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Effect of Feining on bleomycin-induced pulmonary injuries in rats

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ABSTRACT

Ethnopharmacological relevance: The flowers of *Gentiana veitchiorum* has been widely used in decoction form in the traditional medicine of Tibet against tussis, tracheitis, angina for their anti-inflammatory, antimicrobial and alexipharmic properties.
Aim of the study: The aim of current study was to evaluate the therapeutic effects of Feining, a Chinese herbal formula (national invention patent: ZL200510042636.3) against pulmonary injuries and to clarify the mechanisms involved.
Materials and methods: Experimental pulmonary injuries were induced by bleomycin (BLM) in rats with or without subsequent treatment of Feining or prednisone as positive control. The pulmonary injuries were evaluated by histological analysis. Also, the levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione (GSH) and hydroxyproline (Hyp) in the lung tissue were determined. To clarify one of the possible active principles responsible for Feining, high performance liquid chromatography–diode array detector–mass spectrometry (HPLC–DAD–MS) method was applied to identify the components of *Gentiana veitchiorum*, one of major ingredients of Feining.
Results: Feining significantly improved lung alveolitis scores and reduced the Hyp content of lungs, which is an index of collagen accumulation. Moreover, Feining played a role against the oxidative damages by decreasing the MDA level, whereas increasing SOD and GSH activity, which correlated with oxidation resistance and scavenging of free radicals. In addition, Feining alleviated inflammatory lung injury by decreasing tumor necrosis factor-α (TNF-α) expression. HPLC–DAD–MS analysis revealed that there was 1.97% gentiopicroside in *Gentiana veitchiorum*.
Conclusion: Feining has certain therapeutic effects against pulmonary injuries.

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive interstitial lung disease characterized by increased fibroblastic proliferation and deposition of extracellular matrix (ECM) resulting in a loss of lung function and, eventually, respiratory failure (American Thoracic Society, 2000, 2002). IPF is a devastating disease with a 5-year survival rate of 20–30% and a poor prognosis with a median survival of 3 years from the time of diagnosis (Collard et al., 2004; Martinez et al., 2005; Jeon et al., 2006; Mallick, 2008). To date, the etiology of IPF is still unclear and the pathogenesis of IPF remains incompletely understood due to its complexity. Although corticosteroids, immunosuppressive and cytotoxic agents, in addition to antifibrotic agents have been used in the treatment of IPF, currently there are no consistently effective medical therapies for IPF patients (Selman et al., 1998; Raghu et al., 2004; Jeon et al., 2006). Therefore, the discovery of novel therapeutic agents is critical for the treatment of IPF.

In accordance with the theory of traditional Chinese medicine, a number of herbal drugs have been used in the treatment of lung diseases, which provides a basis for exploring the potential of compounds from natural resources for the treatment of IPF. The plant named *Gentiana veitchiorum* (identified by Prof. Guolian Lei from Shaanxi University of Chinese Medicine), which belongs to the family of Gentianaceae, is a perennial herb about 5–10 cm in height and is indigenous in Tibet and Qinghai in China. It has been widely used in ethnomedicine for eyestrain accompanied by headache, pharyngitis and jaundice (Yang, 1991). With anti-inflammatory, antimicrobial and alexipharmic activity, the flowers of *Gentiana veitchiorum* are used in decoction form in the traditional medicine of Tibet against tussis, tracheitis, smallpox and angina (Ma and Zhao, 1990; Wang et al., 2007). Shiweirongdanfa Particles, a

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Chinese herbal formula, has satisfactory therapeutic effects on the clinical pulmonary infections, and the flowers of *Gentiana veitchiorum*, contained in this formula play a potential role (Dong and Li, 2003; Zhu et al., 2003; Sun, 2004).

Allowing for the activity of Gentiana flowers, we invented a new Chinese herbal formula (national invention patent: ZL200510042636.3), which is composed of Gentiana flowers and *panax notoginseng saponins* (PNS). The current study tested the therapeutic role of Feining by administration after bleomycin-induced pulmonary injuries, and the underlying mechanisms were investigated as well.

2. Materials and methods

2.1. Reagents

Bleomycin was purchased from Nippon Kayaku Co., Ltd., and prednisone from Xi'an Lijun Pharmaceutical Co., Ltd. PNS was the product of Yu Nan Phytopharmaceutical Co., Ltd. The kits for the assay of SOD, MDA, GSH and Hyp were from Nanjing Jiancheng Bioengineering Institute. A murine TNF-α enzyme-linked immunosorbent assay (ELISA) Development Kit from Jingmei Biotech Engineering Co., Ltd. was used for the detection of TNF-α in lung tissues. Prednisone and Feining was dissolved in saline immediately before study at calculated final concentrations and administered intragastrically to the animals.

