Idiopathic pulmonary fibrosis (IPF) is a progressive interstitial lung disease with a poor prognosis, consequently causing high morbidity and mortality.¹,² Unfortunately, there are currently no effective drugs that reverse organ fibrosis,³ therefore, there is an urgent unmet clinical need for novel modulators of pulmonary fibrosis and tissue remodeling.

Although the pathogenesis of IPF still remains unclear, studies demonstrate that IPF is characterized by accumulation of fibroblasts and myofibroblasts and deposition of extracellular matrix protein.⁴,⁵ As one of the most important pro-fibrogenic cell factors, transforming growth factor-β1 (TGF-β1) has been shown to increase connective tissue synthesis and inhibit connective tissue proteases, which contribute to significant pulmonary fibrosis.⁶,⁷ The excess of TGF-β1 or/and the increased susceptibility of fibroblasts to it are possible causes of persistent tissue remodeling.⁸ As a consequence, the expression of alpha-smooth muscle actin (α-SMA) increases when fibroblasts differentiate into myofibroblasts, which results in a highly contractile cell type responsible for increased tissue contraction and production of extracellular matrix (ECM) components in tissue repair and fibrosis. However, another critical aspect in tissue repair is correct bidirectional signaling between fibroblasts and the ECM.⁹ Caveolin-1 (Cav-1) plays an important role in monitoring this homeostasis by connecting various cell signaling events in tissue repair.⁹ Down-regulation of Cav-1 expression significantly lowers its negative regulation effect on multiple signaling pathways such as TGF-β1, Smad etc., and thus accelerates pulmonary fibrosis process.¹⁰,¹¹

Panax Notoginseng Saponins (PNS), the biologically active constituent extracted from the root of Panax notoginseng (Araliaceae), have been well known to have anti-tumor, anti-ischemia, anti-ageing and anti-atherosclerosis activities. Recent evidence implied that PNS significantly attenuated lung inflammation and prevented the progress of pulmonary fibrosis in rat model.¹² However, PNS consist of 20 saponins and it is unclear which component is the most effective saponin against pulmonary fibrosis. Ginsenoside Rg1 (Rg1) is the major active molecules in PNS¹³ and has been reported to play protective roles in hepatic fibrosis and renal interstitial fibrosis in rat models. Therefore, in the present study, we assessed the influence of Rg1 in a rat model of bleomycin-induced pulmonary fibrosis and investigated its potential mechanism.

**MATERIALS AND METHODS**

**Animals** Male Sprague-Dawley rats weighing 210 to 230 g were purchased from Henan Experimental Animal Centre (Henan, China). The rats were fed a standard laboratory chow and maintained at 23±1°C, constant humidity (50–60%) with a 12-h light/dark cycle, and free access to food and water. All animals used in the study received humane care in compliance with the Xinxiang Medical University Institutional Animal Care and Use Committee that follows the Guidance to Treat Experimental Animal published by the Ministry of Science and

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Experimental Protocol

The rats were randomly divided into the following six groups (10 rats in each group): Control group, BLM group, BLM+prednisone acetate (5 mg/kg), BLM+Rg1 groups at the dosages of 18, 36, 72 mg/kg, respectively. The rats in all groups were administrated by gavage daily with a volume of liquid of 5 mL/kg. In the control and BLM groups, normal saline were given. The rats in the BLM+Rg1 three groups were given Rg1 (purity 99%, Chengdu Herbpurity Co., China) at a dosage of 18, 36, or 72 mg/kg, respectively. After being administered drugs for 7 d, 5 rats from each group were anesthetized and then euthanized, and other rats were sacrificed after giving drug continuously for 28 d. Both lungs were quickly excised, washed, and stored.

