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Milkvetch root improves immune function in patients with acute exacerbation of COPD

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Abstract. Milkvetch root as a medicine has been used for more over 2000 years in China, can strengthen immune function, protect liver, promote urination, resist aging and stress, reduce blood pressure and extensively resist bacterium. This study explored the effects of milkvetch root on the immune function of patients with a definitive diagnosis of acute exacerbation of chronic obstructive pulmonary disease (COPD). The patients were randomly assigned to either the experimental or control group. All patients received conventional clinical therapy; those in the experimental group were also administered milkvetch root. The serum levels of cytokines including tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8), IL-1β, and IL-32 and immunocytes including T helper (Th), cytotoxic T (Tc), natural killer (NK), regulatory T (Treg) and B cells were measured 1 day before treatment and 7 and 14 days post-treatment. After bronchodilator inhalation, pulmonary function was evaluated at these same time points. The serum TNF-α, IL-8, IL-1β, and IL-32 levels were significantly lower in the experimental group than in the control group 14 days post-treatment. The Th/Tc ratio and NK cell ratio was significantly higher but the Treg cell ratio was significantly lower in the experimental group than in the control group. The forced expiratory volume in 1 second (FEV1) and FEV1/forced vital capacity (FVC) were significantly higher in the experimental group than in the control group 14 days post-treatment. These results indicate that milkvetch root can improve the immune function of patients with acute exacerbation of COPD.

Keywords: Chronic obstructive pulmonary disease, milkvetch root, immune function

1. Introduction

Chronic obstructive pulmonary disease (COPD) is induced by chronic bronchitis and emphysema. It is characterized by reversible airflow obstruction with clinical progression and represents a serious public health concern worldwide [1, 2]. Chronic inflammation involving the lung parenchyma, airways, and pulmonary vessels is the basis of disease progression in patients with COPD. This is particularly evident in acute exacerbation of COPD, which is characterized by substantial activation of inflammatory cells and release of inflammatory factors and media. Improvement in the quality of life

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of patients with COPD is dependent upon taking effective therapeutic measures, controlling the inflammatory reaction, and improving pulmonary function [3-5]. Many clinicians practicing modern medicine believe that milkvetch root can improve the body's immune function [6-8]. Based on traditional Chinese medicine theory, this study combined milkvetch root and conventional treatment methods in patients with acute exacerbation of COPD and observed the effects of milkvetch root on stabilization of the patients' immune status.

2. Patients and methods

2.1. Patients

In total, 82 patients with acute exacerbation of COPD diagnosed at the Third Affiliated Hospital of Nantong University in China from April 2012 to October 2013 were enrolled in this study. Each patient was randomly assigned to either the control group (n = 41; 21 men and 20 women; age, 51–90 years) or the experimental group (n = 41; 22 men and 19 women; age, 46–93 years) based on their sequence of hospital admission. The inclusion criteria were as follows: the diagnosis of acute exacerbation of COPD was achieved in accordance with the Guideline for Diagnosis and Treatment of COPD (2007 Revised Edition) formulated by the Chinese Society of Respiratory Diseases of the Chinese Medical Association [9]; local and systemic hormones had not been used within 3 months prior to enrollment; and pulmonary function could be measured at the time of admission. The exclusion criteria were as follows: respiratory system diseases other than COPD; left ventricular systolic or diastolic dysfunction; hemopathy; malignant tumors; systemic autoimmune disease; other infectious disease; severe endocrine, liver, or kidney disease; or recent surgery. This study was performed in accordance with medical ethical standards and was approved by our institution's Hospital Ethics Committee. All patients provided written informed consent to participate.

2.2. Therapeutic regimen

Antispasmodic, expectorant, antiasthmatic, and anti-infection treatments were performed in accordance with Guideline for Diagnosis and Treatment of COPD (2007 Revised Edition). The experimental group also received 15 mg of astragalus granules (JiangyinTianjiang Pharmaceutical Co., Ltd.) with water twice a day for 14 consecutive days.

