

Effect of Dexamethasone on TGF- β 1/Smad3 Signalling Pathway in Airway Remodelling Model of Asthmatic Rats

Yan Feng¹, Chang Yang², Wen Yan¹ and Tao Jiang¹

ABSTRACT

Objective: To determine the effect of dexamethasone on regulating the TGF- β 1/Smad3 signalling pathway in airway remodelling model of asthmatic rats.

Study Design: An experimental study.

Place and Duration of Study: Department of Pathology, The First Hospital of Wuhan, Hubei Province, China, from February 2017 to April 2018.

Methodology: Thirty male SPF Sprague-Dawley rats were randomly divided into the control group, the model group and the dexamethasone group, with 10 rats in each group. Rats were sensitised and excited by ovalbumin (OVA) to establish the model of bronchial asthma. The bronchial basement membrane perimeter (Pbm), the bronchial wall thickness (Wat), the smooth muscle thickness (Wam), collagenous fiber thickness (Wac) and other airway remodelling indices were measured and calculated by image analysis system. The expressions of TGF- β 1 and Smad3 mRNA and protein in lung tissue of each group of rats were detected by RT-PCR and Western blot.

Results: Wat/Pbm, Wai/Pbm and Wam/Pbm in the model group were higher compared with those in the control group (all $p < 0.001$); Wat/Pbm, Wai/Pbm, and Wam/Pbm in the dexamethasone group were significantly lower than those in the model group (all $p < 0.001$). Relative expression levels of TGF- β 1, Smad3 mRNA and protein in the model group were higher than those in the control group (all $p < 0.001$); the relative expression levels of TGF- β 1, Smad3 mRNA and protein in the dexamethasone group were lower than those in the model group (all $p < 0.001$).

Conclusion: Dexamethasone may antagonize airway remodeling by regulating TGF- β 1/Smad3 signaling pathway, which likely to play a role in the treatment of bronchial asthma.

Key Words: Dexamethasone, Rat, Bronchial asthma, Airway remodelling, TGF- β 1/Smad3 signalling pathway.

INTRODUCTION

Bronchial asthma is a chronic respiratory disease characterised by varying degrees of chronic inflammation of the airways and airway remodelling.^{1,2} The pathological changes of airway remodelling mainly include airway epithelial damage, smooth muscle cell hypertrophy and so on.³ It has been found that transforming growth factor beta (TGF- β 1)/Smad3 signalling pathway is one of the important signalling mechanisms leading to the formation of asthmatic airway remodelling.^{4,5} TGF- β 1 is considered the main regulator of airway remodelling in asthma, directly affecting the deposition of collagen in the airway wall and promoting the formation of fibrosis.⁶ The Smads protein is an important member of the TGF- β signal transduction system, and Smad3 may be the signal transduction molecule that plays a leading role in the TGF- β signal transduction pathway.⁷ Inhaled corticosteroids, which can reduce chronic inflammation of the

airways, are currently the most effective treatment for bronchial asthma. Dexamethasone is a commonly used glucocorticoid that not only improves asthmatic symptoms, but also reduces airway inflammation. There are studies available where effect of dexamethasone on the different modulator of respiratory airways have been demonstrated.⁸ However, whether dexamethasone plays a therapeutic role by regulating TGF- β 1/Smad3 signalling pathway is unclear.

The objective of this study was to investigate the effect of dexamethasone on the TGF- β 1/Smad3 signalling pathway in the airway remodelling model of rats with bronchial asthma, in order to provide reference for the treatment of bronchial asthma.

METHODOLOGY

This study was conducted at the Department of Pathology, The First Hospital of Wuhan, Hubei Province, China, from February 2017 to April 2018, after approval by the Committee on Animal Research and Ethics. Thirty healthy male SPF Sprague-Dawley rats were randomly divided into the control group, the model group, and the dexamethasone group by random number table, with 10 rats in each group. On the 1st and 8th day, 1 mL of ovalbumin (OVA) (1 mg) + Al (OH)₃ (100 mg) saline solution was intraperitoneally injected for sensitisation, and the rats were placed in a semi-closed container.