Lung Coefficient Measurement

Lung coefficient ([lung wet weight (mg)/body weight (g)]×100) is an index for evaluating lung edema. The lungs were removed as described in the Li report with slight modifications. After the lungs were cleaned with ice-cold phosphate buffered saline (PBS) solution, the lungs were blotted with a piece of filter paper and other lungs were cleaned with ice-cold phosphate buffered saline (PBS) and then weighing. A midline incision was made in the neck and the trachea was exposed by blunt dissection. BLM (Nippon Kayaku Co., Japan) was quickly injected into the trachea at a dose of 5 mg/kg (50 µL in volume). Control rats were given a single intratracheal dose of saline alone.

Animal Model of Bleomycin-Induced PF

Pulmonary fibrosis model was induced in rat by single endotracheal injection of bleomycin (BLM) with dosage of 5 mg/kg as previously described with minor modifications. Briefly, rats were anesthetized with intraperitoneal injection of 300 mg/kg of chloral hydrate and weighted. A midline incision was made in the neck and the trachea was exposed by blunt dissection. BLM (Nippon Kayaku Co., Japan) was quickly injected into the trachea at a dose of 5 mg/kg (50 µL in volume). Control rats were given a single intratracheal dose of saline alone.

Hematoxylin and Eosin (HE) Staining

HE staining was used to assess pathological changes and determine the classification of alveolitis after 7 d of drug administration. After dehydration by graded ethanol, right upper lobe of the lung was put into dimethylbenzene until completely transparent. The specimens were then immersed in melted mixed wax and put into an incubator at 75°C for 4 h to be embedded. A 4.5-mm of right upper lobe was cut by coronal section. Paraffin sections (8 µm) were put on glass slides and stained with hematoxylin for 5–10 min and eosin for 2–3 min after routine dewaxing. A high-definition image analyzer was used for histopathological examination. The remained were maintained at −80°C for next analyses.

Masson's Trichrome Stains

Masson's trichrome stains were used to assess pathological changes and the classification of PF after 28 d of drug treatment. The paraffin section of 5 µm was made from right middle lobe of the lung and stained with Masson trichrome blue. Paraffin sections were cut on glass slides and stained with hematoxylin for 6 min, Masson compound staining fluid for 5 min, 5% phosphotungstic acid for 5 min and 2% aniline blue for 5 min after routine dewaxing. A high-definition image analyzer was used for histological examination of the specimen. The more is the color of blue, the heavier is the degree of pulmonary fibrosis. The classification of pulmonary fibrosis was as follows: no pulmonary fibrosis (−), light degree of pulmonary fibrosis (+, lesions range <20% in the whole lung), moderate pulmonary fibrosis (+++, lesions range ca. 20–50% in the whole lung), and severe pulmonary fibrosis (++++, lesions range >50% in the whole lung, accompanied by alveolar fusion and lung parenchyma structural changes). The degree of PF was recorded as 0, 1, 2 and 3 points, which was correlated with −, +, ++ and ++++, respectively.

The Measurement of Collagen Biomarkers in PF

The contents of hydroxyproline (HyP) and alpha smooth muscle-actin (α-SMA) in lung tissue were respectively determined using enzyme-linked immunosorbent assay (ELISA) method. A piece of right lower lobe of lung tissue weighing 0.2 g and 1.8 mL normal saline of 0°C was centrifuged for 10 min (1000 r/min) at −4°C. Supernatant was removed and stored at 4°C. The protein content of each sample was determined with coomassie brilliant blue. The regression equation and correlation coefficient (r) of HyP and α-SMA were determined respectively by the concentration and absorbance values of standard protein. The regression equation and correlation coefficient (r) of HyP and α-SMA were determined separately based on the concentration and absorbance values of standard protein. HyP: Y=0.0022X−0.1068, R^2=0.9490; α-SMA: Y=0.035X−0.1134, R^2=0.9528. The contents of HyP and α-SMA were detected respectively by HyP kit and α-SMA kit according to the manufacturer’s protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The absorbance of colored products of HyP and α-SMA in each sample was measured at 450 nm using MULTISKAN (Mk3, Thermo, U.S.A.). The concentration of the sample was calculated using the optical density (OD) value of the sample. The greater the OD values of the sample, the higher the protein content.

