



Soufeng Yuchuan decoction mitigates the ovalbumin-induced lung damage in a rat model of asthma



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ABSTRACT

Airway remodeling is a key feature of asthma. Extracellular matrix synthesis and vascular remodeling respectively regulated by transforming growth factor (TGF- β 1) and vascular endothelial growth factor (VEGF), are important for the airway remodeling. This study aimed to investigate the effect of Soufeng Yuchuan (SFYC) decoction, a Traditional Chinese Medicine, on airway remodeling and expression of VEGF and TGF- β 1 in asthma model rats. A rat model of asthma was induced by ovalbumin (OVA) treatment. The results showed that SFYC decoction improved general conditions and reduced the damage in lung tissues in asthma model rats. Furthermore, SFYC decoction significantly reduced the OVA-induced levels of VEGF and TGF- β 1 in sera and in bronchoalveolar lavage fluid. Moreover, SFYC decoction decreased the OVA-induced VEGF mRNA and protein levels in lung tissues in asthma model rats. Interestingly, SFYC with high dose was more potent in reducing TGF- β 1 level in rat sera and BALF than dexamethasone (positive control). In summary, SFYC decoction effectively mitigates lung damage in OVA-induced asthma model rats, which was associated with inhibition of VEGF and TGF- β 1.

1. Introduction

Asthma is one of the most common chronic airway diseases in children, imposing an increasingly consistent burden on health system [1]. Glucocorticoids have been considered as the first-line agents for asthma treatment for decades. However, their clinical applications are limited by low efficacy in severe asthma and by undesired side effects associated with high dose or prolonged use. Therefore, it is extremely urgent to develop better medications to manage asthma.

Up to date, several traditional Chinese medicines have been shown to ameliorate asthmatic symptoms in asthma animal models [2–4]. On the basis of learning and drawing lessons from the existing research, our research group proposed the new idea of "collaterals obstructed by potential Feng stasis and phlegm" for asthma pathogenesis. Accordingly, the Soufeng Yuchuan (SFYC) decoction was formulated. SFYC decoction is composed of 12 traditional Chinese herbs (details in Methods below): Baijiangcan (*Bombyx Batryticatus*), Dilong (*Ground Dragon*), Dangshen (*Codonopsis Pilosula*), Xingren (*Almond*), Zhipipaye (*Roasted Folium Eriobotryae*), Mengshi (*Mica Colorata*), Laifuzi (*Semen Raphani*), Juluo (*Orange Collaterals*), Honghua (*Safflower*), Chantui

(*Periostracum Cicadae*), Zhimahuang (*Roasted Ephedra*), and Gancao (*Licorice*). Some of these herbs have been shown to suppress inflammation, to relieve cough, and to expel phlegm. However, the exact role of SFYC decoction in asthma needs to be elucidated.

Airway remodeling, also known as airway reconstruction, is one of the main pathological features of asthma [5]. Vascular endothelial growth factor (VEGF) and transforming growth factor- β 1 (TGF- β 1) are the two critical factors in the pathogenesis of airway remodeling. VEGF can be induced by allergic reaction and mediate vascular remodeling and angiogenesis, which is associated with airway remodeling [6]. TGF- β 1 has been shown to be involved in the synthesis of the matrix molecules related to airway remodeling [7,8]. Thus, the modulation of VEGF and TGF- β 1 expression may also play an important role in regulating airway remodeling in asthma.

The present study therefore attempted to explore the role of SFYC decoction on general condition and lung histopathology in a rat model of asthma, and to investigate whether its possible underlying molecular mechanisms were associated with its regulation of VEGF and TGF- β 1 expression.

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2. Materials and methods

2.1. Preparation of SFYC decoction

Upon chemical authentication, herbarium voucher specimen of *Herba Cistanches* was deposited at the First Affiliated Hospital of Henan University of Traditional Chinese Medicine (Zhengzhou, Henan, China). SFYC decoction was prepared from conventional decoction of 12 kinds of Chinese medicinal materials including Baijiangcan (10 g), Dilong (10 g), Dangshen (10 g), Xingren (10 g), Zhipipaye (10 g), Mengshi (10 g), Laifuzi (10 g), Juluo (6 g), Honghua (6 g), Chantui (6 g), Zhimahuang (6 g), and Gancan (6 g). These materials were purchased from the First Affiliated Hospital of Henan University of Traditional Chinese Medicine (Zhengzhou, Henan, China).

