Danggui Buxue Tang ameliorates bleomycin-induced pulmonary fibrosis in rats through inhibiting transforming growth factor-β1/Smad3/plasminogen activator inhibitor-1 signaling pathway


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Abstract

OBJECTIVE: To investigate the effect of Danggui Buxue Tang (DBT), a decoction from Traditional Chinese Medicine, on bleomycin-induced pulmonary fibrosis in rats, and to propose the possible underlying mechanism.

METHODS: Forty male Sprague-Dawley rats were randomly divided into sham group, model group, prednisone group and DBT group. Pulmonary fibrosis rat model was established by intratracheal injection with bleomycin. Body weight and lung index were monitored. Histopathologic examination and collagen deposition were determined using Hematoxylin and eosin (HE) and Masson’s trichrome staining. Immunohistochemistry staining was applied to observe the expression of alpha-smooth muscle actin (α-SMA), mRNA expression of α-SMA, collagen I and collagen III were measured by real-time fluorescence quantitative PCR (RT-qPCR). Inflammatory cytokines, including tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and IL-1β in serum were detected by Enzyme-linked immunosorbent assay. Alkaline hydrolysis method was conducted to investigate the content of hydroxyproline (HYP). Transforming growth factor-β1 (TGF-β1), Smad3 and plasminogen activator inhibitor-1 (PAI-1) protein level were examined by Western blot assay.

RESULTS: DBT significantly reduced the severity of bleomycin-induced pulmonary fibrosis and inflammation as indicated by minimizing the lost of weight, and by lowering the levels of lung index, inflammation score, Ashcroft score, collagen volume fraction (%), HYP, α-SMA, collagen I, collagen III, TNF-α, IL-6, IL-1β, TGF-β1, Smad3 and PAI-1, consistent with the effect of prednisone.

CONCLUSION: Our findings suggest that DBT is able to ameliorate the pulmonary fibrosis, the possible mechanism may involve inhibition of pulmonary inflammation and collagen deposition, possibly via suppressing TGF-β1/Smad3/PAI-1 signaling pathway.

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**Keywords:** Pulmonary fibrosis; Transforming growth factor beta 1; Smad3 protein; Plasminogen inactivators; Danggui Buxue Tang

**INTRODUCTION**

Pulmonary fibrosis, especially idiopathic pulmonary fibrosis, is a chronic lung disease and is characterized by excessive deposition of extracellular matrix (ECM) to mesenchymal transition, and then leads to a progressive impairment of lung function, ultimately resulting in respiratory failure and death. Several studies have observed that environmental pollution, bacterial infections, smoking, gastroesophageal reflux and some drugs include bleomycin are closely related to the onset of idiopathic pulmonary fibrosis. In recent years, the mortality rate of idiopathic pulmonary fibrosis is considerably increased, and it substantially threatens human health. However, current treatments for idiopathic pulmonary fibrosis are limited by low efficacy and severe side effects. The two drugs, nintedanib and pirfenidone, are approved to treat idiopathic pulmonary fibrosis by the US Food and Drug Administration (FDA) recently, which are contributed to stabilize patients’ conditions, but they do not reverse the progression of fibrosis. Therefore, it is necessary that further research is to be performed to explore new treatment methods for idiopathic pulmonary fibrosis.

Danggui Buxue Tang (DBT) is an ancient Chinese herbal decoction from traditional Chinese medicine, which was first recorded in Nei Wai Shang Bian Huo Lun during the Jin dynasty (1247 BC). Recent pharmacological studies confirmed that DBT had the ability to improve bone loss, immune modulation and fibrosis. For example, Zhou et al demonstrated that DBT alone and in combinations with SERMs could exert bone protective effects in vitro and in vivo. Moreover, this prescription promotes the adhesion and migration of bone marrow stromal cells via the focal adhesion pathway in vitro, and can ameliorate chronic fatigue syndrome through immune modulation and may act to normalize cytokines and their related signaling pathways. Furthermore, DBT can suppress high glucose-induced proliferation and ECM accumulation of mesangial cells via inhibiting Long non-coding RNA (lncRNA) PVT1. Additionally, DBT attenuates tubulointerstitial fibrosis via suppressing NOD-like receptor protein 3 (NLRP3) inflammasome in a rat model of unilateral ureteral obstruction. However, the role and molecular mechanism of DTB on bleomycin-induced pulmonary fibrosis remain unclear.