2.2. Plant materials and preparation of Feining

The medical herbals, *Gentiana veitchiorum* were purchased from a local market in Tibet of China and identified by Prof. Guolian Lei from Shaanxi University of Chinese Medicine. Voucher specimen has been deposited in the Department of Pharmacology, Fourth Military Medical University (No. GLM081424). Briefly, the plants were extracted with the 20 times their weight of distilled water by refluxing for 1.5 h in the first, second and third extractions. The extracts were filtered, the combined filtrates were concentrated under normal pressure. The density of the final extracts was 1.2 g/ml (50 °C) (Huang et al., 2006), and the concentration of crude drug was 85.21%. For preparation of Feining, 7.62% of PNS were incorporated into the aforementioned extracts.

2.3. Animal groups and treatment

Sprague–Dawley rats (130 ± 10 g), 50% male and 50% female, were purchased from the Laboratory Animal Center, Fourth Military Medical University, Xi'an, China. All animals were maintained in a 12:12 h light–dark cycle at 23 °C with food and water available ad libitum for at least 1 week before starting the experiments. The study was approved by the Fourth Military Medical University Animal Care and Use Committee and followed the national and institutional rules on animal experiments.

All rats were randomly assigned into normal control group, Bleomycin (BLM)-treated group, prednisone group (dose, 6 mg/kg body weight), and three Feining groups at a dose of 1, 2 and 4 g crude drug/kg body weight, respectively. Pulmonary alveolitis was induced by endotracheal injection of BLM (Thrall et al., 1987; Lazenby et al., 1990; Lazo et al., 1990; Osanai et al., 1991; Zia et al., 1992; Izbicki et al., 2002) at the dose of 5 mg/kg body weight (1 mg of BLM in 0.01 ml 0.9% saline) except in the normal control group. The normal control group received equal amounts of 0.9% saline. Every day during the experimental period, rats in the Feining or the prednisone groups were administered Feining or prednisone by gavage, respectively, and the normal control rats and BLM model rats received equal amounts of 0.9% saline. On the time points of days 7, 14 and 56, six rats in each group were sacrificed and the lungs were harvested. The right lungs were fixed in 10% formaldehyde for one day, paraffin-embedded and sliced for histopathological studies (HE staining). The left lungs were frozen in liquid nitrogen and stored at −80 °C prior to assessment of SOD activity and MDA, GSH, Hyp and TNF-α measurements.

2.4. Histological analysis

After hematoxylin & eosin (H&E) staining, the sections of lung tissues were analyzed qualitatively under light microscopy. Also, a semi-quantitative grading system described by Szapiel et al. (1979) was applied for the evaluation of alveolitis severity. The scores of alveolitis in lung specimens were graded from − to +++ and correspondingly numbered from 0 to 3. To minimize sources of bias, the three investigators did not know which group they were analyzing.

2.5. Determination of the levels of SOD, MDA, GSH, Hyp and TNF-α in lung tissue homogenate

Lung tissue samples were homogenized in cold Tris–HCl buffered saline (pH 7.4, 10 mmol/l Tris–HCl, 0.1 mmol/l EDTA-2Na, 10 mmol/l saccharose, 0.8% sodium chloride solution) at 4 °C with a homogenizer (Gong et al., 2005). The tissue homogenate was 10% (w/v). Samples were centrifuged at 3000 × *g* for 10 min at 4 °C, and the supernatant was used to measure the levels of SOD, MDA, GSH, Hyp and TNF-α according to the protocols of the manufacturers.

2.6. HPLC-ESI/MS (high performance liquid chromatography-the electricity sprays fog/mass spectroscopy) analysis

The HPLC-ESI/MS system used in this experiment consisted of an Agilent 1100 series LC/MSD Trap (Agilent Technologies, USA) equipped with a quaternary pump (G1311A), an automatic sample injector (G1313A), a photodiode array detector (G1315B-DAD), and a thermostated column compartment (G1316A). The column was a reverse-phase column (C_{18}, 5 μm, 150 mm × 0.5 mm I.D. Agilent), and an ion-trap mass spectrometer (Agilent, USA), Chromatography Data System (Agilent Chemstation) was used to control the system and to analyze the data.