These materials were directly mixed with distilled water to 0.315 g/mL, 0.63 g/mL, and 1.26 g/mL, respectively. The detailed produces were as follows: Mengshi was soaked in 200 mL water and boiled for 30 min, after which other herbs which had been soaked in 800 mL water for 1 h were added and boiled together under high heat, and then decocted for 30 min under low heat, the yielded liquid (named L1) was then poured out. Then 1000 mL of water was added and boiled again following the above-mentioned decoction method, and the liquid (named L2) was also poured out. The obtained L1 and L2 were then poured into a casserole and condensed to 80 mL under heat. The concentration of the drug solution was 1.26 g/mL. The corresponding concentrations of 0.315 g/mL and 0.63 g/mL were obtained by dilution with distilled water.

2.2. Animals

Male Sprague-Dawley (SD) rats (6-week-old, 180–200 g) were purchased from the Experimental Animal Center of Henan province (Zhengzhou, Henan, China; license number: SCXK (Lu) 20140007) and maintained under specific pathogen-free conditions. All animals were fed a standard diet with controlled condition (temperature, 25 °C ± 1 °C; humidity, 50%). The animal experimental protocols were approved by the Ethics Committee of the First Affiliated Hospital of Henan University of Traditional Chinese Medicine (Zhengzhou, Henan, China). All experimental procedures that involved animals were approved according to the guidelines of the Care and Use of Laboratory Animals by the National Institute of Health, China.

2.3. Model establishment, grouping, and drug treatment

After one week of acclimatization, rats were randomly divided into the following six groups (n = 10 per group): Control + vehicle, OVA + vehicle, OVA + Dex, OVA + LSFYC, OVA + MSFYC, and OVA + HSFYC group. The rats in the Control + vehicle group were given an intraperitoneal injection of normal saline and received airway nebulization. The rats in the other groups were intraperitoneally atomized with ovalbumin (OVA) liquid prepared on the same day to establish a rat asthma model.

On the first day of the experiment, each rat, except for that in the Control + vehicle group, was intraperitoneally injected with 1 mL of 10 % OVA solution (Shanghai Xinyu Biotechnology Pharmaceutical Co., Ltd., Shanghai, China) containing 100 mg of dry powder of aluminum hydroxide (Shanghai Xinyu Biotechnology Pharmaceutical Co., Ltd., Shanghai, China). Two weeks after sensitization, these rats were administered 1 % OVA solution (dissolved in physiological saline) by nebulization to induce asthma, which began for three consecutive days, and then was stimulated once every other day for 20 min. If the rats developed respiratory acceleration, sneezing, perioral cyanosis, abdominal spasm and nodding breathing, it was suggested that asthma was successfully stimulated in rats.

The OVA-sensitized rats were administered with drugs 30 min before aerosol inhalation challenge. The rats in the Control + vehicle

group were treated with normal saline, and the rats in the OVA + Dex group were given dexamethasone (Dex; 0.75 mg/kg; Tianjin Tianyao Pharmaceutical Co., Ltd, Tianjin, China) daily for 21 days. The rats in the LSFYC, MSFYC, and HSFYC group were administered 0.315 g/mL (low dose), 0.63 g/mL (middle dose) or 1.26 g/mL (high dose) of the SFYC via gastric perfusion at a dose of 0.02 mL/g body weight once a day for 21 days, whereas rats in the Control + vehicle and OVA + vehicle group were treated with the same volume of physiological saline as vehicle once a day for 21 days. During the experiment, the general condition of each rat was observed.

2.4. Analysis of serum samples

Blood was aseptically obtained from the abdominal aortas of the rats 6 h after the final challenge, and the serum were isolated by centrifugation of the blood at 1000 × g for 10 min. The serum was collected and stored at −20 °C for further analysis. The serum levels of VEGF and TGF-β1 were detected by their commercially available enzyme-linked immunosorbent assay (ELISA) kits (USCN, Wuhan, China) according to the manufacturer's instructions.