Transforming growth factor β1 (TGF-β1) is regarded as a key regulator of cell proliferation, differentiation, migration, immune regulation and ECM transformation in fibrosis diseases. The key regulator can contribute to fibrosis through both Smad-based and non-Smad-based signaling pathways, leading to activation of myofibroblasts, over-production of ECM and inhibition of ECM degradation. However, the knowledge of the efficacy of DBT on TGF-β1/Smad signaling pathway in blocking pulmonary fibrosis remain uncovered. The present study is to elucidate the effect of DBT on bleomycin-induced pulmonary fibrosis and to identify that DBT performs a critical role in the regulation on TGF-β1/Smad signaling pathway.

**METHODS**

**Animals**

A total of 40 adult (6-8 weeks) male Sprague Dawley rats weighing about 180-200 g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China, SPF grade, Certificate number: SCXK2012-0001). All rats were handled according to the National Institutes of Health Guide for Care and Use of Laboratory Animals and experiments were approved by the Ethics Committee for Animal Experiments of Hebei University of Chinese Medicine. All rats were maintained at 20-24°C with a relative humidity of 45%-55% and normal photoperiods (12 h light/12 h dark). The rats were given a normal pelleted diet and tap water ad libitum.

**Preparation of the DBT**

DBT was prepared with two Chinese medicinals: Huangqi (Radix Astragali Mongolici) and Danggui (Radix Angelicae Sinensis) in a ratio of 5:1. The prescription is in the form of formula granules and was obtained from Guangdong Yifang Pharmaceutical Co., Ltd. (Foshan, China) (Table 1).

**Animal grouping and modeling**

After adaptively reared for 1 week, animals were randomly divided into 4 equal groups (n = 10 per group): sham group, model group, prednisone group and DBT group. Pulmonary fibrosis rat model was established by infusing bleomycin (NIPPON KAYAKU, Tokyo, Japan) through intratracheal injection (5 mg/kg). Gavage administration was conducted on 2nd day after surgery: sham group and model group were gavaged with normal saline (1 mL·100 g⁻¹·d⁻¹), prednisone group was gavage with prednisone aqueous solution (0.5 mg·100 g⁻¹·d⁻¹); the tablet was obtained from Zhejiang...

<table>
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<th>Granule name</th>
<th>Lot number</th>
<th>Packingsize (g/package)</th>
<th>Equivalent to crude drug (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huangqi (Radix Astragali Mongolici)</td>
<td>8017093</td>
<td>200</td>
<td>1000</td>
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<tr>
<td>Danggui (Radix Angelicae Sinensis)</td>
<td>8032653</td>
<td>200</td>
<td>660</td>
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Xianju Pharmaceutical Co., Ltd., Xianju, China), and DBT group was gavage with DBT decoction (0.081 g · 100 g ⁻¹ · d⁻¹, calculated according to 60 kg body weight for adults and equal to 0.375 g · 100 g ⁻¹ · d⁻¹ crude drugs). The cycle of gavage administration was 28 d. Body weight changes were measured on days 0, 7, 14 and 28. On day 28 after intragastric administration, the rats were anesthetized with intraperitoneal injection of 2% pentobarbital sodium (4 mg/100 g body weight), and then their lungs were removed and weighted to compute pulmonary index [weight of wet pulmonary (mg)/weight of body (g) × 100%]. The left lungs were fixed with 4% paraformaldehyde for histopathological examination and immunohistochemistry staining. Meanwhile, the right lungs were stored at −80 °C for real-time quantitative polymerase chain reaction (RT-qPCR) assay and Western blot assay.

Histopathology examination
Pulmonary specimens were sectioned into 4 μm thick slices and then deparaffinized in xylene for 15 min (5 min for per time), hydrated in gradient alcohol, and then rinsed with 1% phosphate buffered saline (PBS) three times. Hematoxylin and eosin (HE) and Mason’s trichrome staining were carried out for morphologic detection. Alveolitis inflammation (Spaziol score system) and degree of lung fibrosis (collagen volume fraction and Ashcroft score system) were quantified as described previously. Collagen volume fraction (%) = area of collagen fiber/area of total view.

