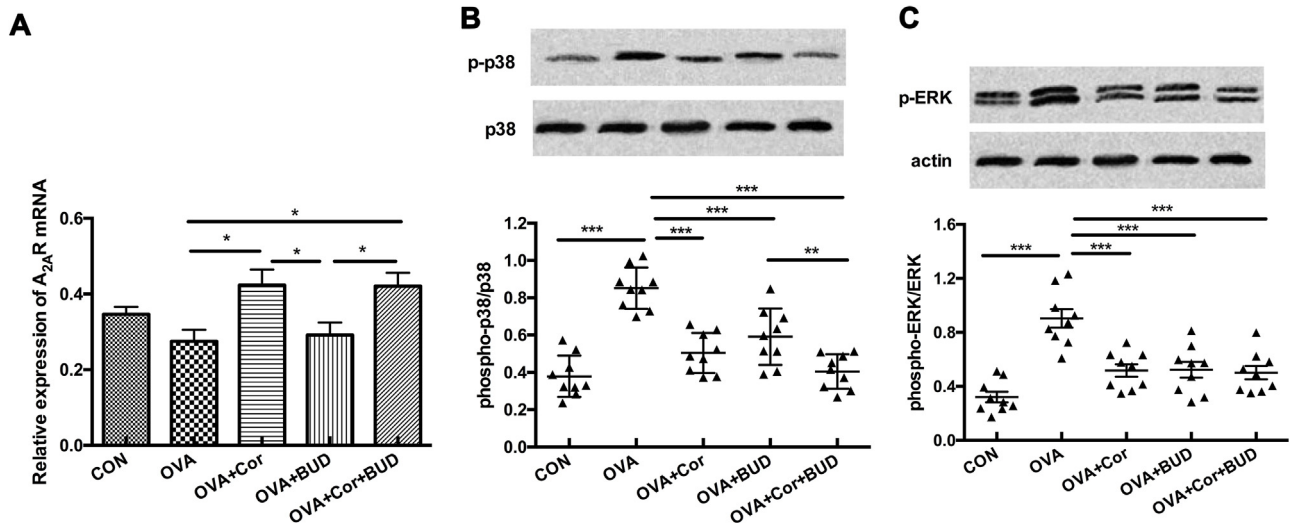


Fig. 5. Expressions of A_{2A}AR mRNA and proteins in lung tissues. RT-PCR analysis for A_{2A}AR mRNA (A) and western blot analysis of ratios of phosphorylated p38MAPK to total p38MAPK (B) and phosphorylated ERK to total ERK (C) in lung tissues of rats from different groups. Data are expressed as means $\pm$ SEM for $n = 10$ for each group. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

treatment groups were higher than that in the control group, but lower than OVA challenged group and the difference reached statistical significance (Fig. 5C); whereas the differences among all the treatment groups did not reach statistical significance (Fig. 5C).

4. Discussion and conclusion

Airway remodeling has been proposed as a mechanism which could explain many clinical features of asthma, such as persistent bronchial hyperresponsiveness, impaired lung function and glucocorticoids insensitivity. Therefore, we investigated whether Cor plays a protective role in airway remodeling. Using an OVA-induced rat model of asthma, we showed that Cor delayed the secretion of IgE, TNF- α and TGF- β_1 as well as Th2-associated cytokines, including IL-5 and IL-13. Cor also ameliorated OVA-driven eosinophils, which is the cardinal pathophysiological symptom of allergic airway diseases. The molecular mechanism by which Cor blocks the expression of these inflammatory mediators appeared to involve the disturbances of MAPKs signaling cascade pathways. Moreover, Cor increased A_{2A}AR mRNA expression. Cor significantly reduced peribronchial fibrosis, as assessed by measuring the TGF- β_1 deposits and decreased thickness of bronchial wall, as compared with the OVA challenged

rats. These findings verify the inhibition by Cor of airway remodeling in chronic asthma. In addition, combination of Cor and BUD shows synergistically profound efficacy in regulating the above biomarkers.

In asthmatics, the levels of TGF- β_1 are increased, and the expression of TGF- β_1 is correlated with the severity of the disease and the degree of airway remodeling [17–19]. Our observations suggest that the reduction of TGF- β_1 by Cor may lead to the decrease in the thickness of bronchial wall induced by repeated OVA challenge. Cor was proved to be beneficial for asthma control by minimizing the airway thickness. Regardless of TGF- β_1 , there are cytokines, chemokines, and growth factors released from inflammatory and structural cells in the airways in the development of remodeling [7], for example, TNF- α , IL-5 and IL-13. In addition, an overabundance of eosinophils, mast cells, activated T helper lymphocytes, neutrophils, macrophages and dendritic cells also participate in the process.

Eosinophil is an important hallmark of asthma and is blamed for the chronicity of the disease. Authors have shown that eosinophil recruitment is induced following challenged to OVA in asthmatic mouse models [20]. Previous reports demonstrated that depletion of eosinophils protects mice from developing mucus accumulation, AHR and/or airway remodeling [21,22]. Moreover, as

an important proinflammatory cytokine, TNF- α is involved in recruitment of eosinophils and increase of cytokine release [23]. To explore the beneficial effects of Cor, we examined whether the rise in the numbers of eosinophils and TNF- α level in BALF could be inhibited. As expected, the present results clearly demonstrated that Cor significantly reduced eosinophil numbers and TNF- α level in the BALF.