2.5. Analysis of bronchoalveolar lavage fluid (BALF) samples

On the 36th day of the experiment, rats were intraperitoneally anesthetized with 10 % chloral hydrate and euthanized by exsanguination. An intragastric needle was quickly inserted into the trachea, and the rat right main bronchus was dissociated. Bronchoalveolar lavage was performed by washing the lungs three times with 8 mL saline at room temperature. After centrifugation at 400 × g for 10 min at 4 °C, the cell-free BALF (supernatant) was collected and stored at −20 °C for further analysis. The levels of VEGF and TGF-β1 in the BALF from the rats were examined using their commercially available ELISA kits (USCN, Wuhan, China).

2.6. Histological examination

The lung tissues were isolated from rats, washed with normal saline, fixed in 4 % paraformaldehyde solution, dehydrated with gradient alcohol, and then embedded in paraffin before being cut into 4-μm thick sections. After deparaffinization and rehydration, the sections were stained with hematoxylin and eosin (H&E) and observed using a light microscope (Olympus Inc., Tokyo, Japan). Airway wall thickness was analyzed using image analysis software ImagePro Plus 6.0.

2.7. Immunohistochemistry

The lung sections were dewaxed, rehydrated, and treated with trypsinization to retrieve the antigen. After that, 3 % H₂O₂ was added to quench endogenous peroxidase activity for 15 min. Subsequently, the sections were incubated with a primary antibody against VEGF (1:100; Abcam, Cambridge, MA, USA) overnight at 4 °C, followed by a horse-radish peroxidase-conjugated secondary antibody IgG at room temperature for 20 min. Then sections were stained with diaminobenzidine, counterstained with hematoxylin, dehydrated, and then embedded in paraffin. The sections were analyzed using a light microscope (Olympus Inc., Tokyo, Japan). IPP6.0 software was used to analyze the expression of VEGF through measuring the average optical density (OD) from 3 randomly selected high-power fields at ×400 magnification.

2.8. RNA extraction and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from lung tissues by using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) and was reverse transcribed into cDNAs using HiScript II Reverse Transcriptase (Vazyme Biotech, Nanjing, China). The cDNA template

was synthesized through qRT-PCR using SYBR Green/Fluorescein qPCR Master Mix (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The mRNA expression of VEGF was calculated by the $2^{-\Delta\Delta Ct}$ method and normalized to the internal control β -actin. The primers used were as follows:

VEGF-F, 5'-CGTCTACCAGCGCAGCTATTG-3'
 VEGF-R, 5'-CTCCAGGGCTTCATCATTGC-3'
 β -actin-F, 5'-CACGATGGAGGGGCGGACTCATC-3'
 β -actin-R, 5'-TAAAGACCTCTATGCCAACACAGT-3'

2.9. Statistical analysis

All statistical analyses were performed using SPSS 23.0 (SPSS, Inc., Chicago, IL, USA). The data are presented as the mean $\pm$ standard deviation (SD) from three independent experiments. One-way analysis of variance (ANOVA) was used to analyze differences among multiple groups. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. SFYC decoction improved general conditions in asthma model rats

The rats in the Control + vehicle group had glossy hair, good appetite and spirit. Except for the Control + vehicle group, the rats in the other groups had varying degrees of decreased appetite, sneezing, nose scratching, irritability, shortness of breath, cyanosis of lips and abdominal spasm, indicating that the asthma model was constructed successfully. Of note, the rats in the OVA + Dex group and OVA + SFYC groups (especially in the OVA + Dex group and the OVA + HSFYC group) also showed irritability and dyspnea in the early stage of excitation. However, with the continuation of drug administration, the respiratory rate was slowed down, and the cyanosis symptoms were improved.

