Experimental Study on Effect of Compound Biejia Ruangan Prescription (复方鳖甲软肝方) on High-Resolution Computerized Tomographic Images in Bleomycin Induced Pulmonary Fibrosis Rats*

ZHANG Dong-wei(张东伟), WANG Ji-feng(王继峰), NIU Jian-zhao(牛建昭), GAO Bao-hua(高宝华), and LI Gong-yu(李贡宇)

ABSTRACT  Objective: To study the therapeutic effect of Compound Biejia Ruangan prescription (CBRP) on rat model with pulmonary fibrosis induced by bleomycin. Methods: Fifty-four male Sprague-Dawley rats were randomly divided into 6 groups (9 rats in each group). From the first day to the 28th day of the experiment, except to those in the sham-model control group that were treated with normal saline, the same amount of bleomycin injection as the normal saline given to the control group was given through endotracheal instillation to all the rats in all the other groups. From the 29th day of the modeling, CBRP solution of different dosages was respectively injected into the rats in the high, moderate and low CBRP dose group, while equal volume of normal saline was given to those in the sham-model control group and the model control group, and an equal volume of prednisone solution was given to rats in the prednisone group. On the 80th day, the high-resolution computerized tomographic (HRCT) images were observed on an equal footing, and HRCT-pathology was correlatively studied. Results: Different HRCT pathological changes were shown in the rats with pulmonary fibrosis, such as lung consolidation, thickening of interlobular septum and interlobular mesenchyma as well as lobular deformation, nodule shadow, abnormal brochiovascular tract, thickened pleura with irregular junction and polished glass-like dense shadows. Honeycomb lung was observed in some cases. Pathological sections showed fibrotic proliferation of lung tissues and noticeable pulmonary interstitial fibrosis. CBRP could improve HRCT images of rats with pulmonary fibrosis, and lower fibrotic proliferation of the lung tissue. Conclusion: CBRP plays its therapeutic role possibly through its effect on the structure of the lung in rats with pulmonary fibrosis.

KEY WORDS  Compound Biejia Ruangan prescription, rats, pulmonary fibrosis, high-resolution computerized tomography

Experimental WORK AND RESEARCH

Idiopathic pulmonary fibrosis (IPF) is a disease characterized by diffuse alveolitis and alveolar structural derangement leading to pulmonary interstitial fibrosis. Its pathogenic cause is still unknown, and the prognosis is unfavourable. For cases at the primary stage, even if they positively respond to steroid therapy, the survival time is about 5 years. The currently used therapeutic methods mainly by adrenocortical hormones and immunosuppressants have proved to be unsatisfactory because of significant adverse reactions (1). In recent years, great progress has been achieved when TCM drugs are used in the treatment of pulmonary fibrosis, and Compound Biejia Ruangan Prescription (CBRP) has been found to have a definite clinical effect on liver fibrosis. To probe into the therapeutic effect of CBRP on pulmonary fibrosis, we, by means of high-resolution computerized tomography (HRCT), observed the effect of CBRP on pathological histology of the bleomycin-induced rats with pulmonary fibrosis so as to provide a new drug in clinical treatment of the disease.

METHODS

Drugs

CBRP, consisting of Carapax Trionycis, Radix Angelicae sinensis, Radix Notoginseng, Cordyceps, Radix Astragali, Radix Isatidis, Fructus Forsythiae, Radix Peoniae rubra, Radix Codonopsis pilosulae, Placenta Hominis and Rhizoma Zedoariae, was provided by Inner Mongolia Furui Pharmaceutical Factory, Ltd., each tablet containing 5g of powdered raw materials with no subsidiary materials (Batch No. 20020501). Prednisone acetate, used as a positive-control drug in the experiment, is product of Guangdong Huanan Pharmaceutical Factory, Ltd., each tablet containing 5g of powdered raw materials with no subsidiary materials (Batch No. 20020501). Prednisone acetate, used as a positive-control drug in the experiment, is product of Guangdong Huanan Pharmaceutical Factory with the batch number of

* This study was supported by National Funds of Natural Science (No. 30130220) and Administration of Education against SARS(No. 15)

The Cell and Biochemistry Laboratory of Beijing University of Chinese Medicine, Beijing (100029)

Correspondence to: Dr. WANG Ji-feng, Tel.: 010-64286995; Fax: 010-64286995; E-mail: wangjifengbeijin@163.com
Bleomycin Hydrochloride (BLM) injection is produced by Nippon Kayaku Co. Ltd. (REG. NO: X20000349).