¹ Department of Pathology, The First Hospital of Wuhan, Hubei Province, 430022, China

² Department of Respiratory, The Third People's Hospital of Hubei Province, 430033, China

Correspondence: Chang Yang, Department of Respiratory, The Third People's Hospital of Hubei Province, 430033, China
E-mail: inlm28@163.com

Received: September 01, 2018; Accepted: December 24, 2018

Ultrasonic fog was started on the 15th day to excite the rats with 1% OVA atomisation, 30 min each time, once every other day for 8 weeks. When the typical episodes of asthma such as irritability, cough, shortness of breath, rapid breathing and decreased activity were observed, the bronchial asthma model was successfully constructed. The excitation stage of the dexamethasone group was the same as that of the model group. In the excitation phase, 0.5 mg/kg dexamethasone was intraperitoneally injected half an hour before the excitement, and the inhalation excitation was also carried out in a closed container with 1% OVA saline. In the sensitisation and excitation phases of the control group, the OVA/Al (OH)₃ mixture or the 1% OVA atomisation solution was replaced by normal saline.

Each group was sacrificed within 24 hours after the last excitation, and the thoracic cavity was opened immediately to expose the lung tissue. The trachea was opened and intubated with a 5.5 scalp needle to rapidly separate the lung tissue, and the lung tissue was quickly placed in liquid nitrogen for storage.

Three small bronchioles with intact structures in the left lung tissue sections of each rat were selected, and the bronchial basement membrane perimeter (Pbm), the bronchial wall area (Wat), the inner wall area (Wai), the airway smooth muscle area (Wam), and other airway remodelling indices were measured by Image-pro Plus 6.0 pathological image analysis software. Wat, Wai, and Wam were normalised with Pbm, and Wat/Pbm, Wai / Pbm, Wam / Pbm respectively indicating the thickness of each layer of the corresponding wall.

The mRNA levels of TGF-β1 and Smad3 in lung tissue of each group were detected by RT-PCR. Appropriate amount of right lung tissue was grounded under liquid nitrogen. RNA was extracted by adding RNA extraction reagent trizol, and the instructions were carried out in strict accordance with the kit instructions. The primer sequences of GAPDH, TGF-β1 and Smad3 are shown in Table I. The RT-PCR reaction conditions were as follows: cDNA synthesis and pre-denaturation at 40°C for 10 min, 94°C for 2 min, PCR amplification at 94°C for 15 s, 58°C for 30 s, 72°C for 45 s, 40 cycles, and finally extension at 72°C for 10 min. Real-time fluorescence

quantitative PCR was used to detect the expression level. Bio-Rad CFX96 real-time quantitative PCR system software automatically generated the cycle value (Ct), and the relative quantification of the target gene was normalised to calculate $\Delta\Delta C_t$. The mRNA levels at TGF-β1, Smad3 were calculated using the $2^{-\Delta\Delta C_t}$ relative quantification method.

Western blot was used to detect the expression of TGF-β1 and Smad3 protein in lung tissue. Appropriate amount of right lung tissue was taken to be washed twice with PBS. Radio-immunoprecipitation assay (RIPA) Lysis Buffer was added and homogenised at low temperature, which was then centrifuged at 12000 xg for 10 min at 4°C. Supernatant was taken and protein was quantified by BCA method to make the protein sample. The sample was loaded and SDS was carried out. After PAGE electrophoresis, the electrophoresis gel was immersed in the transfer membrane for 5 min, and proteins were blotted on the membrane by semidry method, and blocked with PBST containing 5% BSA for 1 hour at room temperature. Primary antibodies (TGF-β1, Smad3 and β-actin) were incubated overnight at 4°C. The next day, the membrane was washed with TBST, and horseradish peroxidase-labelled goat anti-rabbit IgG was added for 2 hours at room temperature. After membrane washing, ECL chemiluminescence solution was added and placed in the gel imaging system to be observed and photographed. The protein gray value analysis was performed by Image J software, and the relative expression of TGF-β1 and Smad3 protein was expressed by the ratio of the target band gray scale to the β-actin gray scale of the internal reference strip.

Data was analysed in SPSS version 21. Mean value±SD was calculated for measurement data. Multiple sample averages were compared using single factor analysis of variance. The p-values less than 0.05 were regarded as significant.

RESULTS

There were significant differences in Wat/Pbm, Wai/Pbm, and Wam/Pbm between the control group, the model group and the dexamethasone group (all p<0.001). Wat/Pbm, Wai/Pbm, and Wam/Pbm in the model group

Table I: Primer sequence.