3.2. SFYC decoction improved lung histological condition in asthma model rats

We then performed H&E staining to evaluate morphological changes in rat lung tissues (Fig. 1A). Compared with the Control + vehicle group, there were an increase in airway smooth muscle mass

(including hypertrophy and hyperplasia), a large number of inflammatory cell infiltration, disorder of airway epithelial structure and narrowing of the airway lumen in the OVA + vehicle group. In addition, compared with the OVA + vehicle group, the OVA + Dex and OVA + SFYC groups (especially the OVA + HSFYC and OVA + MSFYC groups) exhibited significantly reduced infiltration of inflammatory cells, more regular arrangement of airway epithelial cells, and significantly improved lumen stenosis. As shown in Fig. 1B, the thickness of airway wall was noticeably increased in the OVA + vehicle group relative to that in the Control + vehicle group. Compared with the OVA + vehicle group, the thickness of airway wall was notably decreased in the OVA + Dex, OVA + MSFYC, and OVA + HSFYC groups. There were no significant differences in airway wall thickness between OVA + Dex and OVA + MSFYC group or between OVA + Dex and OVA + HSFYC group.

3.3. SFYC decoction decreased VEGF expression in asthma model rats

VEGF is a key angiogenic molecule and can greatly contributes to airway remodeling in asthma [9]. Thus, we sought to determine whether the protective effect of SFYC decoction on asthma model rats was associated with its regulation of VEGF. As shown in Fig. 2A, serum level of VEGF in the OVA + vehicle group was significantly higher than that in the Control + vehicle group. Compared with the OVA + vehicle group, the serum VEGF levels were notably decreased in the OVA + Dex and OVA + SFYC groups. Furthermore, SFYC intervention decreased serum VEGF level in a dose-dependent manner. Similar expression pattern of VEGF was observed in BALF (Fig. 2B). qRT-PCR analysis of VEGF mRNA expression (Fig. 2C) and immunohistochemistry analysis of VEGF protein expression (Fig. 3A and B) in rat lungs further showed similar expression pattern of VEGF in rat sera and BALF. In addition, there was no significant difference in VEGF expression between the OVA + Dex and OVA + HSFYC group.

3.4. SFYC decoction decreased TGF- β 1 expression in asthma model rats

TGF- β 1 is also a crucial mediator of airway remodeling in asthma [10]. Accordingly, we then investigated the effect of SFYC on TGF- β 1 expression. Data revealed that serum level of TGF- β 1 in the OVA + vehicle group was significantly increased when compared with the Control + vehicle group. Furthermore, SFYC intervention notably and

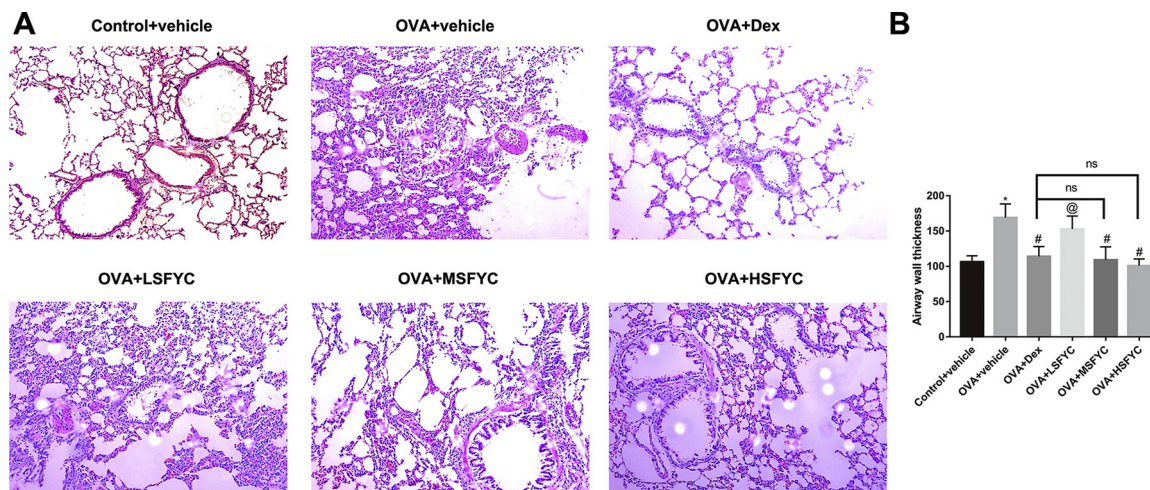


Fig. 1. SFYC decoction improved lung histological condition in asthma model rats.

