



# Effect of Ginkgo biloba extract combined with prednisone on bronchoalveolar lavage fluid related cytokines in patients with IPF

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## ABSTRACT

**Objective:** To explore the effect of Ginkgo biloba extract (EGb) combined with prednisone on bronchoalveolar lavage fluid (BALF) related cytokines in patients with idiopathic pulmonary fibrosis (IPF). **Methods:** A total of 60 patients with IPF who were admitted in our hospital from March, 2015 to March, 2016 were included in the study and randomized into the observation group and the control group with 30 cases in each group. The patients in the two groups were given oxygen inhalation, bronchodilator agents, phlegm dissipating and asthma relieving, anti-infection, and other supporting treatments. The patients in the control group were orally given prednisone (0.5 mg/kg·d), continuously for 4 weeks, then in a dose of 0.25 mg/kg·d, continuously for 8 weeks, and finally the dosage was reduced to 0.125 mg/kg·d. On this basis, the patients in the observation group were given additional EGb, *ie.* ginkgo leaf capsule, 1 g/time, 3 times/d, continuously for 12 weeks. The efficacy was evaluated after 12-week treatment. ELISA was used to detect the levels of TNF- $\alpha$ , IL-4, IL-10, and IFN- $\gamma$  in BALF. The radioimmunoassay was used to determine the levels of serum HA, ColIII, PCIII, and LN. The pulmonary function detector was used to measure TLC, VC, DLCO, and 6MWT. **Results:** After treatment, TNF- $\alpha$  level in the control group was significantly reduced when compared with before treatment ( $P < 0.05$ ), and others were not significantly changed. The levels of TNF- $\alpha$ , IL-4, and IL-10 in the observation group were not significantly reduced, but IFN- $\gamma$  was significantly elevated when compared with before treatment ( $P < 0.05$ ). The difference between the two groups was statistically significant ( $P < 0.05$ ). After treatment, HA, ColIII, PCIII, and LN levels in the control group were not significantly improved ( $P > 0.05$ ), while HA, ColIII, PCIII, and LN levels in the observation group were significantly reduced when compared with before treatment ( $P < 0.05$ ), and the difference between the two groups was statistically significant ( $P < 0.05$ ). After treatment, TLC, VC, DLCO, and 6MWT in the two groups were significantly improved when compared with before treatment ( $P < 0.05$ ), and the difference between the two groups was statistically significant ( $P < 0.05$ ). **Conclusions:** EGb combined with prednisone can effectively enhance the levels of TNF- $\alpha$ , IL-4, IL-10, and IFN- $\gamma$  in BALF in patients with IPF, and improve the pulmonary fibrosis degree in order to improve the pulmonary function and patients' living qualities; therefore, it deserves to be widely recommended.

## 1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a disease characterized by pulmonary interstitial fibrosis caused by alveolar structure disorder, diffused pulmonary alveolitis, and interstitial pneumonia,

with manifestations of progressive and aggravated dyspnea, and restrictive ventilatory function, and can cause cardio-pulmonary failure to death in the advanced stage[1]. The pathogenesis of IPF is not yet clear. With a continuously deep study on cellular and molecular biology, it is argued that due to the damage of pathogenic factors on the alveolar epithelial cells, the inflammatory cells and immunologic effector cells enter the alveoli to release a large amount of cytokines and inflammatory mediators, and promote the extracellular matrix deposition, collagen accumulation, and the activation and proliferation of lung fibroblasts, finally

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resulting in pulmonary interstitial fibrosis[2]. Currently, there is no special treatment for IPF. Anti-fibrosis, glucocorticoids, and immunosuppressants are mainly involved in the treatment of IPF, but the adverse reactions are great, and the treatment compliance is poor[3]. Recent researches demonstrate that EGb can resist the pulmonary interstitial fibrosis, and is widely applied in the clinic, with a satisfactory effect[4]. The study is aimed to explore the effect of EGb combined with prednisone on bronchoalveolar lavage fluid (BALF) related cytokines in patients with IPF.