Instruments
High-resolution computerized tomography (HRCT), Semens Plus Four, made in Germany, was used. Also used was automatic staining instrument (SHANDON).

Grouping and Modeling of Animals
Fifty-four male Sprague-Dawley rats, weighing 206±16g, were purchased from Beijing Vital Laboratory Animal Technology Co. Ltd., and fed with normal granulated forage and enough water. All the rats were randomly divided into 6 groups, 9 in each group. The sham-model group, the model control group, the prednisone group, and the high, moderate and low CBRP groups.

Having been weighed and anesthetized with intraperitoneal injection of 1.5% pentobarbital sodium, 2.5 mg/kg body weight, the rats were fixed and had their trachea exposed. Then 0.3 ml of BLM solution and 0.3 ml of air were injected into the trachea through an incision in the cervical region. Immediately after the injection, the rats were vigorously rolled right and left in the upright position in an attempt to make the drug evenly distributed in the lungs. For the rats in the sham-model group, the same volume of normal saline was given through the above-mentioned method instead. After the modeling, the rats were separately raised.

Drugs Administration
The administration of drug was started from the 29th day of the induction of pulmonary fibrosis, with normal saline, prednisone and CBRP given respectively to the SD rats in different groups. CBRP powder and prednisone acetate tablet were given by dissolving them in distilled water and made into suspension for oral infusion. The dosage of normal saline for rats in the sham-model group and the model control group was 14 ml/kg, and that of prednisone suspension for rats in the prednisone group was 14 ml/kg of 4% prednisone solution. 14 ml/kg CBRP of high (10%), middle (5%) and low (2.5%) concentration was administered to rats respectively in the high, moderate and low CBRP groups.

Drugs were given for six successive days in one week. The successive administration lasted 52 days in total.

High-resolution CT Scanning
HRCT scanning was conducted on the 80th day of the experiment in light of the experimental method recommended by YANG Kui, et al(5), which was improved by authors, and the criteria for observation and evaluation were based on the basic terms reported by Austin(6). After having been anesthetized with intraperitoneal injection of 1.5% pentobarbital sodium (2.5mg/kg), the rats were made to lie in the prone position to perform scanning of chest with the section thickness of CT as 1.0 mm at 12 mm intervals, with windows level appropriate for pulmonary parenchyma (width 1200 Hounsfield units; mean: 700 Hounsfield units). The following images shown in all the rats were recorded: lung consolidation, thickening of interlobular septum and interlobular mesenchyma, lobular deformation, nodule shadow, abnormal bronchiovascular tract, thickened pleura with irregular junction and polished glass-like dense shadows.

Pathological Examination
The right lung was incised to make histological sections, which were stained with Hematoxylin-Eosin (HE) and Masson staining for pathological diagnosis and HRCT-pathology control study. With the method proposed by Dr. Szapiel, et al(7), the extent of alveolitis (graded from 0 to Ⅲ) and fibrosis (graded from 0 to Ⅲ) were determined and scored according to the specific description listed in Table 1.

Statistical Analysis
Chi-square ($\chi^2$) test was performed to analyze the frequency of various signs of HRCT scan, and $P<0.05$ was considered statistically significant. Meanwhile regression analysis was performed to determine the correlation between the total frequencies of the signs of HRCT scan and the scores of the pathological sections in each group. All the results were analyzed by statistical software SPSS 10.0.