Quantitative (q)RT-PCR

The qRT-PCR technology was used to measure the level of TGF-β1 and Cav-1 mRNA expression in lung. After the rats were sacrificed, 100 mg of the left lung were obtained and grinded in liquid nitrogen. The total RNA was extracted with Trizol reagent (Invitrogen, Carlsbad, CA, U.S.A.) and was reverse-transcribed into cDNA using HiScript Q RT SuperMix for qPCR (+gDNA wiper) (Vazyme Science and Technology Co., Nanjing, China). The real-time PCR reactions were performed using AceQTM qPCR SYBR® Green Master Mix (Vazyme Science and Technology Co.) in a 20 µL reaction volume containing 1 µL of cDNA. Thermal cycling and SYBR Green fluorescence detection were done using the real time qPCR (ABI StepOne, U.S.A.). The primers of TGF-β1 (ID: MQP030343), Cav-1 (ID: MQP026708) and reference glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (ID: MQP027158) used in this study were purchased from GeneCopoeia Inc., U.S.A. PCR conditions were as following: At 95°C for 10 min; 95°C for 10 s, 60°C for 20 s, 72°C for 15 s, 40 cycles. A melting curve analysis was made after the final PCR cycle to evaluate the presence of nonspecific PCR products and primer dimers. During the initial optimization run, four-fold serial dilution was used to demonstrate the linear amplification range for each gene. The threshold cycle (Ct) represents the PCR cycle during which an increase in SYBR Green fluorescence above the baseline signal can be detected first. Relative quantitation was performed by normalizing Ct values of each sample gene to Ct values of the housekeeping gene (ΔCt). ΔCt corresponds to the difference between the Ct of the gene of interest and the Ct of the references gene.
Western Blotting The protein concentrations of the extracts were determined using a protein assay reagent kit (Bradford, P0010S, Beyotime Institute of Biotechnology, Beijing, China). Fifty micrograms of proteins was separated by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) electrophoresis and transferred to polyvinylidene difluoride (PVDF) membranes. The membranes were blocked with 5% nonfat milk in 0.01 M PBS at room temperature for 1 h and then incubated overnight at 4°C with the primary antibodies (Abcam Co., U.S.A.), including anti-TGF-β1 antibody (1:800), anti-Cav-1 (1:5000) and anti-β-actin (1:5000). After three washes with Tris buffered saline with Tween (TBST), all membranes were incubated with infrared radiation Dye 800-conjugated goat anti-rabbit secondary antibodies (1:1000) ±Rg1 treatment reduced alveolar inflammation and pulmonary fibrosis scores at 7 and 28 d. Data are expressed as the mean ± S.E.M. *p < 0.01 BLM vs. control; †p < 0.05 or ††p < 0.01 Rg1 treatment vs. BLM; †p < 0.05 Rg1 vs. prednisone. One-way ANOVA followed by LSD method.

RESULTS

Rg1 Treatment Attenuated BLM-Induced Lung Injury, Inflammation and Pulmonary Fibrosis Lung coefficient and the scores of alveolitis may reflect the degree of BLM-induced lung injury. As shown in Fig. 1A, BLM exposure resulted in notable increase of lung coefficient compared to that of control group at 7 and 28 d time point (p < 0.01 vs. control). All 7-d treatment regimens with various doses of Rg1 decreased the lung coefficients. Compared to lung coefficient of 10.45 ± 1.76 in BLM group, Rg1 treatment at 18, 36 and 72 mg/kg decreased lung coefficients to 8.89 ± 1.56, 5.82 ± 1.46 and 4.26 ± 0.60, respectively (p < 0.05 or p < 0.01 vs. BLM). Rg1 treatments at 28 d displayed the similar results (p < 0.01 vs. BLM). Compared with positive control group, Rg1 36 and 72 mg/kg showed significant reduction in lung coefficients 7 and 28 d after drug administration (p < 0.01 or p < 0.05 vs. positive control).