Content of hydroxyproline (HYP) examination
To measure the level of HYP, 100 mg lung tissue was weighed and homogenated. Alkali hydrolysis assay was conducted to investigate the content of HYP, 1 ml hydrolytic liquid was added and the mixture was boiled in water for 20 min in concordance with the instruction manual. The absorbance value was examined at 550 nm on a spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA, USA). HYP detection kit was purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

Immunohistochemistry examination
Expression of α-SMA was examined by immunohistochemistry. The formalin-fixed lung tissue was sliced into 4 μm thick. The sections were immersed in xylene for 15 min (5 min for per time) before being rehydrated in water by using an gradient ethanol. Then the sections were heated in citric acid for 15 min. After the slices were cooled to room temperature, the sections were washed with 1% PBS buffer for 15 min (5 min for per time) before incubated with 3% H₂O₂ for 10 min. Then the sections were blocked with 5% bovine serum albumin (BSA, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) for 10 min. Subsequently, α-SMA mouse monoclonal antibody (Abcam, Cambridge, UK, USA; diluted into 1 : 100) was added and incubated overnight at 4 °C. Then, anti-mouse secondary antibody (1 : 100, Beijing Zhongshan Jingqiao Biological Technology Co., Ltd., Beijing, China) was incubated in 37 °C for 30 min. After that, diaminobenzidine (DAB) color development kit (Beijing Zhongshan Jingqiao Biological Technology Co., Ltd., Beijing, China) was applied. As the slices were dried, the images were viewed by microscope (Olympus Corporation, Tokyo, Japan). Mean optical density (MOD) was measured by Image-Pro Plus 6.0 (MOD = integrated option density / positive area).

Measurement of inflammatory cytokines by Enzyme-linked immunosorbent assay (ELISA)
ELISA was performed to measure the content of tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6) and IL-1β in serum. The rats were sacrificed to collect the blood. Then, the samples were centrifuged (3500 rpm, 4 °C, 15 min) to gather supernatants. The inflammatory cytokines content was examined according to the manufacturer’s protocol. The optical density was determined at 450 nm on a spectrophotometer (Molecular Devices Corporation, Sunnyvale, CA, USA). ELISA kit for TNF-α, IL-6 and IL-1β were provided by IBL International GmbH (Hamburg, Germany).

RT-qPCR examination
The mRNA expression of α-SMA, collagen I and collagen III were measured by RT-qPCR. The total RNA of the lung tissues were extracted by using estep super total RNA extraction kit (Promega Corporation, Madison, WI, USA). Thermo Nanodrop 2000 was used to detect the quality and the concentration of the extracted RNA. The total RNA was added to the reverse transcription reaction system (10 μL), and was reverse transcribed to cDNA by the specific RT primer according to the manufacturer’s instructions (Promega Corporation, Madison, WI, USA). RT-qPCR was performed on a BioRad real-time PCR instrument (BioRad Laboratories, Hercules, CA, USA). β-actin was used as an internal control. The primers shown follows were synthesized by Sangon Biotech (Shanghai, China): α-SMA 5'-GTGCTGTCCTCCCTATGCTTCTGG-3' (Forward), 5'-GGCACGTGTAGTACACATCC-3' (Reverse), 77 bp; collagen I: 5'-TGCCGTCGACCTCAAGATGTCG-3' (Forward), 5'-ACAAGCGTGCTGTAGTGA-3' (Reverse), 462 bp; collagen III: 5'-ACGTAGATGAATTTGGATGCG-3' (Forward), 5'-GGTTGGGGCGACTGTC-3' (Reverse), 154 bp; β-actin 5'-CTCTAGATGACACGTCG-3' (Forward), 5'-ACATCTGCTGGAAGTGG-3' (Reverse), 104 bp. Samples underwent pre-denaturation at 95 °C for 10 min, followed by 45 cycles of 95 °C denaturation for 15 s, and 60 °C annealing and extension for 60 s. Expression level was calculated by using the formula 2-ΔΔCt.