## 2. Materials and methods

### 2.1. General materials

A total of 60 patients with IPF who were admitted in our hospital from March, 2015 to March, 2016 were included in the study, among which 44 were male, and 16 were female; aged from 42 to 70 years old, with an average age of (51.4±9.1) years old. All the patients were in accordance with the diagnostic criteria of IPF[5]. Exclusion criteria: (1) those who had pulmonary fibrosis caused by connective tissue diseases and drugs; (2) those who were merged with heart, liver, kidney, and other serious complications; (3) those who were accompanied by bronchial asthma, bronchiectasis, and other respiratory system diseases. The patients were randomized into the observation group and the control group with 30 cases in each group. The comparison of the general materials between the two groups was not statistically significant ( $P>0.05$ ).

### 2.2. Methods

The patients in the two groups were given oxygen inhalation, bronchodilator agents, phlegm dissipating and asthma relieving, anti-infection, and other supporting treatments. The patients in the control group were orally given prednisone (produced by Zhejiang Xianju Pharmaceutical Co. Ltd, approval No. H33021207), 0.5 mg/kg•d, continuously for 4 weeks, then in a dose of 0.25 mg/kg•d, continuously for 8 weeks, and finally the dosage was reduced to 0.125 mg/kg•d. On this basis, the patients in the observation group were given additional EGb, *ie.* ginkgo leaf capsule (produced by Hunan Hansen Pharmaceutical Co. Ltd, approval No. Z20026289), 1 g/time, 3 times/d, continuously for 12 weeks. The efficacy was evaluated after 12-week treatment.

### 2.3. Observation indicators

The pulmonary lobe lavage was performed with normal saline under the fiber bronchoscope in the sites with dense lesions showed by the chest CT. The lavage fluid was aspirated and centrifuged for the supernatant. ELISA was used to detect the levels of TNF- $\alpha$ ,

IL-4, IL-10, and IFN- $\gamma$  in BALF. The morning fasting venous blood was collected and centrifuged for the serum. The radioimmunoassay was used to determine the levels of serum HA, ColIII, PCIII, and LN. The pulmonary function detector was used to measure TLC, VC, DLCO, and 6MWT.

### 2.4. Statistical analysis

SPSS 18.0 software was used for the statistical analysis. The measurement data were expressed as mean±SD, and *t* test was used. *Chi*-square test was used for the enumeration data.  $P<0.05$  was regarded as statistically significant difference.

## 3. Results

### 3.1. Comparison of BALF related cytokines before and after treatment

After treatment, TNF- $\alpha$  level in the control group was significantly reduced when compared with before treatment ( $P<0.05$ ), and others were not significantly changed. The levels of TNF- $\alpha$ , IL-4, and IL-10 in the observation group were significantly reduced, but IFN- $\gamma$  was significantly elevated when compared with before treatment ( $P<0.05$ ). The difference between the two groups was statistically significant ( $P<0.05$ ) (Table 1).

### 3.2. Comparison of serum pulmonary fibrosis related indicators before and after treatment

After treatment, HA, ColIII, PCIII, and LN levels in the control group were not significantly improved ( $P>0.05$ ), while HA, ColIII, PCIII, and LN levels in the observation group were significantly reduced when compared with before treatment ( $P<0.05$ ), and the difference between the two groups was statistically significant ( $P<0.05$ ) (Table 2).

### 3.3. Comparison of pulmonary function before and after treatment

After treatment, TLC, VC, DLCO, and 6MWT in the two groups were significantly improved when compared with before treatment ( $P<0.05$ ), and the difference between the two groups was statistically significant ( $P<0.05$ ) (Table 3).

## 4. Discussion

IPF is a serious destructive pulmonary disease characterized by excessive synthesis and deposition of extracellular matrix, with main

**Table 1**

Comparison of BALF related cytokines before and after treatment (pg/mL, mean±SD).