A. Representative images of H&E staining showing morphological changes in lung tissues ($\times 100$) from rats in the groups of Control + vehicle, OVA + vehicle, OVA + Dex group, OVA + LSFYC, OVA + MSFYC, and OVA + HSFYC. **B.** Airway wall thickness (μ m) was analyzed using Image Pro-plus 6.0. * $P < 0.05$, vs. Control + vehicle; # $P < 0.05$, vs. OVA + vehicle; @ $P < 0.05$, vs. OVA + Dex; ns, no significance. N = 10/group.

Abbreviations: OVA, ovalbumin; Dex, dexamethasone; LSFYC, low dose of Soufeng Yuchuan; MSFYC, middle dose of Soufeng Yuchuan; HSFYC, high dose of Soufeng Yuchuan.

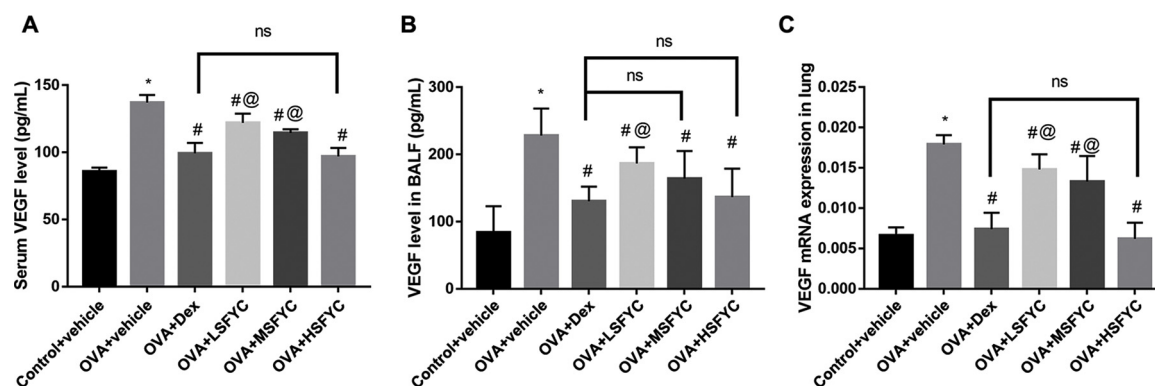


Fig. 2. VEGF expression in rat sera and BALF as well as VEGF mRNA level in rat lungs in each group.

A. Serum VEGF level in rats. B. VEGF level in BALF from rats. C. VEGF mRNA expression in rat lung tissues. * $P < 0.05$, vs. Control + vehicle; # $P < 0.05$, vs. OVA + vehicle; @ $P < 0.05$, vs. OVA + Dex; ns, no significance. N=10/group. Data are presented as the mean $\pm$ SD.

Abbreviations: OVA, ovalbumin; Dex, dexamethasone; LSFYC, low dose of Soufeng Yuchuan; MSFYC, middle dose of Soufeng Yuchuan; HSFYC, high dose of Soufeng Yuchuan; VEGF, vascular endothelial growth factor; BALF, bronchoalveolar lavage fluid.

dose-dependently decreased serum TGF- β 1 level in asthma model rats (Fig. 4A). TGF- β 1 level in BALF showed similar expression pattern (Fig. 4B). In addition, compared with the OVA + Dex group, TGF- β 1 levels in sera and in BALF were significantly decreased in the OVA + HSFYC group (Fig. 4A and B).

4. Discussion

The present study provided evidence that SFYC decoction improved general conditions in asthma model rats, as evidenced by slowed respiratory rate, and improved cyanosis symptoms. Furthermore, SFYC decoction reduced airway smooth muscle mass and inflammatory cell infiltration, and improved disorder of airway epithelial structure and narrowing of the airway lumen in asthma model rats, suggesting that SFYC decoction may inhibit asthmatic airway remodeling. Moreover, the anti-remodeling effect of SFYC decoction was comparable to that of positive control drug Dex.