For preparation, the powdered (passed through a 60 mesh sieve) *Gentiana veitchiorum* flowers were extracted with ligarine under reflux for 30 min (Zhang et al., 1991), and then dried. 200 mg powder sample and 20 ml methanol were added to a 50 ml centrifuge tube in an ultrasonic bath for 40 min, the solution was then filtered and the filtrates placed in a 10 ml volumetric flask. The solution was then filtered through a 0.45 μm syringe filter into an HPLC vial for analysis. Also, 10 mg standard gentiopicroside (purchased from the National Institute for the Control of Pharmaceutical and Biological Products) and methanol were added to a 10 ml volumetric flask in an ultrasonic bath for 30 min, and then 3 ml of the solution and methanol were placed in another 10 ml volumetric flask. The solution was then filtered through a 0.45 μm syringe filter into an HPLC vial for analysis.

Before analysis, the conditions of chromatograph and mass spectrometer were optimized for the sake of accuracy. For chromatograph, the mobile phase consisted of water (A) and 0.5% acetic acid in methanol (B) using a gradient program of 15–20% (B) in 0–6 min, 20–40% (B) in 6–20 min, 40–60% (B) in 20–30 min and 60–35% (B) in 30–35 min. The flow rate was 1.0 ml/min and the column temperature was maintained at 40 °C. The DAD detector was set at 275 nm to obtain chromatograms. UV spectra were acquired from 200 to 400 nm. For mass spectrometer, capillary voltage, nebulizing gas pressure (N_2), drying gas (N_2) flow rate, and drying temperature were set at 3 kV, 15 psi, 5.0 L/min, and 325 °C, respectively. Full scan spectra from m/z 100 to 1000 in the positive ion mode were obtained (scan time 0.1 s).

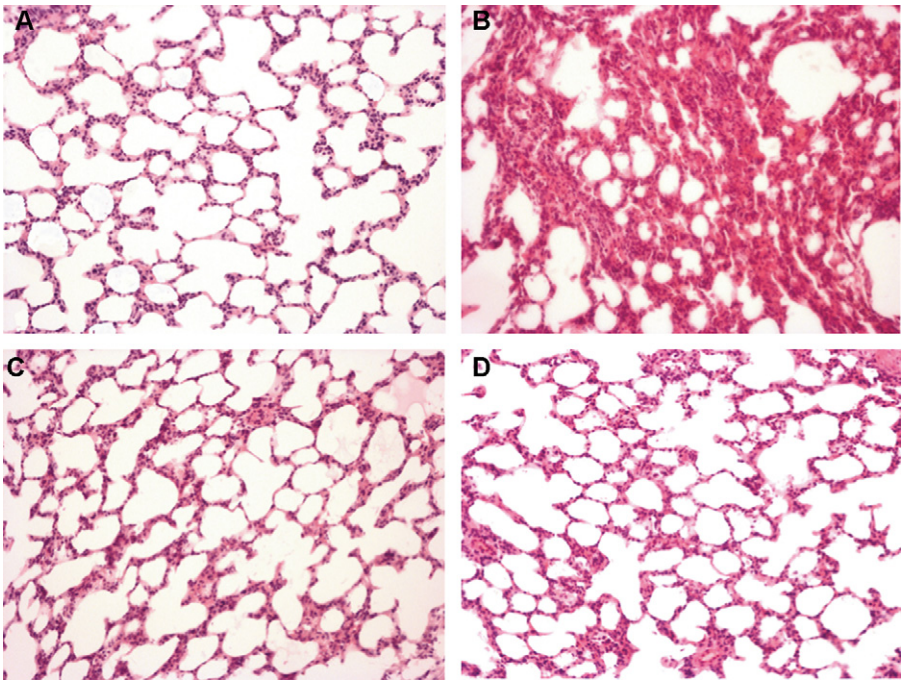

Fig. 1. Effects of Feining on histopathologic changes in BLM-induced lung injury in rats. (A) Normal group; (B) control group; (C) prednisone group; (D) Feining groups (4 g/kg). All panels were stained with H&E and are shown at the same magnification (100×). Abbreviations: BLM—bleomycin, H&E—hematoxylin and eosin.

For analysis, three independent samples (0.2 μl) were injected into the HPLC, each sample solution was injected in triplicate and the area value of the gentiopicroside peaks were interpolated on the calibration curves made with standard gentiopicroside.