RESULTS
General Appearance of the Rats
On the 2nd day after BLM injection, most of the experimental rats were found to have cough and asthma, which lasted 10 days or so. Rats in the sham-model group were in good state, with normal breathing, quickness in movement, tidy and shining fur, normal food intake and increased body weight. But those in other groups, especially rats in the model control group were found 2 days
Table 1. Criteria for Grading and Scoring Alveolitis and Fibrosis

| Grade | Alveolitis                      | Score | Fibrosis                  | Score |
|-------|--------------------------------|-------|---------------------------|-------|
| -     | None                           | 0     | none                      | 0     |
| +     | Mild: thickening of the alveolar septum by mononuclear cell infiltration, with involvement limited to focal, pleural-based lesions occupying less than 20% of the lung and with good preservation of the alveolar architecture. | 1     | Mild: focal regions of fibrosis involving less than 20% of the lung. Fibrosis involved the pleura and the interstitium of subpleura parenchyma with some distortion of alveolar architecture. | 1     |
| ++    | Moderate: a more widespread of alveolitis involving 20 to 50% of the lung, although still predominantly pleurally based. | 2     | Moderate: more extensive fibrosis involving 20 to 50% of the lung and fibrotic region mostly extending inward from pleural and still focal. | 2     |
| +++   | Severe: a diffuse alveolitis involving more than 50% of the lung, with occasional consolidation of intra-alveolar spaces by mononuclear cells and some hemorrhagic areas within the interstitium and/or alveoli. | 3     | Severe: widespread fibrosis, involving more than 50% of the lung, confluent lesions with extensive derangement of parenchymal architecture, including cystic air spaces lined by cuboidal epithelium. | 3     |

Table 2. Pathological Grading/Scoring of Alveolitis and Fibrosis in Different Groups

| Group              | n   | Alveolitis | Score | Fibrosis | Score |
|--------------------|-----|------------|-------|----------|-------|
| Sham-model Control | 9   | - 9 0 0 0 0 | 9     | - 0 0 0 0 | 0     |
| Model Control      | 9   | 0 1 3 5 22 | 0     | 1 3 5 22 | 22    |
| Prednisone         | 9   | 1 5 3 0   | 11    | 2 4 3 0  | 10    |
| High CBRP Dose     | 9   | 1 4 2 2   | 14    | 1 4 3 1  | 13    |
| Moderate CBRP Dose | 9   | 2 5 2 0   | 9     | 2 6 1 0  | 8     |
| Low CBRP Dose      | 9   | 1 5 2 1   | 12    | 1 5 3 0  | 11    |

after BLM injection, with marked polypnea, fine bibasilar inspiration crackles (Velcro rales), listlessness, dullness, anorexia and flushing and withering fur. The condition for rats in various CBRP groups were found to be better to various degrees than that in the model control group.

Pulmonary Histopathology

For findings of the histopathological analysis in rats from various groups, refer to Table 2.

Histopathological appearance of alveolitis and fibrosis under light microscope showed that on the 80th day, the structure of bronchi and terminal bronchiole was normal in rats in the sham-model group with simple columnar epithelium in terminal bronchiole, cilia in epithelia, and intact circular smooth muscle in inherent membrane. There were visible simple columnar epithelium or cuboidal epithelium respiratory bronchiole, a few alveolar outlets in the walls of the respiratory bronchiole, spherical inflation in terminal of alveolar septum and regular structure and a certain amount of type I and II alveolar cells in the walls of pulmonary alveoli.

In rats of the model control group, there were significant thickening of alveolar walls with disorganized alveolar structure and markedly thickened interalveolar septum. Some of the alveolar cavities became bag-shaped with broken alveolar walls, and some of alveolar cavities became atrophic with accumulated exudates, edema or inflammatory infiltration (Fig. 1).