Gene	Upstream primer	Downstream primer
GAPDH	5'-ACTCAGAAGACTGTGGATGG-3'	5'-GTTGCTGTTGAAGTCACAGG-3'
TGF-b1	5'-GGAGACATGAGAGCTGCCAAC-3'	5'-CCAGCAGCATGTCTGAAGATC-3'
Smad3	5'-TGTCCATGAGAGCTCAGCA-3'	5'-TCGTACAGCATGTCTGAATGCC-3'

Table II: Comparison of airway remodelling indices in each group.

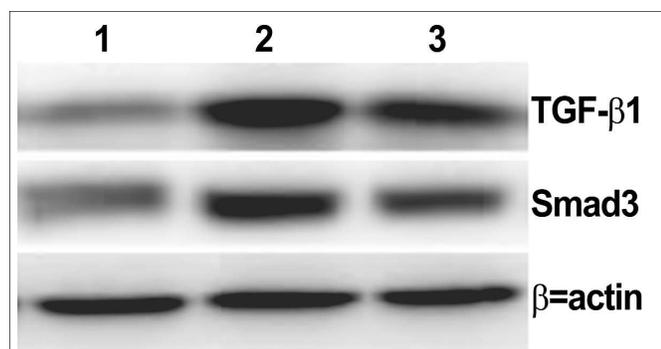
Parameters	Control group (Number of bronchus measured = 30)	Model group (Number of bronchus measured = 30)	Dexamethasone group (Number of bronchus measured = 30)	p-value
Wat/Pbm (μm ² ·μm ⁻¹)	50.73 ±3.20	73.06 ±4.89	61.22 ±3.70	<0.001
Wai/Pbm (μm ² ·μm ⁻¹)	41.92 ±3.84	65.87 ±2.59	52.41 ±3.02	<0.001
Wam/Pbm (μm ² ·μm ⁻¹)	9.17 ±1.34	25.35 ±1.99	17.08 ±2.06	<0.001

Table III: Comparison of relative expression levels of TGF- β 1 and Smad3 mRNA in lung tissue of rats in each group.

Parameters	Control group (Number of rats = 10)	Model group (Number of rats = 10)	Dexamethasone group (Number of rats = 10)	p-value
TGF- β 1 mRNA	0.92 \pm 0.57	1.98 \pm 0.45	1.21 \pm 0.17	<0.001
Smad3 mRNA	0.51 \pm 0.23	1.45 \pm 0.39	0.76 \pm 0.30	<0.001

Table IV: Comparison of relative expression levels of TGF- β 1 and Smad3 protein in lung tissue of rats in each group.

Parameters	Control group (Number of rats = 10)	Model group (Number of rats = 10)	Dexamethasone group (Number of rats = 10)	p-value
TGF- β 1 protein	0.84 \pm 0.18	1.75 \pm 0.38	0.96 \pm 0.26	<0.001
Smad3 protein	0.45 \pm 0.14	1.37 \pm 0.31	0.64 \pm 0.15	<0.001

**Figure 1:** Results of Western blot analysis of TGF- β 1 and Smad3 protein expression:

1: Control group, 2: Model group, 3: Dexamethasone group.

were higher compared with those in the control group (all $p < 0.001$); Wat/Pbm, Wai/Pbm, and Wam/Pbm in the dexamethasone group were significantly lower than those in the model group (all $p < 0.001$, Table II).

Relative expression levels of TGF- β 1 and Smad3 mRNA in the control group, the model group and the dexamethasone group were statistically different (both $p < 0.001$). Relative expression levels of TGF- β 1 and Smad3 mRNA in the model group were higher than those in the control group (both $p < 0.001$); the relative expression levels of TGF- β 1 and Smad3 mRNA in the dexamethasone group were lower than those in the model group (both $p < 0.001$, Table III).

Relative expression levels of TGF- β 1 and Smad3 protein in the control group, the model group and the dexamethasone group were statistically different (both $p < 0.001$). Relative expression levels of TGF- β 1 and Smad3 protein in the model group were higher than those in the control group (both $p < 0.001$); relative expression levels of TGF- β 1 and Smad3 protein in the dexamethasone group were lower than those in the model group (both $p < 0.001$, Figure 1 and Table IV).