We also assessed the scores of alveolitis by HE staining. BLM administration resulted in remarkable increase in alveolitis scores when compared to the control group (p < 0.01 vs. control). Compared to alveolitis scores of 2.53 ± 0.46 in the BLM group, Rg1 treatments 18, 36 and 72 mg/kg reduced the scores of alveolitis to 1.83 ± 0.20, 1.53 ± 0.28, 1.42 ± 0.26 at 7 d with a dose-dependent manner (p < 0.01 vs. BLM, Fig. 1B). In grading of alveolar inflammation, Rg1 36 and 72 mg/kg group significantly decreased the scores of alveolitis compared with positive control group (p < 0.05 vs. positive control group).

The HE staining (Fig. 2) showed normal alveolar structure, intact pulmonary vascularity and absence of inflammation in the lungs of control group at 7 d. BLM administration caused serious lung injury with inflammatory cell infiltration and thickening of the pulmonary interstitium present. Scattered alveolar damage, fusion, bullae formation and accumulation of alveolar exudates were also noted. Rg1 treatment groups...
demonstrated histological improvement. Masson's trichrome staining was used to examine the extent of collagen production in PF. The scores of PF was used to assess the degree of PF. The results (Fig. 2) showed lung sections of control group with normal collagen fiber distribution and small amount of staining of aniline blue (black arrow) at 28 d. BLM administration resulted in extensive collagen fiber deposition and increased staining of aniline blue. Rg1 treatment significantly reduced collagen fiber deposition in the lung interstitium and peribronchiolar areas. Quantitative evaluation of histological findings in Fig. 1B.

![HE staining](image1)

![Masson's staining](image2)

(A) HE staining of the lung tissue (×200). Results showed normal alveolar structure, intact pulmonary vascularity and absence of inflammation in the lungs of control group at 7 d. BLM administration caused serious lung injury with inflammatory cell infiltration and thickening of the pulmonary interstitium present. Scattered alveolar damage, fusion, bullae formation and accumulation of alveolar exudates were also noted. Rg1 treatment groups demonstrated histological improvement.; (B) Masson's staining (the right middle lobe). The results showed lung sections of control group with normal collagen fiber distribution and small amount of staining of aniline blue (black arrow) at 28 d. BLM administration resulted in extensive collagen fiber deposition and increased staining of aniline blue. Rg1 treatment significantly reduced collagen fiber deposition in the lung interstitium and peribronchiolar areas. Quantitative evaluation of histological findings in Fig. 1B.

Rg1 Reduced the Contents of Collagen Biomarkers To further confirm the therapeutic effects of Rg1 on PF, we measured the contents of Hyp and α-SMA in the lung tissue at 28 d (Fig. 3). The contents of Hyp and α-SMA of the lung tissue in BLM group greatly increased compared with the control group (p<0.05 or p<0.01, vs. control). Compared with the Hyp contents of 1.99±0.32 in BLM group, Rg1 treatment at 18, 36 and 72 mg/kg markedly decreased the contents of Hyp to 1.66±0.10, 1.57±0.09, 1.44±0.28 at 28 d with a dose-dependent manner respectively (p<0.05 or p<0.01, vs. BLM). The contents of α-SMA had shown similar results (p<0.05 or p<0.01, vs. BLM). Rg1 72 mg/kg group showed significant decrease in the contents of Hyp and α-SMA than of positive
control group ($p<0.05$ vs. positive control group).