It could also be observed through Masson staining that there were large amount of proliferated fibrosis around bronchi and blood vessels, fibrosis foci in the alveolar cavities and significant interstiti-
Table 3. Frequency of Pathological Signs Shown in HRCT Scanning

| Group             | n | Consolidation | Brochio-vascular Abnormality | Irregular Linear Shadows | Pleura Thickening | Ground Glass Opacity | Nodule Shadow | Honeycomb Lung | Total |
|-------------------|---|---------------|------------------------------|--------------------------|------------------|---------------------|---------------|----------------|-------|
| Sham-model Control| 9 | 0             | 0                            | 0                        | 1                | 0                   | 0             | 0              | 1     |
| Model Control     | 9 | 8             | 6                            | 6                        | 8                | 7                   | 5             | 5              | 45    |
| Prednisone        | 9 | 2             | 2                            | 3                        | 4                | 4                   | 3             | 1              | 19    |
| High CBRP Dose    | 9 | 5             | 5                            | 4                        | 6                | 5                   | 5             | 3              | 33    |
| Moderate CBRP Dose| 9 | 2             | 2                            | 2                        | 3                | 4                   | 2             | 1              | 16    |
| Low CBRP Dose     | 9 | 4             | 4                            | 4                        | 3                | 4                   | 5             | 2              | 26    |

Notes: consolidation, $\chi^2 = 18.125, P=0.003$; brochiovascular abnormality, $\chi^2 = 11.875, P=0.037$; irregular linear shadows, $\chi^2 = 9.815, P=0.081$; pleura thickening, $\chi^2 = 12.813, P=0.025$; ground glass opacity, $\chi^2 = 11.770, P=0.038$; nodule shadow, $\chi^2 = 9.006, P=0.169$; honeycomb lung, $\chi^2 = 10.095, P=0.037$; total times, $\chi^2 = 77.482, P=0.001$.

Compared with the model control group, the extent and severity of alveolitis and fibrosis were milder or even close to normal in the prednisone group and the CBRP groups, indicating that CBRP could significantly lessen the fiber accumulation and antagonize the pulmonary fibrosis. (Fig. 2)

HRCT Image and Corresponding Pathological Changes

Frequency of pathological signs occurred in rats in various groups were listed in Table 3.

Chi-square test showed that the difference among the groups was significant in frequency of consolidation, brochiovascular abnormality, pleura thickening and ground glass opacity, but insignificant in frequency of irregular linear shadows, nodule shadow and honeycomb lung.

HRCT scan showed that rats in the sham-model group had clear pulmonary outline, evenly-distributed lung marking, normal interlobular septum, interlobular mesenchyma and pleura with regular junction, no polished glass-like dense shadow, natural running of the brochiovascular tract with sharp margin running into fine branches gradually from inward to outward. But rats in the model control group showed dramatic change in the pulmonary structure with reduced transmittance, even honeycomb lung in some rats.

Fig. 2 Light Microscopic Picture (Masson, original magnification: 6 X 1.25 X 10) Images of the alveolar structure in rats in the moderate CBRP dose group showing a beginning picture of the alveolar structural change.

Fig. 3 HRCT Image of the Rats in Model Control Group showing increased pulmonary fiber density with disorganized running of bronchiovascular tract and honeycomb changes.

Fig. 4 HRCT Image of the Rats in Moderate CBRP Dose Group showing polished glass-like shadows of a certain degree, which was proved to be significantly improved when compared with the rats in the model control group.

There was a higher frequency of HRCT image changes for rats in the model control group than
those in the sham-model control group. The condition in the CBRP groups was found to be better than that in the model control group (high CBRP dose group, $\chi^2 = 4.072, P = 0.044$; moderate CBRP dose group, $\chi^2 = 29.914, P = 0.001$; low CBRP dose group, $\chi^2 = 10.454, P = 0.001$), suggesting that CBRP could improve the pathological changes induced by BLM. (Fig. 3 and 4).

Correlation Analysis of HRCT Image Changes and Pathological Findings

Fig. 5 and 6 showed respectively the correlation between the frequency of HRCT image changes and the accumulated scores of alveolitis and fibrosis ($r_1 = 0.8159, r_2 = 0.8159$, both $P = 0.001, n = 54$), suggesting that they were highly correlated.