DISCUSSION

Pathological changes in airway wall and airway smooth muscle layer thickening are important pathological features in asthmatic airway remodelling. Studies have found that in asthmatic airway remodelling, there is an increased number of airway smooth muscle cells (ASMCs) or cell hypertrophy, and the smooth muscle

layer is significantly thickened.⁹ Related studies have shown that the bronchial basement membrane perimeter remains relatively unchanged in the airway under different systolic and diastolic states.¹⁰ Accordingly, the bronchial basement membrane perimeter was measured to normalise the total wall area, the inner wall area, and the smooth muscle area to eliminate the effect of different airway contractions on the measurement results. This study found that the intrapulmonary bronchial Watt/Pbm, Wai/Pbm, Wam/Pbm in inhaled OVA-sensitised asthmatic rat models increased significantly compared with those in the control group, which is consistent with other reports.¹¹ Wat /Pbm, Wai /Pbm, and Wam /Pbm can be used to measure the extent of airway remodelling.

At present, the role of inhaled corticosteroids in asthmatic airway remodeling has received increasing attention.¹² Studies have found that glucocorticoid dexamethasone can inhibit ASMC proliferation.¹³ Some scholars have found that dexamethasone can alleviate the symptoms of allergic asthma rats.¹⁴ This study showed that the ratio of Wat/Pbm, Wai/Pbm, and Wam/Pbm in the model group was significantly lower than that in the dexamethasone group. It suggested that dexamethasone can inhibit the increase of airway wall and smooth muscle thickness, posing a positive effect on improving airway remodelling. This conclusion is basically consistent with previous research reports.¹⁵

Airway smooth muscle of asthmatic patients secretes a large amount of TGF- β 1, which in turn will promote hypertrophy of smooth muscle cells and goblet cells, promote collagen and fibronectin synthesis and deposit in extracellular matrix, thereby causing airway lumen narrowing and irreversible lung function changes.¹⁶

Ojiaku *et al.* revealed a potential link between airway injury-repair responses and bronchial hyperreactivity in asthma, and define TGF- β 1 signalling as a potential target to reduce AHR in asthma.¹⁷ Studies have also shown that TGF- β 1 is significantly increased in airway epithelium and submucosa of asthmatic patients, and its expression is positively correlated with airway basement membrane thickness, number of fibroblasts, and severity of asthma attacks.¹⁸ Smads family of proteins is by far the only proven substrate acted by the TGF- β 1 receptor.

Smad3 is a receptor-regulated Smad protein. When phosphorylated with Smad2 by a TGF- β 1 type I receptor, it forms a heteromultimer with Smad4 and is transferred into the nucleus to bind to DNA to activate DNA transcription, regulating the expression of the target protein. Lund *et al.* found that the development of atopic asthma in children with early rhinovirus-induced wheezing was associated with DNA methylation changes at several genomic sites in chromosomal regions previously linked to asthma; and the strongest changes in atopic asthma were detected in the promoter region of Smad3 gene at chr 15q22.33 and introns of DDO/METTL24 genes at 6q21.¹⁹ Le *et al.* suggested that Smad3 signalling is required for allergen-induced airway remodelling, as well as allergen-induced accumulation of myofibroblasts in the airway.²⁰ Studies indicate that there is a possible pathway of mouse pulmonary fibrosis model through TGF- β 1/Smad3 pathway.²¹ The results of this experiment show that dexamethasone can effectively reduce the expression of TGF- β 1, Smad3 mRNA and protein in the lung tissue of asthmatic rats, as well as delay airway remodeling.

CONCLUSION

Dexamethasone may antagonize airway remodelling by regulating TGF- β 1/Smad3 signalling pathway, likely to play a role in the treatment of bronchial asthma.