The accumulation, migration, and prolonged survival of eosinophils in the lung is regulated by a number of different cytokines such as IL-5 [24,25]. IL-5-deficient mice chronically challenged with allergen had significantly less peribronchial fibrosis, thickness of the smooth muscle layer, and numbers of peribronchial TGF- β positive cells compared with wild type mice [25]. In addition, anti-IL-5 significantly reduced levels of airway eosinophils, eosinophil TGF- β_1 mRNA⁺ cells, and levels of BALF TGF- β_1 [18] suggesting an important role for eosinophil derived TGF- β_1 in airway remodeling and IL-5 plays an essential role in airway remodeling formation. Overexpression of IL-13 within the lungs results in IgE production, inflammation and eosinophilia. Several previous studies have demonstrated that inhibiting IL-13 revealed significantly decreased airway inflammation, and airway responsiveness in mouse models of asthma [26], while more recent studies have demonstrated that IL-13 contributes to features of airway remodeling [27–30]. And IL-13 is believed to be the central mediator of allergic asthma remodeling via reducing the TGF- β_1 activation [31]. Current data showed that Cor also suppressed the OVA-challenged IL-5 and IL-13 levels in BALF.

The Mitogen-activated protein kinase (MAPK) family is serine/threonine family of kinases, which includes p38MAPK and extracellular regulating kinase (ERK) protein kinase pathways. Reportedly, the MAPK family is involved in mediating numerous biological functions, including cell proliferation, differentiation, apoptosis, and inflammation [32]. Activation of the p38MAPK pathway is important in the development of the inflammatory response, AHR and airway remodeling caused by various stimuli [33–35]. In present study, we investigated the effects of Cor on the MAPKs signaling cascade, and our results showed that monotherapy of Cor or corticosteroid abolished OVA-stimulated inflammatory mediators by regulating p38MAPK and ERK, but the combined administration revealed the synergistic effect mainly by down-regulating p38MAPK but not ERK signaling pathway, indicating the benefit effects of Cor rely more on p38MAPK signaling pathways. The reasonable interpretation is likely that Cor targets not only the p38 signaling pathways, but also other regulatory pathways, such as Akt and other specific chemokine-associated pathways [36,37]. To some extent, our results showed that Cor markedly suppressed the OVA-induced expression of inflammatory mediators in the lungs, which may be due to the inactivation of the p38MAPK signaling pathways and the reduced levels of IgE. However, additional research would be worthwhile to address this issue.

At present, therapy using inhaled corticosteroids is recognized as one of the most important strategies to prevent airway remodeling. Therefore, in the present study, we design BUD as the positive control group and combination treatment group, to observe the effects of Cor and to investigate whether it has synergistic effect with corticosteroids. Our findings have elucidated that the airway thickness decreased significantly in monotherapy of Cor group, compared with the OVA-challenged group, but it is still inferior to the effects of glucocorticoids. Whereas there was no significant difference between monotherapy of glucocorticoid group and combination treatment group, indicating that Cor had effects of improving airway remodeling to some extent, but less than the effects of glucocorticoids. When respect to the expression of TGF- β_1 in lung tissue, there were no significant differences between both monotherapy of Cor group and

glucocorticoid group and the controlled group, but combination treatment group had remarkable synergistic effect.

Subepithelial fibrosis in the basement membrane region of the airways is a typical feature of airway remodeling in asthmatic patients. Thus, we further assessed the airway wall thickness. The findings that bronchial wall thickening in OVA-induced asthma is decreased by Cor treatment are of particular interest. But whether A_{2A}R signaling pathways correlated closely with bronchial wall thickening needs further study.

Previous study revealed that allergen inhalation decreased adenosine receptor (AR) expression in sputum and blood of asthma patients [38]. The A_{2A}AR is expressed on virtually all cells that are implicated in many immune inflammatory cells such as neutrophils, monocytes, eosinophils, epithelium, endothelium, lymphocytes and NK cells [39], suggesting that the receptor may play a role in the inflammatory process. The study demonstrated that A_{2A} receptor activation has been shown to suppress the pro-inflammatory signaling mechanisms of cytokines [40–43]. Our results indicated that Cor had effects of increasing the transcription of A_{2A}ARmRNA, and suppressing the expression of TGF- β_1 , suggesting that Cor may inhibit the airway remodeling of asthma by up-regulating the transcription of A_{2A}ARmRNA to diminish the expression of TGF- β_1 , but whether and how the transcription of A_{2A}ARmRNA regulate TGF- β_1 expression need further investigations. Another aspect of interest in our study is the fact that the levels of A_{2A}ARmRNA expression were not significantly changed in BUD-treated group in comparison with the OVA-challenged group, but lower than the control group, a possible explanation for this finding may be that glucocorticoid inhibits airway remodeling not via A_{2A}AR signaling pathway, instead, may suppress A_{2A}ARmRNA expression. It is generally accepted that there are some severe asthmatic patients are insensitive to glucocorticoids treatment. Our present study may therefore provide an additional explanation that the reduction of A_{2A}ARmRNA expression promotes airway remodeling, in turn accounting for the corticosteroid resistance in asthma. Furthermore, our findings also showed that there was no significant difference between monotherapy of glucocorticoid and combination therapy in the bronchial wall thickening. Collectively, these findings demonstrate a novel mechanism that A_{2A}AR signaling pathway plays a certain but not the main role in the airway remodeling of asthma. This may therefore account for the synergistic effects in decreasing TGF- β_1 in combination therapy. These discoveries also provide a foundation for developing new therapeutic strategies for the corticosteroid insensitivity in asthma.

In Conclusion, Cor may play a potential role in inhibiting the airway remodeling of asthma by up-regulating the transcription of A_{2A}ARmRNA, down-regulating the synthesis of TGF- β_1 , and suppressing the activation of the p38MAPK signaling pathways. The results of this study provide novel insight into the disease mechanism underlying asthma, demonstrating that Cor is a promising new candidate for the treatment of allergic asthma.

Disclosures

The authors have no financial or commercial conflicts of interest to disclose in relation to publication of the manuscript.

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