2.7. Statistical analysis

Mean and standard error (S.E.M.) values were calculated for each group of results and significant differences between the means were determined by one-way analysis of variance (ANOVA). If significant, group means were compared using least significant difference (LSD) methods for multiple comparisons of means. The difference between groups at the $P<0.05$ level was considered statistically significant. Alveolitis scores of lung tissue were evaluated using Kruskal–Wallis H test and Mann–Whitney U methods, $\alpha' = 0.01$, the difference between groups at the $P<0.01$ level was considered statistically significant. All statistical analyses were performed using SPSS, version 11.0 (SPSS Science, Chicago, IL, USA).

3. Results

3.1. Feining alleviated pulmonary injuries in rats

To elucidate the effects of Feining on BLM-induced pulmonary injuries, sections of lung tissues were stained with H&E for identification, and the severity of alveolitis was individually accessed using the semi-quantitative grading system. As a result, sections from the control group displayed normal structure with no pathologic changes under a light microscope (Fig. 1A). In the model group, marked histopathologic changes, such as inflammatory cell infiltration and collapsed alveolar spaces in peribronchial regions were observed (Fig. 1B). Although inflammatory lesions were also seen in the prednisone groups (Fig. 1C) and Feining group (Fig. 1D), the extent of inflammation was significantly less severe compared with that in the model group. The alveolitis scores in the lung sections of the Feining groups were significantly decreased compared with those of the model group ($P<0.01$; Table 1).

As an index of collagen accumulation, hydroxyproline level closely relates with lung fibrosis. Analyses of hydroxyproline contents were conducted to evaluate the effect of Feining. As shown in Fig. 2, after 56 days, the hydroxyproline content of the lungs in the model group increased significantly compared with that in the control groups. Treated with Feining, rats showed a significant decrease of hydroxyproline levels in lung tissue compared with the model group rats. As expected, prednisone treatment also induced significantly reduction compared with that in the model group ($P<0.01$).

As all known, the extent of lipid peroxidation was indicated by measuring lung MDA level for each treatment group. SOD, GSH levels in lung tissue indicated oxidation resistance ability and scav-

Table 1
Effects of Feining on the degree of lung alveolitis induced by BLM in rats ($n=6$).

Group	7 days					14 days				
	–	+	++	+++	Integral	–	+	++	+++	Integral
Control	4	2	0	0	0.3 ± 0.5	4	2	0	0	0.3 ± 0.5
Model	0	0	2	4	2.7 ± 0.5*	0	0	3	3	2.5 ± 0.5*
Feining (1 g kg^{-1})	0	3	3	0	1.5 ± 0.5	0	4	2	0	1.3 ± 0.5
Feining (2 g kg^{-1})	0	4	2	0	1.3 ± 0.5#	1	4	1	0	1.0 ± 0.6#
Feining (4 g kg^{-1})	1	4	1	0	1.0 ± 0.6#	1	5	0	0	0.8 ± 0.4#
Prednisone	0	4	2	0	1.3 ± 0.5#	0	5	1	0	1.2 ± 0.4#

Abbreviations: BLM—bleomycin.
* $P<0.01$ *vs.* control.
# $P<0.01$ *vs.* model.

Table 2
The content of SOD, MDA, GSH, and TNF-α in pulmonary homogenates ($x \pm s$, $n = 6$).

Group	Dose ($g\,kg^{-1}$)	7 days				14 days			
		SOD (nkat/g)	MDA (μmol/g)	GSH (mg/g)	TNF-α (ng/l)	SOD (nkat/g)	MDA (μmol/g)	GSH (mg/g)	TNF-α (ng/l)
Control	–	756 ± 94	0.53 ± 0.12	37.8 ± 2.9	226 ± 5	764 ± 61	0.69 ± 0.07	47.7 ± 3.0	197 ± 5
Model	–	592 ± 94[a]	0.84 ± 0.16[a]	30.1 ± 3.1[a]	248 ± 14[a]	662 ± 25[a]	0.85 ± 0.06[a]	39.4 ± 3.0[a]	216 ± 16[a]
Feining	1	495 ± 39[a]	0.69 ± 0.10[a,b]	27.1 ± 1.9[a]	202 ± 11[a,b]	755 ± 33[b]	0.65 ± 0.09[a,b]	59.1 ± 4.7[a,b]	213 ± 13[a]
Feining	2	528 ± 82[a]	0.56 ± 0.06[b]	29.4 ± 2.9[a]	200 ± 14[a,b]	809 ± 51[b]	0.61 ± 0.11[a,b]	60.5 ± 4.2[a,b]	201 ± 6[b]
Feining	4	535 ± 96[a]	0.50 ± 0.09[b]	50.6 ± 2.5[a,b]	189 ± 8[a,b]	832 ± 72[b]	0.58 ± 0.12[a,b]	60.9 ± 4.6[a,b]	192 ± 10[b]
Prednisone	0.006	511 ± 55[a]	0.81 ± 0.12[a]	44.7 ± 7.2[a,b]	208 ± 19[a,b]	809 ± 37[b]	0.64 ± 0.07[b]	60.6 ± 4.1[a,b]	209 ± 11[a]