Western blot analysis
Protein expression of TGF-β1, Smad3 and plasmino-
The lung tissues in the sham group showed the intact chrome staining of pulmonary tissue were performed. Reduced pulmonary fibrosis in rat, HE and Masson’s tri-histopathological lesion and pulmonary inflammation were intratracheal instillation with or without DBT treatment in bleomycin-induced rat. Changes in body weight and pulmonary index after DBT treatment in bleomycin-induced rat

The scores of inflammation and pulmonary fibrosis in model group were increased compared with that in sham group, while DBT and prednisone treatment significantly suppressed the progression of pulmonary fibrosis (Figure 2C, 2D).

Efficacy of DBT on collagen biomarkers in bleomycin-induced pulmonary fibrotic rat

In order to explore the possible mechanism of DBT on alveolar wall, clear alveolar cavity and no obvious inflammatory cells infiltration, whereas bleomycin injured lungs exhibited collapse alveolar space, destroyed and disordered alveoli, collapsed and destructed, emphysema, and infiltration of inflammatory cells (Figure 2A). The lung tissue specimens of the bleomycin-induced rat treated with DBT displayed obviously improvements in inflammation, which is similar to that of treatment with prednisone (Figure 2A). Masson’s trichrome staining showed a higher collagen volume fraction (Figure 2E), obliteration of interalveolar septum, and damage of lung structure in model group, and the level of destruction of pulmonary interstitium and severity of collagen fibrosis in the alveolar septa were reduced in groups of DBT and prednisone (Figure 2B). The scores of inflammation and pulmonary fibrosis in model group were increased compared with that in sham group, while DBT and prednisone treatment significantly suppressed the progression of pulmonary fibrosis (Figure 2C, 2D).
pulmonary fibrosis, based on the inhibitory effect of it on collagen deposition in lung tissue of rat, α-SMA were detected by immunohistochemical staining (Figure 3A). The result observed that bleomycin caused a significant increase in α-SMA MOD level, and was reduced by DBT and prednisone treatment (Figure 3B). We next measured the α-SMA mRNA, collagen I mRNA, collagen III mRNA by RT-qPCR and the level of HYP to further determine the therapeutic efficacy of DBT in bleomycin-induced pulmonary fibrotic rat. As shown in Figure 3C-3E, the level of α-SMA mRNA, collagen I mRNA and collagen III mRNA in lung tissues from bleomycin-induced rat was significantly enhanced compared with the sham group, while DBT and prednisone could significantly inhibit upregulation of α-SMA, collagen I and collagen III mRNA level in lung tissues of bleomycin-induced rat. Additionally, the HYP content of model group was remarkably higher than the sham group, and DBT exhibited a decrease in the HYP contents compared with model group (Figure 3F).

Table 2 Changes of body weight in each group (g ± s)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 28</th>
</tr>
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<tbody>
<tr>
<td>Sham</td>
<td>6</td>
<td>216.57±6.61</td>
<td>245.72±19.16</td>
<td>327.77±8.71</td>
<td>383.67±12.30</td>
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<tr>
<td>Model</td>
<td>6</td>
<td>214.95±2.94</td>
<td>189.28±23.29</td>
<td>241.77±20.8</td>
<td>275.30±21.95</td>
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<tr>
<td>Prednisone</td>
<td>6</td>
<td>216.22±3.27</td>
<td>208.32±9.27</td>
<td>273.63±25.38</td>
<td>337.88±12.73</td>
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<tr>
<td>DBT</td>
<td>6</td>
<td>218.47±5.13</td>
<td>219.95±7.65</td>
<td>279.33±41.24</td>
<td>345.70±14.99</td>
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</table>

Notes: sham group: intratracheal instillation with physiological saline and gavaged with normal saline (1 mL·100 g-1·d-1); model group: intratracheal instillation with bleomycin (5 mg/kg) and treated with normal saline (1 mL·100 g-1·d-1); prednisone group: intratracheal instillation with bleomycin (5 mg/kg) and gavaged with prednisone aqueous solution (0.5 mg·100 g-1·d-1); DBT group: intratracheal instillation with bleomycin (5 mg/kg) and gavaged with DBT (0.081 g·100 g-1·d-1). All treatment was intragastric administration. Compared with sham group, *P < 0.01; compared with model group, †P < 0.01. DBT: Danggui Buxue Tang group.