Groups	n	Time	TNF- $\alpha$	IL-4	IL-10	IFN- $\gamma$
Observation group	30	Before treatment	35.73±5.41	9.71±2.15	22.52±2.36	8.59±1.12
		After treatment	15.26±4.17 <sup>#</sup>	4.24±0.61 <sup>#</sup>	10.35±1.37 <sup>#</sup>	18.78±3.54 <sup>#</sup>
Control group	30	Before treatment	35.48±4.89	9.56±2.08	22.71±2.02	9.17±1.24
		After treatment	14.37±3.11 <sup>*</sup>	9.25±1.53	21.63±2.15	9.51±1.13

<sup>\*</sup>P<0.05, when compared with before treatment; <sup>#</sup>P<0.05, when compared with the control group.**Table 2**

Comparison of serum pulmonary fibrosis related indicators before and after treatment (mean±SD).

Groups	n	Time	HA ( $\mu$ g/L)	ColIII (mg/L)	PCIII ( $\mu$ g/L)	LN ( $\mu$ g/L)
Observation group	30	Before treatment	136.83±37.18	123.47±22.18	105.62±23.41	147.62±33.41
		After treatment	90.67±32.31 <sup>#</sup>	89.93±20.57 <sup>#</sup>	85.28±21.49 <sup>#</sup>	113.16±28.27 <sup>#</sup>
Control group	30	Before treatment	135.95±38.72	123.35±20.81	104.73±24.55	146.59±31.53
		After treatment	134.66±34.62 <sup>*</sup>	124.14±15.43 <sup>*</sup>	105.18±23.36 <sup>*</sup>	146.32±30.71 <sup>*</sup>

<sup>\*</sup>P<0.05, when compared with before treatment and the control group; <sup>#</sup>P>0.05, when compared with before treatment.**Table 3**

Comparison of pulmonary function before and after treatment (mean±SD).

Groups	n	Time	TLC (L)	VC (L)	DLCO (mL·min <sup>-1</sup> ·mHg <sup>-1</sup> )	6 MWT (m)
Observation group	30	Before treatment	3.17±0.65	2.34±0.22	11.17±2.75	196.15±59.65
		After treatment	3.95±0.46 <sup>#</sup>	2.58±0.34 <sup>#</sup>	17.12±3.28 <sup>#</sup>	278.48±53.62 <sup>#</sup>
Control group	30	Before treatment	3.18±0.66	2.33±0.25	11.15±3.21	198.03±46.34
		After treatment	3.57±0.41 <sup>*</sup>	2.47±0.31 <sup>*</sup>	12.25±3.56 <sup>*</sup>	211.16±43.26 <sup>*</sup>

<sup>\*</sup>P<0.05, when compared with before treatment; <sup>#</sup>P<0.05, when compared with the control group.

pathological changes of inflammatory reaction, immune response, alveolar injury, and pulmonary fibrosis. Damage on the endothelial and epithelial cells of alveolar capillaries by various factors can cause the activation of alveolar macrophages, and the infiltration of eosinophils, lymphocytes, monocytes, and neutrophils, and the released cytokines and inflammatory mediators can induce early lung damage[6], in which there were various inflammatory cells in the alveolar space, the alveolar wall is thickened, the alveolar cavity is transformed, the pulmonary parenchyma is not damaged, and the pathological change is reversible; therefore, effective measures should be taken to prevent a further progression of pulmonary fibrosis, which can affect the pulmonary function[7].

Long-term chronic inflammation stimulation can change the lung tissue structure and promote the proliferation of fibroblasts. The proliferation and fibrosis of fibroblasts are independent of inflammation. The cell apoptosis and proliferation are out of balance. The cell apoptosis is beneficial to the formation of pulmonary fibrosis[8]. Recent researches demonstrate that various cytokines are involved in the occurrence and development of pulmonary fibrosis. The cytokines are mutually regulated and restricted, forming the intra-pulmonary cytokine network, which plays an important role in the occurrence and development of IPF[9]. TNF- $\alpha$  is mainly produced by the activated alveolar macrophages, and its pro-inflammatory cytokine can increase the function of eosinophils and neutrophils, produce a toxic effect on the peripheral cells, mediate the expression of various cytokines and inflammatory factors, promote the synthesis of collagen, and stimulate the proliferation of fibroblasts in order to promote the occurrence of pulmonary