Rg1 Treatment Reduced TGF-$\beta$1 Expression in Rat Lungs

TGF-$\beta$1 was known to be critical factors for PF. Therefore, the levels of TGF-$\beta$1 mRNA and its protein of lung tissue in each group were separately determined by the qRT-PCR and Western blot methods at 7 and 28d time point, which these quantitative results showed in Fig. 4. The contents of TGF-$\beta$1 mRNA and protein significantly added in the BLM

Fig. 3. Rg1 Decreased the Contents of Hyp and \(\alpha\)-SMA in the Lung

Hyp and \(\alpha\)-SMA in the right lower lobe of lung were respectively determined using ELISA method. (A) Rg1 treatment decreased the content of Hyp at 28d. (B) Rg1 treatment decreased the content of \(\alpha\)-SMA at 28d. The values are presented as the mean±S.E.M., $n=10$. *$p<0.05$ or **$p<0.01$ BLM vs. control; #$p<0.05$ or ##$p<0.01$ treatment vs. BLM; ***$p<0.05$ Rg1 vs. prednisone. One-way ANOVA followed by Dunnett’s T3 method.

Fig. 4. Effects of Rg1 on the TGF-$\beta$1 mRNA and Protein Expression in the Lung

The 72mg/kg of Rg1 dose was the best on the PF, and therefore was further research on the TGF-$\beta$1 expression by RT-PCR and Western blot. Histogram representing the quantitative analysis of TGF-$\beta$1 level normalized to \(\beta\)-actin level. (A) The quantitative analysis of TGF-$\beta$1 mRNA level at 7d. (B) The quantitative analysis of TGF-$\beta$1 protein level at 28d. (C) The quantitative analysis of TGF-$\beta$1 mRNA level at 7d. (D) The quantitative analysis of TGF-$\beta$1 protein level at 28d. The values are presented as the mean±S.E.M., $n=10$. *$p<0.05$ or **$p<0.01$ BLM vs. control; #$p<0.01$ Rg1 treatment vs. BLM; ***$p<0.01$ Rg1 vs. prednisone. One-way ANOVA followed by LSD method.
groups compared with the control group (p<0.05 or p<0.01, vs. control). Compared with the TGF-β1 mRNA and protein expression of 3.43±0.13 and 192.06±25.25 in BLM group, Rg1 treatment at 72 mg/kg distinctly inhibited TGF-β1 mRNA and protein expression to 0.90±0.11 and 30.06±5.21 at 7 d in the lung tissue respectively (p<0.01, vs. BLM, Figs. 4A, C). Rg1 treatments at 28 d showed the same trend (p<0.01, vs. BLM, Figs. 4B, D). Compared with positive control group, Rg1 72 mg/kg groups in protein expression showed a more overtly reduction after 28 d drug administration (p<0.01)

**Rg1 Treatment Increased Cav-1 Expression in Rat Lungs**

The lack of Cav-1 plays an important role in PF process. However, pretreatment with Cav-1 adenovirus was shown to prevent characteristic changes induced by fibrotic agents like bleomycin or TGF-β1.20,21 Here, we observed the regulation function of Rg1 on Cav-1 gene and protein expression (Fig. 5). The results showed that the levels of Cav-1 mRNA and protein markedly reduced in the BLM groups compared with the control group (p<0.01, vs. BLM). Compared with the Cav-1 mRNA and protein expression of 0.29±0.23 and 0.22±0.02 in BLM group, Rg1 treatment at 72 mg/kg distinctly elevated Cav-1 mRNA and protein expression to 0.88±0.20 and 2.44±0.40 at 7 d in the lung tissue respectively (p<0.01, vs. BLM, Figs. 5A, C). Rg1 treatments at 28 d showed the similar results (p<0.01, vs. BLM, Figs. 5B, D). Rg1 72 mg/kg group in the 7 and 28 d showed significant increase on the contents of Cav-1 mRNA and protein than of positive control group (p<0.05 vs. positive control group).