Several traditional Chinese medicines have been shown to

ameliorate airway responsiveness, inflammation and remodeling in asthma animal models [2–4]. SFYC decoction is composed of 12 traditional Chinese herbs, some of which have been shown to suppress inflammation, to relieve cough, and to expel phlegm. Since SFYC decoction is a mixture of 12 herbs, it is difficult to prove which exact compounds contribute the most to the therapeutic effect of SFYC decoction in asthma. However, in general, the multiple mixture medication should be more dialectical, more conciliatory, and more balanceable [4]. From this point of view, SFYC decoction should apparently have an advantage over a single traditional herb *in vivo*.

VEGF and TGF- β 1 are both mediators of airway remodeling in asthma [10,11]. VEGF is a key angiogenic molecule and can stimulate the proliferation and migration of vascular endothelial to form new vascular, and greatly contributes to airway inflammation and airway remodeling in asthma [9]. On the other hand, TGF- β 1 contributes mainly to abnormal airway smooth muscle function in asthma by inducing airway smooth muscle cell proliferation and hypertrophy and by promoting the release of angiogenic, fibrogenic and inflammatory

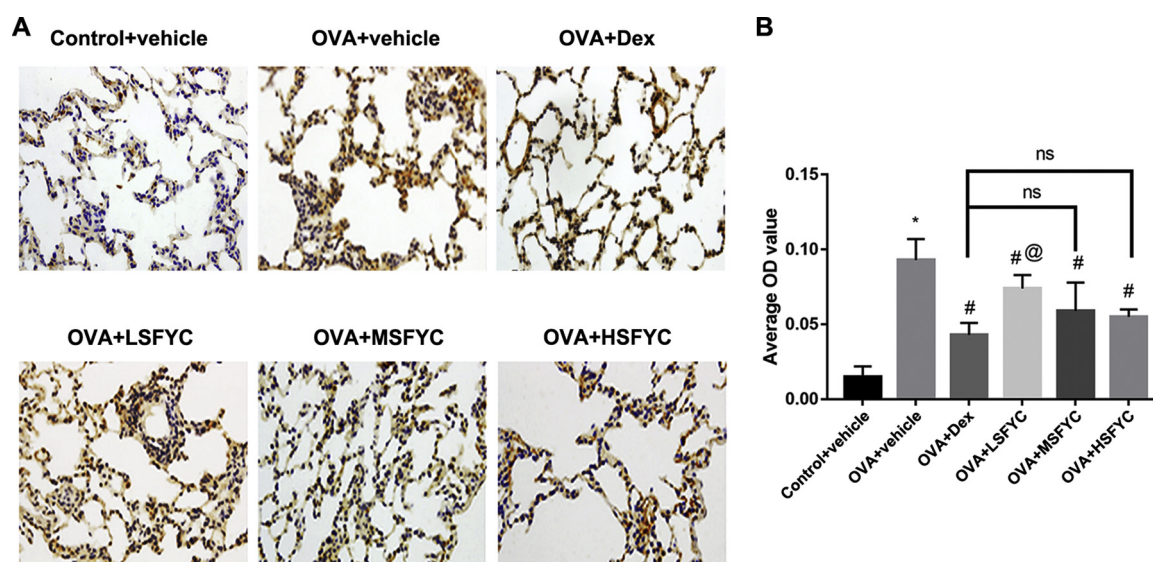


Fig. 3. Immunohistochemical analysis of VEGF expression in rat lungs in each group.

A. Representative images of VEGF immunohistochemical staining in lung tissues from rats in the groups of Control + vehicle, OVA + vehicle, OVA + Dex group, OVA + LSFYC, OVA + MSFYC, and OVA + HSFYC. B. IPP6.0 was used to analyze VEGF expression through measuring average OD values from 3 randomly selected fields at $\times 400$ magnification. * $P < 0.05$, vs. Control + vehicle; # $P < 0.05$, vs. OVA + vehicle; @ $P < 0.05$, vs. OVA + Dex; ns, not significant. N=10/group. Data are presented as the mean $\pm$ SD.

Abbreviations: OVA, ovalbumin; Dex, dexamethasone; LSFYC, low dose of Soufeng Yuchuan; MSFYC, middle dose of Soufeng Yuchuan; HSFYC, high dose of Soufeng Yuchuan; VEGF, vascular endothelial growth factor; OD, optical density.