Abbreviations: SOD—superoxide dismutase; MDA—malondialdehyde; GSH—glutathione; TNF-α—tumor necrosis factor-α.
[a] $P < 0.05$ *vs.* control group.
[b] $P < 0.05$ *vs.* model group.

enging of free radicals ability. As shown in Table 2, lung MDA level in the model group increased significantly compared with the control group, lung SOD activity and lung GSH level in the model group were lower than that in the control group ($P < 0.05$). Treated with Feining, rats showed a significant decrease of the MDA levels compared with that in the model group ($P < 0.05$). On day 7, the GSH levels in the Feining group (dose, 4 g crude drug/kg body weight) were increased significantly compared with the model group and the control group ($P < 0.05$). On day 14, lung SOD activity was increased significantly compared with the model group, the GSH levels were increased significantly compared with the model group and the control group ($P < 0.05$).

The pro-inflammatory cytokine TNF-α in lung tissue was examined by ELISA. As shown in Table 2, the TNF-α level in lung tissue of the model group increased significantly compared with the control groups ($P < 0.05$). On day 7, the levels of TNF-α in the Feining groups and prednisone group were significantly reduced compared with those in the model and control groups ($P < 0.05$). On day 14, the levels of TNF-α in the Feining groups (2 g/kg, 4 g/kg) were significantly reduced compared with those in the model group ($P < 0.05$).

3.2. Identification and quantitative analysis of gentiopicroside in *Gentiana veitchiorum*

Feining is composed of PNS and extracts of *Gentiana veitchiorum*. The composition of PNS is well known. However, the extracts of *Gentiana veitchiorum* are mixtures, and the composition is not well understood. Therefore, an HPLC–DAD–MS method was employed to analyze the components of *Gentiana veitchiorum*. By comparing the retention times, UV and MS spectral data of the peaks with that of the standard compound, gentiopicroside was unambiguously identified. As to quantitative analysis the samples were separated under HPLC conditions. Through the calibration curves, we can quantitative analysis of gentiopicroside in the extracts of *Gentiana veitchiorum*. The percentage of gentiopicroside was 1.97% (one point external standard method), the analytical results are shown in Table 3.

For quality control, precision was measured using three different concentrations of the standard solutions, each standard solution was determined five times on the same day. The relative standard deviation (RSD) values were found to be below 1.3%, which is well within the acceptable level. Repeatability was measured using five different samples, which were run on the same equipment on different days. The RSD values were below 1.56% and were within acceptable limits. The stability was evaluated by determining gentiopicroside in the sample solution under the optimized conditions six times within one day. The measurements were conducted for 2 h over 12 consecutive hours. The RSD values were 0.67%. For the recovery test, measurements were conducted on four different samples, in three samples gentiopicroside was added. The recovery was 100%.

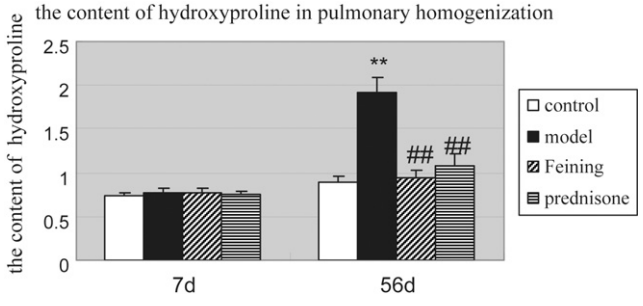

Fig. 2. Analysis of the content of Hp in pulmonary homogenization (μg/mg) ($x \pm s$, $n = 5$). $^{**}P < 0.01$ *vs.* control group. $^{\#\#}P < 0.01$ *vs.* model group.

4. Discussion

The BLM-induced animal model is widely used in the assessment of potential anti-fibrotic agents. Histological markers seen in BLM-treated rats, such as intra-alveolar buds, mural incorporation of collagen and obliteration of the alveolar space are similar to those in IPF patients (Usuki and Fukuda, 1995). Following BLM administration, an acute inflammatory response lasting up to 8 days appears first, followed by fibrogenic changes resulting in the deposition of matrix and distortion of lung structure at 28 or 35 days (Moeller et al., 2008). In the present study, the normal group showed very slight pulmonary alveolitis, and BLM instillation resulted in significant histopathological changes, such as thickened alveolar wall, collapse of alveolar spaces and infiltration of inflammatory cells, and resulted in the hydroxyproline content of the lungs increased significantly which is an index of collagen accumulation, an indicator of pulmonary fibrosis.