DISCUSSION

The major findings of the study focus on five areas. First, Rg1 decreased lung coefficient and the scores of alveolitis and PF. Second, Rg1 improved histopathology of lung in HE and Masson’s trichrome staining. Third, Rg1 lowered the contents of Hyp and α-SMA in the lung tissues. Forth, Rg1 was showed to down-regulate the TGF-β1 mRNA and protein expression of lung in IPF rats. Fifth, Rg1 also showed to up-regulates Cav-1 mRNA and protein expression of lung in IPF rats.

The bleomycin animal model has been widely used to study IPF and remains the best experimental tool available.22 In our rat models induced by endotracheal bleomycin administration, two processes appeared at different time points. Alveolus inflammation was prominent at 7 d after giving bleomycin. At 28 d, significant fibrosis appeared. IPF features induced in our animal models are consistent with other research.18,23 Various doses of Rg1 were given for different periods of time to study its protective effect on IPF. After 7 d of treatment with Rg1, the lung coefficients and scores of alveolitis in rats are significantly decreased. This suggests that anti-IPF effect of Rg1 be related to its anti-inflammatory function. In other study, Rg1 lowered the levels of pro-inflammatory cytokines including interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α.24 However, there is some controversy regarding the efficacy of anti-inflammatory drugs in the treatment of IPF.25,26 Compared with traditional anti-inflammatory drugs and corticosteroids, Rg1 may possess unique anti-inflammation...
tory function. Its mechanism of action has not been clearly elucidated.

After 28 d of Rg1 administration, the lung coefficients and scores of PF in rats were markedly reduced in all the treatment groups, and histopathology of lung tissues was also improved. Treatment with Rg1 cut down the contents of Hyp and α-SMA in lung tissues compared with model group. As two major biomarkers of collagen, Hyp and α-SMA played important roles in deposition and remodeling of ECM. 27, 28) Rg1 may exert anti-fibrosis effect through modifying deposition of collagen and ECM in IPF.

Our experimental results show that Rg1 72 mg/kg dosage proves the most obvious effect. Therefore, the dosage of Rg1 was selected for the further research in molecular level. Results showed that Rg1 72 mg/kg groups decreased the contents of TGF-β1 mRNA and protein, and increased the contents of Cav-1 mRNA and protein in lung tissues compared with model group at 7 and 28 d after treatment. Compared with positive control group, Rg1 at the high dose showed a remarkable effect in lowering the level of TGF-β1 and increasing the contents of Cav-1. TGF-β1 is the central mediator of tissue fibrosis, and the intervention of TGF-β1 expression or its signaling would be the most promising therapeutic targets for treatment of fibro-proliferative diseases. 29) A study showed that the suppression of Cav-1 expression in lung fibroblasts contributes to IPF pathogenesis by promoting TGF-β1 profibrotic effects. Our results suggest that anti-fibrotic effect of Rg1 may be related to the lower level of TGF-β1 and higher level of Cav-1.

However, it is not yet clear how Rg1 affects the down-regulation of TGF-β1 and up-regulation of Cav-1. The several lines of evidence supporting our results can be found from the literature. For instance, persistent activation of nuclear factor-kappaB (NF-κB) is central to the pathogenesis of many inflammatory lung disorders, including cystic fibrosis, asthma, and chronic obstructive pulmonary disease. 30) NF-κB can regulate TGF-β secretion in cell culture models. 31) More recently, we noticed that Rg1 suppressed Aβ25–35-induced toxicity in primary cultured rat cortical neurons in a NF-κB-dependent manner. 32) Thus, NF-κB molecule is probably involved in Rg1 dependent TGF-β1 down-regulation. Additionally, Rg1 has been reported to possess anti-inflammatory activities, for example, Rg1 down modulates shear induced pro-inflammatory cytokine monocyte chemoattractant protein-1 (MCP-1) gene expression and monocytes adhesion without potential cell toxicity via the suppression of mitogen-activated protein kinase (MAPK) pathway, 33) meanwhile, p38/MAPK signaling has been confirmed to mediate an activation of transcription factor 2 (ATF2) transcription of TGF-β1 via cyssteinyl-leukotriene receptor 1 (CysLT1R). 34) It is attractive to speculate p38/MAPK signaling also mediates Rg1 down regulated TGF-β1 expression.