Figure 2 Efficacy of DBT on bleomycin-induced pulmonary fibrosis in vivo
A1-A4: representative images of HE staining of lung sections (× 20); B1-B4: representative images of Masson’s trichrome staining of lung sections (× 20); A1, B1: sham group; A2, B2: model group; A3, B3: Pre group; A4, B4: DBT group. C: inflammation scores analyzed by Szapiel score system; D: aschcroft scores analyzed by Ashcroft score system; E: collagen volume fraction measured by Image-Pro Plus 6.0 and calculated through area of collagen fiber/area of total view.

Sham group: intratracheal instillation with physiological saline and gavaged with normal saline (1 mL·100 g-1·d-1); Model group: intratracheal instillation with bleomycin (5 mg/kg) and treated with normal saline (1 mL·100 g-1·d-1); Prednisone group: intratracheal instillation with bleomycin (5 mg/kg) and gavaged with prednisone aqueous solution (0.5 mg·100 g-1·d-1); DBT group: intratracheal instillation with bleomycin (5 mg/kg) and gavaged with DBT (0.081 g·100 g-1·d-1). All treatment was intragastric administration. Each bar represents a mean value, and vertical hashes represent the standard deviation. Scale bars = 50 μm for all images. *P < 0.01, compared with sham group; †P < 0.01 compared with model group. Sham: sham group; Model: model group; Pre: prednisone group; DBT: Danggui Buxue Tang group; HE: hematoxylin-eosin staining.
Efficacy of DBT on inflammatory cytokines in bleomycin-induced pulmonary fibrotic rat

For the purpose of probing the mechanisms of DBT on curbing pulmonary inflammation, inflammatory cytokines such as TNF-α, IL-6 and IL-1β in serum of pulmonary fibrotic rat were examined by ELISA. The results prompt that a higher content of TNF-α, IL-6 and IL-1β in model rats compared with sham group. However, in prednisone and DBT group, the levels of TNF-α, IL-6 and IL-1β were significantly lowered (Figure 4A-4C).

DBT blocks TGF-β1/Smad3/PAI-1 signaling pathway in lung tissues of bleomycin-induced pulmonary fibrotic rat

To develop the possible molecular mechanism of DBT for inhibiting the process of pulmonary fibrosis, we detected the protein expression of TGF-β1, Smad3 and PAI-1 in lung tissues of each groups. The results showed that bleomycin-treated model group had a significant increase in the levels of TGF-β1, Smad3 and PAI-1. DBT and prednisone significantly inhibited the increase of TGF-β1, Smad3 and PAI-1 levels caused by bleomycin (Figure 5).

DISCUSSION

Idiopathic pulmonary fibrosis, the most common and devastating form of lung fibrosis has a mortality rate that even exceeds that of many cancers, but the treatment options for pulmonary fibrosis are remain limited. Our study showed that DBT significantly reduced bleomycin-induced pulmonary fibrosis through improving histopathological lesion and body weight loss as well as decreasing pulmonary index visibly. In addition, we observed that DBT decreased the increased level of α-SMA, collagen I and collagen III and the content of HYP in lung fibrosis rats modeled by bleomycin. Furthermore, DBT attenuates pulmonary inflammation via repressing the secretion of inflammatory cytokines. Moreover, we demonstrated that the molecular mechanism of DBT for suppressing pulmonary fibrosis was evaluated through TGF-β1/Smad3/PAI-1 signaling pathway in rat.

![Figure 3 Efficacy of DBT on collagen biomarkers in bleomycin-induced pulmonary fibrotic rat](image-url)
bleomycin (5 mg/kg) and gavaged with DBT (0.081 g·100 g⁻¹·d⁻¹), all treatment was intragastric administration. Each bar represents a mean value, and vertical hashes represent the standard deviation. Sham: sham group; Model: model group; Pre: prednisone group; DBT: Danggui Buxue Tang group; ELISA: Enzyme-linked immunosorbent assay; TNF-α: tumor necrosis factor alpha; IL-6: interleukin-6; IL-1β: interleukin-1 beta. *P < 0.01, compared with sham group; †P < 0.01 compared with model group.