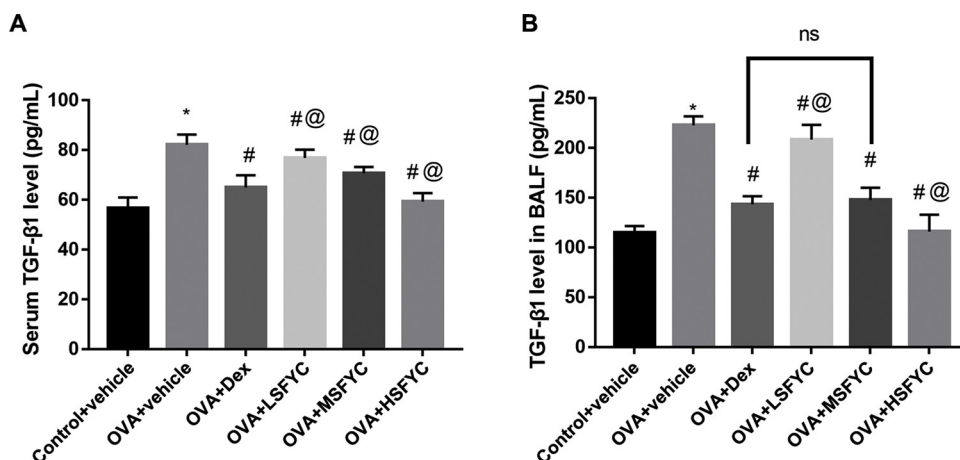


Fig. 4. TGF-β1 expression in rat sera and BALF in each group.

A. Serum TGF-β1 level in rats. **B.** VEGF level in BALF from rats. * $P < 0.05$, vs. Control + vehicle; # $P < 0.05$, vs. OVA + vehicle; @ $P < 0.05$, vs. OVA + Dex. ns, no significance. $N = 10$ /group. Data are presented as the mean $\pm$ SD. Abbreviations: OVA, ovalbumin; Dex, dexamethasone; LSFYC, low dose of Soufeng Yuchuan; MSFYC, middle dose of Soufeng Yuchuan; HSFYC, high dose of Soufeng Yuchuan; TGF-β1, transforming growth factor-β1; BALF, bronchoalveolar lavage fluid.

mediators [12]. Furthermore, levels of VEGF and TGF-β1 have been shown to be increased in chronic lung diseases such as asthma [10,13]. Consistent with this, in the present study, we observed an increase in levels of VEGF and TGF-β1 in OVA-induced asthma model rats. The important role of VEGF and TGF-β1 in mediating airway remodeling prompts us to raise the idea that the modulation of VEGF and TGF-β1 expression may play an important role in regulating airway remodeling and therefore affect the progress of asthma. The interesting findings in this study were that, SFYC decoction significantly reduced the OVA-induced levels of VEGF and TGF-β1 in asthma model rats. These data suggest that inhibition of VEGF and TGF-β1 may be involved in the anti-airway remodeling effect of SFYC decoction in asthma.

Evidence has also shows the interaction between VEGF and TGF-β1 in asthma. It has been reported that inhibition of VEGF blocks TGF-β1 production in a murine model of allergic airway disease [11]. On the other hand, TGF-β1 time-dependently facilitates the release of VEGF by airway smooth muscle cells [14]. These findings indicated a close relationship between VEGF and TGF-β1 in regulating airway remodeling in asthma. However, the mechanism underlying SFYC decoction-mediated suppression of airway remodeling, which seems to be due to an inhibition of VEGF and TGF-β1, indeed requires further investigation.

This study has several limitations. One is that there is no exact composition of the SFYC decoction, which needs further study. In addition, further investigation is required to clarify whether upregulation of VEGF and TGF-β1 could rescue the protective effect of SFYC decoction on asthma.

In summary, the increased expression of VEGF and TGF-β1 in asthma model rats indicates that VEGF and TGF-β1 may be involved in airway remodeling. Early use of SFYC decoction in the treatment of asthma may reduce expression of VEGF and TGF-β1 and therefore ameliorate airway remodeling. Our findings support a possible application of SFYC decoction as a therapeutic drug for asthma patients.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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