The overproduction of free-radical reactions plays a key role in BLM-induced pulmonary inflammation and fibrosis. Free radicals target biomacromolecules, such as deoxyribonucleic acid (DNA),

Table 3
Measurement of gentiopicroside in the flowers of *Gentiana veitchiorum*.

Sample	Volume (μl)	Amount (ng)
1	0.20	39
	0.20	39
	0.20	39
2	0.20	40
	0.20	39
	0.20	40
3	0.20	39
	0.20	39
	0.20	39

protein and lipids, with ultimate progression of lipid peroxidation, resulting in lung damage. On the other hand, recruitment and activated inflammatory cells (e.g. alveolar macrophages or neutrophils) initiated by epithelial damage in the lung produce reactive oxygen species (ROS), which also contribute to the oxidative damage of the lung induced by BLM (Yamazaki et al., 1998). In this study, BLM induced a significant increase in lipid peroxidation as reflected by the MDA level in the model group, and treated with Feining significantly decreased the level of lipid peroxidation caused by BLM. Meanwhile, SOD and GSH levels were increased significantly compared with the model group. It demonstrate Feining can resist oxidation, scavenge free radicals and alleviate lipid peroxidation.

The inflammatory response is the initial response following injury challenges and fibrosis is generally the final outcome of the inflammatory process in the lung. The response includes migration and activation of both resident and circulating inflammatory cells. Inflammatory cells release cytokines and growth factors, stimulate multiplication, migration, secretory activities and collagen production by fibroblasts. Although many cytokine interactions associated with inflammation and fibrosis have been reported in the course of IPF (Maher et al., 2007), TNF-α has been found to play an important role (Gauldie et al., 1993; Song et al., 1998; Fujita et al., 2003). In our study, BLM instillation clearly increased the expression of TNF-α in lungs. Although the TNF-α expression level decreased with time, treated with BLM significantly elevated its expression compared with the normal group. This suggested that the overexpression of TNF-α participates in acute inflammatory lung injury and the chronicity of the inflammatory process induced by BLM.

It is known that the capacity to ameliorate pulmonary fibrosis is often associated with attenuation of inflammatory cell recruitment and excessive collagen deposition. Our study showed that Feining significantly improved lung alveolitis scores; decreased the inflammatory response induced by BLM in the acute phase and lowered the hydroxyproline content of lungs which is an index of collagen accumulation, an indicator of pulmonary fibrosis. We can say Feining alleviated pulmonary inflammatory response by lowering the MDA level which directly correlated with lipid peroxidation; increasing SOD activity and GSH level which correlated with oxidation resistance and scavenging of free radicals. In addition, Feining alleviated acute inflammatory lung injury and the chronicity of the inflammatory process by decreasing TNF-α expression. Above results suggested that the anti-inflammatory response and resistance to collagen accumulation effects of Feining contribute to its protective effect against pulmonary fibrosis.

Previously, Wang et al. (2008) identified gentiopicroside in *Gentiana veitchiorum*. However, they did not make quantitative analysis, thus the concentration of gentiopicroside in *Gentiana veitchiorum* was unknown. In the current we not only made qualitative identification, but also performed quantitative analysis of gentiopicroside in *Gentiana veitchiorum*. Accounting for a variety of bioactivities including analgesic and anti-inflammatory activities, liver-protection and the promotion of bile secretion (Kondo et al., 1994; Liu et al., 2002; Chen et al., 2008), gentiopicroside might be one of the possible active principles responsible for Feining, a composite Chinese herbal formula contains extracts of *Gentiana veitchiorum* and PNS. It is reported that PNS have the effects of reducing lipid peroxidation, inhibiting hepatic fibrosis (Zhang et al., 2000) and alleviating experimental pulmonary fibrosis (Li and Cui, 2002). Therefore, it suggests that gentiopicroside and PNS produce effects together against pulmonary injuries in rats. Whereas, further biological studies are required to give direct evidences, and this work is underway.

Taken together, Feining has certain anti-inflammatory and anti-fibrotic effects on lung tissues. Thus, administration of Feining might be an effective therapy approach to cure pulmonary fibrosis.