DISCUSSION

IPF is a common pathological change of various pulmonary diseases in the late stage. With the pathogenic causes unknown, the disease is of very low curative rate and survival rate. Researches have shown that Carapax Trionycis possesses such actions as enhancing immunity of organism$^{(5)}$, subsidizing edema, anti-inflammation, inhibiting fibroblastic proliferation and suppressing adverse reactions of cytokines$^{(6)}$; Radix Astragali has the actions of regulating immunity$^{(7)}$ and improving haemorrhheologic state$^{(8)}$; Cordyceps is effective in regulating the immunity of the body$^{(9)}$ and promoting degeneration of collagen type I & III$^{(10)}$. With excellent effect of anti-inflammation and anti-free radicals, Radix Angelicae Sinensis can remarkably abate alveolitis and interstitial fibrosis$^{(11-13)}$; Radix Notoginseng can play a role in antagonizing inflammation and regulating immunity$^{(14,15)}$; Radix Isatis can antagonize viruses and bacteria$^{(16)}$, hence, the preparation of CBRP has a therapeutic effect on pulmonary fibrosis.

HRCT is helpful to remarkably elevate the diagnosis level of IPF with its image correlatively related to histomorphology$^{(17)}$. Gay, et al$^{(18)}$ discovered in their studies that HRCT images had a fairly high exactness in evaluating the patients’ response to drugs and their prognosis. The exactness of HRCT in diagnosing pulmonary fibrosis was also confirmed by Ganesh, et al$^{(18)}$, though with the sensitivity lower than that of lung biopsy. Moreover, Mogulkoc, et al$^{(19)}$ indicated that HRCT image, which related to pulmonary physiological indexes to a certain extent, was an objective criterion for determining the necessity of performing lung transplantation in the patient with pulmonary fibrosis. Ganesh, et al$^{(20)}$ discovered that, though the sensitivity of HRCT image was lower than that of open biopsy of the lung, it has rather higher accuracy in the diagnosis of pulmonary fibrosis. To sum up, HRCT image is superior to the routine CT and X-ray chest film in diagnosing pulmonary fibrosis, because it is possible to make an objective iconographic evaluation pulmonary functions and pathological changes in the lung, and hence provides an effective guidance for clinical treatment and an objective basis for clinical evaluation of certain drugs.

When BLM was used to induce pulmonary fibrosis in rats, it induces firstly the alveolitis with inflammatory exudate in alveolar cavities. Along with the development of the disease, there reveals a decrease of inflammatory cell exudation and infiltration, and an increase of proliferation of fibroblast and collagenous fibers, which thickens alveolar walls, deforms and destroys alveoli. It might involve alveolar ductules and bronchiole to cause thickening and structural changes in interlobular septum and interlobular mesenchyma, and abnormal bronchovascular bundle. Due to gradually weakened air exchange function and finally total exhaustion of air of alveoli, pulmonary consolida-
tion resulted. Polished glass-like shadows are the signs of alveolitis and inflammatory infiltration. Interstitial alveolitis and proliferated fibrosis are also the pathological bases of nodule shadow and abnormal bronchiovascular bundle. Irritative inflammation usually activates fibroblasts, and these fibrotic changes can also be verified by pathological examination.

The extent and severity of alveolitis and fibrosis were found, in this study, to be much milder in the prednisone group and the CBRP groups than those in the model control group, or to be even close to normal, suggesting that CBRP can significantly lessen the fiber accumulation and antagonize the pulmonary fibrosis, showing an excellent effect in improving structural changes in the lung induced by BLM, and favourable to be used to treat pulmonary fibrosis. However, the exact mechanism of CBRP in treating pulmonary fibrosis remains to be further studied.

(Acknowledgements: Sincere thanks is due to Dr. ZHANG Xue-zhe, Dr. WANG Wu and Dr. WU Wen-li from the Radiologic Department of China-Japan Friendship Hospital, and to Dr. CHEN Jian from the Department of Respiratory Diseases and Dr. WANG Ren-gui from Department of Medical Images, First Hospital of Peking University, who rendered us devoted support in our experimental studies.)