The rat model of bleomycin-induced pulmonary fibrosis was widely performed to develop the pathogenesis and evaluate new therapeutic strategies for idiopathic pulmonary fibrosis in human.19,20 In our study, bleomycin caused a significant lung injury, as revealed by significantly increased pulmonary index, pulmonary inflammatory score, pulmonary fibrosis score, collagen volume fraction and reduced body weight at day 28 post bleomycin injection. Treatment with DBT remarkably attenuated the effects of bleomycin on pulmonary fibrosis.

Inflammation is an important trigger for pulmonary fibrosis. Up to now, many reports have described that the molecular mechanisms of pulmonary fibrosis are involved in the excessive release of inflammatory cytokines.21 In bleomycin induced fibrosis, inflammatory cells mainly include neutrophils, macrophages and lymphocytes.22 Macrophages would excrete chemokines which recruit inflammatory cells and pro-fibrotic cytokines like TNF-α, IL-6 and IL-1β to create a fibrotic microenvironment.23,24 Here, ELISA results reveal that secretions of TNF-α, IL-6 and IL-1β contribute to the
pulmonary inflammation, while DBT can relieve the bleomycin caused lung tissue injury via inhibiting the secretion of inflammatory cytokines. ECM deposition is the main pathological features of pulmonary fibrosis. As the lung was injured, under the stimulation of the inflammatory cytokines, the fibrocyte transferred to myo-fibroblast (MyoFb) to produce large amounts of ECM. As a major component of vascular smooth muscle cells, α-SMA is a specific marker protein of MyoFb activation. Collagen I, collagen III and HYP are the main component in extracellular collagen and the major biomarkers of fibrosis, regarded as the indicator of pulmonary fibrosis.\(^3\) Our study showed that bleomycin treatment increased the level of α-SMA, collagen I, collagen III and HYP content in lung tissues, whereas they were reduced by DBT. These results suggest that DBT could prevent bleomycin-induced pulmonary inflammation and fibrosis.

TGF-β1 mediated Smad3 signaling pathway has a pivotal role among various factors that regulate lung fibrosis.\(^2\) When TGF-β1 binds with its receptor II (TβRII), the kinase of TGF-β receptor I (TβRI) was activated. TβRII gets phosphorylated and then phosphorylates Smad2 and Smad3 which bind to Smad 4 to constitute a Smad complex. The complex is then shifted to the nucleus to regulate the transcription of target genes which is involved in the initiation and progression of fibrosis and the deposition of collagen I and collagen III. Consequently, TGF-β1/Smad3 pathway may be a therapeutic target for the treatment of lung fibrosis. For example, ponatinib ameliorates pulmonary fibrosis by suppressing TGF-β1/Smad3 pathway, and all-trans-retinoic acid ameliorates bleomycin-induced pulmonary fibrosis by downregulating the TGF-β1/Smad3 signaling pathway in rats.\(^2\) PAI-1 is one of the most important target genes in the TGF-β1/Smad signaling pathway, which can hinder the degradation of ECM composition.\(^2\) Levels of PAI-1 are obviously enhanced in lung tissues and the ATII cells of patients with diverse lung diseases, its most severe clinical counterpart idiopathic pulmonary fibrosis, cigarette smoke and bleomycin-induced lung injuries.\(^3\) Huang et al.\(^3\) reported that a PAI-1 inhibitor obviously inhibited the TGF-β1-induced expression of α-SMA and fibronectin in human lung fibroblasts, suggesting that targeting PAI-1 as a downstream effector of TGF-β can become a promising therapeutic strategy for pulmonary fibrosis.\(^3\) Here, we showed that bleomycin-treated rat increased Smad3 and PAI-1 expression, and DBT treatment significantly inhibited the increase of Smad3 and PAI-1 levels caused by bleomycin. These results suggest that DBT was able to inhibit TGF-β1/Smad3/PAI-1 signaling in bleomycin-induced rats.

In conclusion, our findings suggest that DBT may be a potential therapeutic agent for the treatment of patients with idiopathic pulmonary fibrosis. The mechanism behind it might involve the inhibition of the pulmonary inflammation and ECM deposition, and the molecular mechanism may involve inflammatory cytokines and TGF-β1/Smad3/PAI-1 pathway.

REFERENCES

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