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References

American Thoracic Society, 2000. Idiopathic pulmonary fibrosis: diagnosis and treatment: international consensus statement: American Thoracic Society (ATS), and the European Respiratory Society (ERS). American Journal of Respiratory and Critical Care Medicine 161, 646–664.

American Thoracic Society, 2002. ATS/ERS international consensus classification of idiopathic interstitial pneumonias. American Journal of Respiratory and Critical Care Medicine 165, 277–304.

Chen, L., Wang, H.B., Sun, X.L., Sun, W.J., 2008. Study on the analgesic and anti-inflammatory activities of gentiopicroside. Natural Product Research and Development 20, 903–906.

Collard, H.R., Ryu, J.H., Douglas, W.W., Schwarz, M.I., Curran-Everett, D., King Jr., T.E., Brown, K.K., 2004. Combined corticosteroid and cyclophosphamide therapy does not alter survival in idiopathic pulmonary fibrosis. Chest 125, 2169–2174.

Dong, H.Z., Li, Y.H., 2003. Effect evaluation of Shiweirongdanfa particles therapy 300 cases of child bronchitis. Chinese Journal of Medical Writing 10, 924–925.

Fujita, M., Shannon, J.M., Morikawa, O., Gauldie, J., Hara, N., Mason, R.J., 2003. Overexpression of tumor necrosis factor-alpha diminishes pulmonary fibrosis induced by bleomycin or transforming growth factor-beta. American Journal of Respiratory Cell and Molecular Biology 29, 669–676.

Gauldie, J., Jordana, M., Cox, G., 1993. Cytokines and pulmonary fibrosis. Thorax 48, 931–935.

Gong, L.K., Li, X.H., Wang, H., Zhang, L., Chen, F.P., Cai, Y., Qi, X.M., Liu, L.L., Liu, Y.Z., Wu, X.F., Huang, C.G., Ren, J., 2005. Effect of Feitai on bleomycin-induced pulmonary fibrosis in rats. Journal of Ethnopharmacology 96, 537–544.

Huang, Y.G., Wang, X.J., Gu, Y., Tian, Q., Wang, R., 2006. Extraction process of getiana veitchiorum with orthogonal design. Pharmaceutical Journal of Chinese People's Liberation Army 5, 359–361.

Izbicki, G., Segel, M.J., Christensen, T.G., Conner, M.W., Breuer, R., 2002. Time course of bleomycin-induced lung fibrosis. International Journal of Experimental Pathology 83, 111–119.

Jeon, K., Chung, M.P., Lee, K.S., Chung, M.J., Han, J., Koh, W.J., Suh, G.Y., Kim, H., Kwon, O.J., 2006. Prognostic factors and causes of death in Korean patients with idiopathic pulmonary fibrosis. Respiratory Medicine 100, 451–457.

Kondo, Y., Takano, F., Hojo, H., 1994. Suppression of chemically and immunologically induced hepatic injuries by gentiopicroside in mice. Planta Medica 60, 414–424.

Lazenby, A.J., Crouch, E.C., McDonald, J.A., Kuhn, C.D., 1990. Remodeling of the lung in bleomycin-induced pulmonary fibrosis in the rat. An immunohistochemical study of laminin. Type IV collagen and fibronectin. American Review of Respiratory Diseases 142, 206–214.

Lazo, J.S., Hoyt, D.G., Sebti, S.M., Pitt, B.R., 1990. Bleomycin: a pharmacologic tool in the study of the pathogenesis of interstitial pulmonary fibrosis. Pharmacology and Therapeutics 47, 347–358.

Liu, Z.W., Chen, C.X., Jin, R.M., Shi, G.Q., Song, C.Q., Hu, Z.B., 2002. Studies on liver-protection and promoting bile secretion of gentiopicroside. Chinese Traditional and Herbal Drugs 33, 47–50.

Li, X.J., Cui, S.H., 2002. The mechanisms and effects of panax notoginside and methylprednisolone in a rat model of pulmonary fibrosis. Chinese Journal of Tuberculosis and Respiratory Diseases 25, 520–523.

Martinez, F.J., Safrin, S., Weycker, D., Starko, K.M., Bradford, W.Z., King, T.E., Flaherty, K.R., Schwartz, D.A., Noble, P.W., Raghu, G., Brown, K.K., 2005. The clinical course of patients with idiopathic pulmonary fibrosis. Annals of Internal Medicine 142, 963–967.

Maher, T.M., Wells, A.U., Laurent, G.J., 2007. Idiopathic pulmonary fibrosis: multiple causes and multiple mechanisms? European Respiratory Journal 30, 835–839.