REFERENCES

1. Nagao T, Nagai S, Hiramoto Y, et al. Serial evaluation of high-resolution computed tomography findings in patients with idiopathic pulmonary fibrosis in usual interstitial pneumonia. Respiration 2002;69(5) : 413−419.
2. YANG K., WANG JL, XlONG MH. High resolution CT evaluation of idiopathic pulmonary fibrosis. Journal of Clinical Radiology 2001;20(1) : 46−47.
3. Austin JM, Muller NL, Fredman PJ, et al. Glossary of terms for CT of the lungs: Recommendations of the nomenclature committee of the Fleischner society. Radiology, 1996;200(2) : 327−331.
4. Szapiel SV, Elson NA, Fulmer JD, et al. Bleomycin-induced interstitial pulmonary disease in the nude, athymic mouse. Am Rev Respir Dis 1979; Oct; 120(4) : 893−899.
5. XU GZ, LING XM, ZHANG YJ, et al. Protective effect of Carapax Trionycis extract on immune function of mice undergoing large dose X-ray radiation. Chinese Journal of Public Health 1996;15(3) : 170−171.
6. ZENG FB, YAN JJ, WAN B, et al. Pharmacological study on Biejiajian Pills. Chinese Traditional Patent Medicine 2002;24(7) : 529−532.
7. JIN RM, ZHANG XC, CHEN CX, et al. Studies on pharmacological functions of hairy root of Astragalus membranaceus. China Journal of Chinese Material Medica 1999;24(10) : 61.
8. LI H. Influence of Naomaibao on platelets morphology and haemorhheology in rats after ischemia/reperfusion. Journal of Microcirculation 1998;8(2) : 3−5.
9. WANG DH, YAN Y. Study on immune regulation of Dongchong Xiaocao nutrient liquid. China Public Health 2001;17(5) : 417.
10. MA X., QIU DK, XU J, et al. Experimental study on anti-liver fibrosis function of Dongchong Xiaocao polysaccharide liposome. Chinese Journal of Basic Medicine in TCM 1999;5(9) : 28−31.
11. BAO MY, LIANG XY, TIAN SX, et al. Study on Influence of Danggui Buxue Decoction on mice with immuno-deficiency and the model selection. Lisiong Journal of Traditional Chinese Medicine 1998; 25 (3) : 138−139.
12. ZHANG CF, SUN FZ. Current research on action of Danggui in respiratory system. Chinese Traditional and Herbal Drugs 1999;30(4) : 311−313.
13. WU HP, KONG LD. Free radicals scavenging and anti-lipid peroxidation effects of different preparation of Danggui. China Journal of Chinese Material Medica 1996;21 (10) : 599−601.
14. LI SH, CHU Y. Anti-inflammation effects of total saponins of Panax notoginseng (English). Acta Pharmacological Sinica 1999; (6) : 551−554.
15. WANG JM, BAI MP, CHEN ZM. Influence of notoginseng on immune function of mice. China Journal of Traditional Chinese Medicine and Pharmacy 1989; 4 (4) : 29−30.
16. ZHANG DZ, YI JH. Study on pharmacological effect of Isatidis extract in antagonizing influenza virus. China Pharmaceutica 2001;10(5) : 52−53.
17. Schettino IA, Ab'Saber AM, Vollmer R, et al. Accuracy of high resolution CT in assessing idiopathic pulmonary fibrosis histology by objective morphometric index. Pathol Res Prac 2002;198(5) : 347−354.
18. Gay SE, Kazerooni EA, Toews GB, et al. Idiopathic pulmonary fibrosis: predicting response to therapy and survival. Am J Respir Crit Care Med 1998;157(4 Pt1) : 1063−1072.
19. Mogulkoc N, Brutsche MH, Bishop PW, et al. Pulmonary function in idiopathic pulmonary fibrosis and referral for lung transplantation. Am J Respir Crit Care Med 2001;164(1) : 103−108.
20. Ganesh Raghu, Yolanda N. Mageto, Diane Lockhart, et al. The accuracy of the clinical diagnosis of new-onset idiopathic pulmonary fibrosis and other interstitial lung disease: A prospective study. Chest 1999; 116 (5) : 1168−1174.

(Received June 6, 2003)