REFERENCES

- Khan SW, Hamid A, Siddiqi FA, Bakhtawar M. Frequency of allergic asthma and common aeroallergens sensitization in Pakistani patients of bronchial asthma. *J Pak Med Assoc* 2018; **68**:1217-21.
- Naseem A, Liaqat J, Zaidi SB, Iftikhar R. Sputum neutrophilia in severe persistent asthmatics. *J Coll Physicians Surg Pak* 2014; **24**:420-3.
- Brasier AR. Therapeutic targets for inflammation-mediated airway remodeling in chronic lung disease. *Expert Rev Respir Med* 2018; **12**:931-9.
- Michalik M, Wójcik-Pszczola K, Paw M, Wnuk D, Koczurkiewicz P, Sanak M, *et al.* Fibroblast-to-myofibroblast transition in bronchial asthma. *Cell Mol Life Sci* 2018; **75**: 3943-61.
- Shan L, Kang X, Liu F, Cai X, Han X, Shang Y. Epigallocatechin gallate improves airway inflammation through TGF- β 1 signaling pathway in asthmatic mice. *Mol Med Rep* 2018; **18**: 2088-96.
- Ojaku CA, Yoo EJ, Panettieri RA. Transforming growth factor β 1 function in airway remodeling and hyperresponsiveness: The missing link? *Am J Respir Cell Mol Biol* 2017; **56**:432-42.
- van der Kraan PM. Differential role of transforming growth factor-beta in an osteoarthritic or a healthy joint. *J Bone Metab* 2018; **25**:65-72.
- Schwartz JT, Becker S, Sakkas E, Wujak LA, Niess G, Usemann J, *et al.* Glucocorticoids recruit Tgfr3 and Smad1 to shift transforming growth factor- β signaling from the Tgfr1/Smad2/3 axis to the Acvr1/Smad1 axis in lung fibroblasts. *J Biol Chem* 2014; **289**:3262-75.
- Rhee CK, Kim JW, Park CK, Kim JS, Kang JY, Kim SJ, *et al.* Effect of imatinib on airway smooth muscle thickening in a murine model of chronic asthma. *Int Arch Allergy Immunol* 2011; **155**:243-51.
- James AL, Hogg JC, Dunn LA, Paré PD. The use of the internal perimeter to compare airway size and to calculate smooth muscle shortening. *Am Rev Respir Dis* 1988; **138**: 136-9.
- Tang X, Nian H, Li X, Yang Y, Wang X, Xu L, *et al.* Effects of the combined extracts of herba epimedii and fructus ligustrilucidi on airway remodeling in the asthmatic rats with the treatment of budesonide. *BMC Complement Altern Med* 2017; **17**:380.
- Jang TY, Jung AY, Kwon S, Kim YH. Hypergravity enhances the therapeutic effect of dexamethasone in allergic asthma and rhinitis animal model. *PLoS One* 2018; **13**:e0197594.
- Dixon ER, Weinberg JA, Lew DB. Effect of dexamethasone on bovine airway smooth muscle cell proliferation. *J Asthma* 1999; **36**:519-25.
- Wang ZR, Wang Q, Sui Y, Zhang ZL, Jia FJ, Fan J, *et al.* Dexamethasone alleviates allergic asthma immature rat through toll like receptor 4. *Eur Rev Med Pharmacol Sci* 2018; **22**:184-9.
- Niu C, Wang T, Zou W, Jie H, Ying L, Zhang M, *et al.* Enhanced pause correlates with airway neutrophils and airway-epithelial injury in asthmatic mice treated with dexamethasone. *J Asthma* 2018; 1-35.
- Tian X, Tian X, Huo R, Chang Q, Zheng G, Du Y, *et al.* Bacillus calmette-guerin alleviates airway inflammation and remodeling by preventing TGF- β 1 induced epithelial-mesenchymal transition. *Hum Vaccin Immunother* 2017; **13**: 1758-64.
- Ojaku CA, Cao G, Zhu W, Yoo EJ, Shumyatcher M, Himes BE, *et al.* TGF- β 1 Evokes human airway smooth muscle cell shortening and hyperresponsiveness via Smad3. *Am J Respir Cell Mol Biol* 2018; **58**:575-84.
- Okona-Mensah KB, Shittu E, Page C, Costello J, Kilfeather SA. Inhibition of serum and transforming growth factor beta (TGF- β 1)-induced DNA synthesis in confluent airway smooth muscle by heparin. *Br J Pharmacol* 1998; **125**:599-606.
- Lund RJ, Osmala M, Malonzo M, Lukkarinen M, Leino A, Salmi J, *et al.* Atopic asthma after rhinovirus-induced wheezing is associated with DNA methylation change in the SMAD3 gene promoter. *Allergy* 2018; **73**:1735-40.
- Le AV, Cho JY, Miller M, Mcelwain S, Golgotiu K, Broide DH. Inhibition of allergen-induced airway remodeling in smad3-deficient mice. *J Immunol* 2007; **178**:7310-6.
- Shi K, Jiang J, Ma T, Xie J, Duan L, Chen R, *et al.* Dexamethasone attenuates bleomycin-induced lung fibrosis in mice through TGF- β 1, Smad3 and JAK-STAT pathway. *Int J Clin Exp Med* 2014; **7**:2645-50.