Ma, J., Zhao, R.N., 1990. Resources of Tibetan herbal medicine Gentianaceae in Gansu Province. Journal of Gansu College of Traditional Chinese Medicine 7, 18–20.

Mallick, S., 2008. Outcome of patients with idiopathic pulmonary fibrosis (IPF) ventilated in intensive care unit. Respiratory Medicine 102, 1355–1359.

Moeller, A., Ask, K., Warburton, D., Gauldie, J., Kolb, M., 2008. The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis? The International Journal of Biochemistry and Cell Biology 40, 362–382.

Osanai, K., Takahashi, K., Sato, S., Iwabuchi, K., Ohtake, K., Sata, M., Yasui, S., 1991. Changes of lung surfactant and pressure—volume curve in bleomycin-induced pulmonary fibrosis. Journal of Applied Physiology 70, 1300–1308.

Raghu, G., Brown, K.K., Bradford, W.Z., Starko, K., Noble, P.W., Schwartz, D.A., King Jr., T.E., 2004. A placebo-controlled trial of interferon gamma-1b in patients with idiopathic pulmonary fibrosis. The New England Journal of Medicine 350, 125–133.

Selman, M., Carrillo, G., Salas, J., 1998. Colchicine, d-penicillamine, and prednisone in the treatment of idiopathic pulmonary fibrosis: a controlled clinical trial. Chest 114, 507–512.

Song, M., He, B., Qiu, Z., 1998. Expressions of TNF alpha, PDGF in alveolar type II epithelial cells of rats with bleomycin-induced pulmonary fibrosis. Chinese Journal of Tuberculosis and Respiratory Diseases 21, 221–223.

Sun, Z.Z., 2004. Clinical observation of Shiweirongdanfa particles in the treatment of community-acquired pneumonia. Modern Journal of Integrated Traditional Chinese and Western Medicine 13, 890–891.

Szapiel, S.V., Elson, N.A., Fulmer, J.D., Hunninghake, G.W., Crystal, R.G., 1979. Bleomycin-induced interstitial pulmonary disease in the nude, athymic mouse. American Review Respiratory Diseases 120, 893–897.

Thrall, R.S., Swendsen, C.L., Shannon, T., Kennedy, C.A., Frederich, D.S., Grunze, M.F., Sulavik, S.B., 1987. Correlation of changes in pulmonary surfactant phospholipids with compliance in bleomycin-induced pulmonary fibrosis in the rat. American Review of Respiratory Diseases 136, 113–118.

Usuki, K., Fukuda, Y., 1995. Evolution of three patterns of intra-alveolar fibrosis produced by bleomycin in rats. Pathology International 45, 552–564.

Wang, W.p., Zhang, Z.H., Yang, T.M., He, H., Cheng, L.P., Ma, J., 2007. Resources of medicinal plants Gentianaceae in Gansu Province. Journal of Lanzhou University (Medical Sciences) 33, 34–44.

Wang, E.J., Ma, Y.B., Zhang, X.M., Jiang, Z.Y., Chen, J.J., 2008. Five alkaloids from vine stems of *Diploclisia affinis*. China Journal of Chinese Materia Medica 33, 2505–2507.

Yamazaki, C., Hoshino, J., SekiguchI, T., Hori, Y., Miyauchi, S., Mizuno, S., Horie, K., 1998. Production of superoxide and nitric oxide by alveolar macrophages in the bleomycin-induced interstitial pneumonia mice model. The Japanese Journal of Pharmacology 78, 69–73.

Yang, Y.C., 1991. The Records of Tibet Medicine, first ed. Qinghai, China.

Zhang, J.S., Tian, Z.X., Lou, Z.C., 1991. Simultaneous determination of five bitter secoiridoid glycosides in nine Chinese gentiana species used as the Chinese drug "Long Dan" by high performance liquid chromatography. Acta Pharmaceutica Sinica 26, 864–870.

Zhang, R.H., Zhou, Z.C., Hong, D.L., 2000. Efects of panax notogenseng on CCl_4-induced liver fibrosis in rats. Acta Academiae Medicinae Militaris Tertiae 22, 307–310.

Zhu, Q.S., Peng, L.J., Liu, Y., 2003. Efficacy of Shiweinongdanhua granule in the treatment of chronic bronchitis. Chinese Journal of New Drugs 12, 214–216.

Zia, S., Hyde, D.M., Giri, S.N., 1992. Development of bleomycin hamster model of subchronic lung fibrosis. Pathology 24, 155–163.