Cav-1 was reduced in affected systemic sclerosis lungs and skin and in idiopathic pulmonary fibrosis lung tissues and fibroblasts. Increasing Cav-1 expression markedly improved bleomycin-induced pulmonary fibrosis. Its level, contrary to TGF-β1 expression, decreases considerably in active fibrotic areas, and negative regulation of Cav-1 by TGF-β1 via accelerating Cav-1 degradation has been confirmed in many studies. In Wang’s research, they administered TGF-β1 to human primary pulmonary fibroblasts and MRC-5, a human pulmonary fibroblast cell type, and analyzed the expression of Cav-1, and the result showed the mRNA of Cav-1 markedly decreased in primary fibroblasts after 1 d of treatment with TGF-β1 and Cav-1 protein levels decreased after TGF-β1 treatment in a time- and dose-dependent manner, suggesting TGF-β1 might be one of the negative regulators of Cav-1 expression. 35) Currently, it is yet unknown how Rg1 restores Cav-1 molecules, based on our results of down regulated TGF-β1 by Rg1, we speculate that Rg1 up-regulates Cav-1 molecules probably through acting on TGF-β1 molecules itself and its downstream of signaling pathway, since it is reported that Rg1 as a specific inhibitor of MAPK/extracellular signal-regulated kinase (ERK) kinase suppressed ERK1/2 as well as α-SMA in TGF-β1-induced nasal polyp-derived fibroblasts (NPDFs). 36) However, work from Shivshankar demonstrated that bleomycin induced much less fibrosis in Cav-1−/− mice relative to that in wild type (WT) animals, most likely because the cascade of fibrotic processes initiated after injury was not promoted in this mode. 37) These indicate that the role of Cav-1 in the regulation of fibrosis is controversial in animal models, possibly which depends on the stages of the diseases and different model. Therefore, we should further investigate the mechanisms of Rg1 regulating TGF-β1 and Cav-1 in the future studies.

The mechanism of Rg1 can be very complicated, which can also involve the activation of glucocorticoid receptor (GR). The relationship between Rg1 and GR has been extensively studied, such as anti-inflammatory function of Rg1, anti-apoptosis, and neural differentiation and so on. 38) Therefore, the unwanted effects of Rg1 should be concerned, especially osteoporosis. However, work from Du study has clearly indicated that Rg1 effectively inhibits acute and chronic inflammation in vivo, while it does not cause hyperglycemia or osteoporosis as seen with dexamethasone. 39) Moreover, it has been reported that Rg1 exhibits an estrogen-like activity, 40) by which it probably protects bone destruction against dexamethasone-induced osteocyte apoptosis. 41) Therefore, we did not examine the osteoporosis conditions in rats at 7 and 28 d after Rg1 treatments. However, we examined the body weight of rats because of the lung coefficient determination in our study. Our result shows that compared with the sham group, the body weight of rats in model group were significantly reduced, while the Rg1 treatments appear to increase body weight slightly, but there is no statistical significance.

In summary, the anti-IPF effects of Rg1 were evident in all three Rg1 groups, and the dose–effect relationship was also present. This study demonstrates that Rg1 possesses protective effects against bleomycin-induced histological changes in the lungs. Besides, we believe Rg1 produce a good beneficial effect, and it does not induce unwanted effects. Its molecular mechanism of anti-fibrosis may be related to up-regulation of Cav-1 and down-regulation of TGF-β1 mRNA and protein expression in the lung tissues.

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Conflict of Interest The authors declare no conflict of interest.
REFERENCES

37. Cho JS, Moon YM, Um JY, Moon JH, Park IH, Lee HM. Inhibi-