interstitial fibrosis[10]. IL-4 can expand the inflammatory reaction, stimulate the proliferation of fibroblasts, and promote the thickening of pulmonary alveolar basement membrane and the development of fibrosis, resulting in pulmonary structure remodeling[11]. IL-10 is a multiple-effect Th2 cytokine. In the lung tissues with fibrosis, the pulmonary fibroblasts, alveolar macrophages, and the proliferated alveolar epithelial cells can secrete IL-10 in a high level[12]. IFN- $\gamma$ , Th1-like cytokine, can inhibit the aggregation of collagen and the proliferation of fibroblasts, and resist IL-4, IL-10, and other Th2-like cytokines[13]. It is reported that IL-4 and IFN- $\gamma$  are the important immune regulating system, are involved in the pulmonary fibrosis, and play an important role in the development of the disease[11]. It is also reported that TNF- $\alpha$ , IL-4, and IL-10 in patients with IPF are highly expressed and are significantly reduced after effective interventions; therefore, it is argued that TNF- $\alpha$ , IL-4, and IL-10 are closely associated with the development and outcome of IPF[12]. The inflammation and repairing process are accompanied after lung tissue injury. When there is a large or recurrent tissue injury, the alveolar cell components are progressively damaged, the interstitial collagen is ruined, and the fibrous tissue are largely increased, resulting in the formation of fibrosis and scars, and the irreversible change. In the repairing stage, the pro-fibrosis growth factors can stimulate the proliferation of fibroblasts and the synthesis of collagen, promote the deposition of HA, collagen, and LN in the extracellular matrix, and reduce the degradation of extracellular matrix[13, 14].

Glucocorticoid is mainly involved in the treatment of IPF in that it can effectively inhibit the inflammatory immune reaction, restrain the differentiation and proliferation of fibroblasts, and delay the

progression of fibrosis, but its toxic and side effect is large. In recent years, various researches demonstrate that EGb in the treatment of IPF has a significantly efficacy, can effectively improve the lung function and enhance the living quality[15]. EGb, an effective component extracted from the dry leaves of Ginkgo biloba, mainly including flavonoid, terpene lactones, and organic acids, has various and complex biological effects, can effectively regulate the immune function, improve the microcirculation, resist the oxidation, and eliminate the oxygen free radicals[16]. Some researches demonstrate that EGb can effectively regulate the pulmonary function, improve PaO<sub>2</sub>, reduce the exudation of inflammatory cells, and improve the expression of cell immune factors and the clinical symptoms[17]. Some scholars study the clinical efficacy of EGb in the treatment of IPF, and it is found that EGb can preferably improve the pulmonary function PaO<sub>2</sub>, reduce the expressions of TNF- $\alpha$  and IL-10, and improve the clinical symptoms; therefore, it is argued that EGb in the treatment of IPF has an important application value[10].

The results in the study showed that after treatment, TNF- $\alpha$  level in the control group was significantly reduced when compared with before treatment ( $P < 0.05$ ), and others were not significantly changed, the levels of TNF- $\alpha$ , IL-4, and IL-10 in the observation group were significantly reduced, but IFN- $\gamma$  was significantly elevated when compared with before treatment ( $P < 0.05$ ), the difference between the two groups was statistically significant ( $P < 0.05$ ); after treatment, HA, ColIII, PCIII, and LN levels in the control group were not significantly improved ( $P > 0.05$ ), while HA, ColIII, PCIII, and LN levels in the observation group were significantly reduced when compared with before treatment ( $P < 0.05$ ), and the difference between the two groups was statistically significant ( $P < 0.05$ ); after treatment, TLC, VC, DLCO, and 6MWT in the two groups were significantly improved when compared with before treatment ( $P < 0.05$ ), and the difference between the two groups was statistically significant ( $P < 0.05$ ), indicating that EGb combined with prednisone in the treatment of IPF has an accurate efficacy, and can effectively improve the expression levels of cytokines and the pulmonary function.

In conclusion, EGb combined with prednisone can effectively enhance the levels of TNF- $\alpha$ , IL-4, IL-10, and IFN- $\gamma$  in BALF in patients with IPF, and improve the pulmonary fibrosis degree in order to improve the pulmonary function and patients' living qualities; therefore, it deserves to be widely recommended.

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