2.3. Evaluation of pulmonary function

All patients in both the experimental and control groups inhaled 5 mg of the bronchodilator terbutaline on the day of admission and in the morning at 7 and 14 days after admission in the conscious state. Fifteen minutes later, pulmonary function parameters were measured using a spirometer (MasterScreen, Jaeger, Germany), and the forced vital capacity (FVC), forced expiratory volume in 1 second (FEV1), and FEV1/FVC ratio were recorded. FEV1 and FEV1/FVC are adopted as diagnostic indicators for the determination of the airflow limitation in patients with COPD; FEV1/FVC can be used to determine a mild airflow limitation while the percentage of FEV1 in a predicted value is used as a good index for the determination of midrange or severe airflow limitation. They are the routine testing items in clinic for COPD pulmonary function examination.

2.4. Measurement of cytokines and immunocytes

After the patients had fasted, 5 ml of venous blood was collected on the day of admission and in the morning at 7 and 14 days after admission in both the experimental and control groups. The blood samples were placed in an unheated drying tube (serum was isolated within 2 hours and stored at –80°C, and inflammatory cytokines were measured) and in a tube containing the anticoagulant ethylenediaminetetraacetic acid (immunocytes were evaluated the same day). The inflammatory cytokines measured were tumor necrosis factor alpha (TNF-α), interleukin-8 (IL-8), IL-1β, and IL-32. The ELISA kit used in this study was purchased from eBioscience (San Diego, CA, USA), and the SpectraMax 340 microplate reader was purchased from Molecular Devices, Inc. (Sunnyvale, CA, USA). The immunocytes measured were T helper (Th) cells ($CD3^+/CD4^+$ cells), cytotoxic T (Tc) cells ($CD3^+/CD8^+$ cells), natural killer (NK) cells ($CD3^-/CD16/56^+$ cells), regulatory T (Treg) cells ($CD4^+/CD25^+/Foxp^{3+}$ cells), and B cells ($CD3^-/CD19^+$ cells). Flow cytometry was performed (FACSCalibur; BD Biosciences, San Jose, CA, USA). All procedures were conducted in precise accordance with the manufacturer's instructions.

2.5. Statistical methods

All data were analyzed using SPSS 10.0 software (SPSS Inc., Chicago, IL, USA). Intragroup comparisons at different time points in both groups were performed using the two-sample t-test. Intergroup comparison at the same time point was performed using the two-independent-samples t-test. Qualitative data in both groups were compared using the chi-squared test in four-fold table. The testing standard for all hypothesis tests was set at $\alpha=0.05$. A value of $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Quantitative analysis of patients

Of all 82 patients with COPD, 6 from the control group and 3 from the experimental group were excluded because of data loss, replacement therapy, or early discharge. In total, 73 patients (35 in the control group and 38 in the experimental group) were included in the final analysis.

3.2. Comparison of baseline data between the two groups

No significant differences in age (73.18 ± 10.79 years vs. 74.17 ± 10.20 years; $t = 0.388$, $P = 0.699$) or sex (male/female: 20/18 vs. 16/19; $\chi 2 = 0.349$, $P = 0.555$) were detected between the experimental and control groups (all $P > 0.05$). Additionally, no significant differences in FEV1 (52.34% ± 7.13% vs. 51.57% ± 7.53%; $t = 0.443$, $P = 0.659$) or FEV1/FVC (56.75% ± 9.44% vs. 57.49% ± 9.08%; $t = 0.341$, $P = 0.734$) were detected between the experimental and control groups (all $P > 0.05$). Finally, no significant differences in the immunological parameters were observed before treatment between the experimental and control groups (all $P > 0.05$) (Table 1).

Table 1

Comparison of immunological parameters before treatment in the experimental and control groups

Group	Case	IL-8(ng/L)	IL-1β(ng/L)	TNF-α(ng/L)	IL-32(ng/L)
Control	35	59.25±9.31	12.22±3.72	67.39±9.58	32.34±4.69
Experimental	38	58.18±8.72	12.68±3.39	68.49±10.04	31.61±5.19
t		0.510	0.550	0.481	0.630
P		0.612	0.584	0.632	0.531
Group	Case	Treg cell ratio (%)	Th/Tc ratio (%)	NK cell ratio (%)	B cell ratio (%)
Control	35	8.42±1.79	1.15±0.33	12.08±4.83	10.72±2.41
Experimental	38	8.55±1.68	1.11±0.32	11.47±4.10	10.61±2.21
t		0.311	0.542	0.581	0.219
P		0.756	0.590	0.563	0.828

Note: IL: interleukin, TNF: tumor necrosis factor, Treg: regulatory T cell, Th: T helper cell, Tc: cytotoxic T cell, NK: natural killer cell

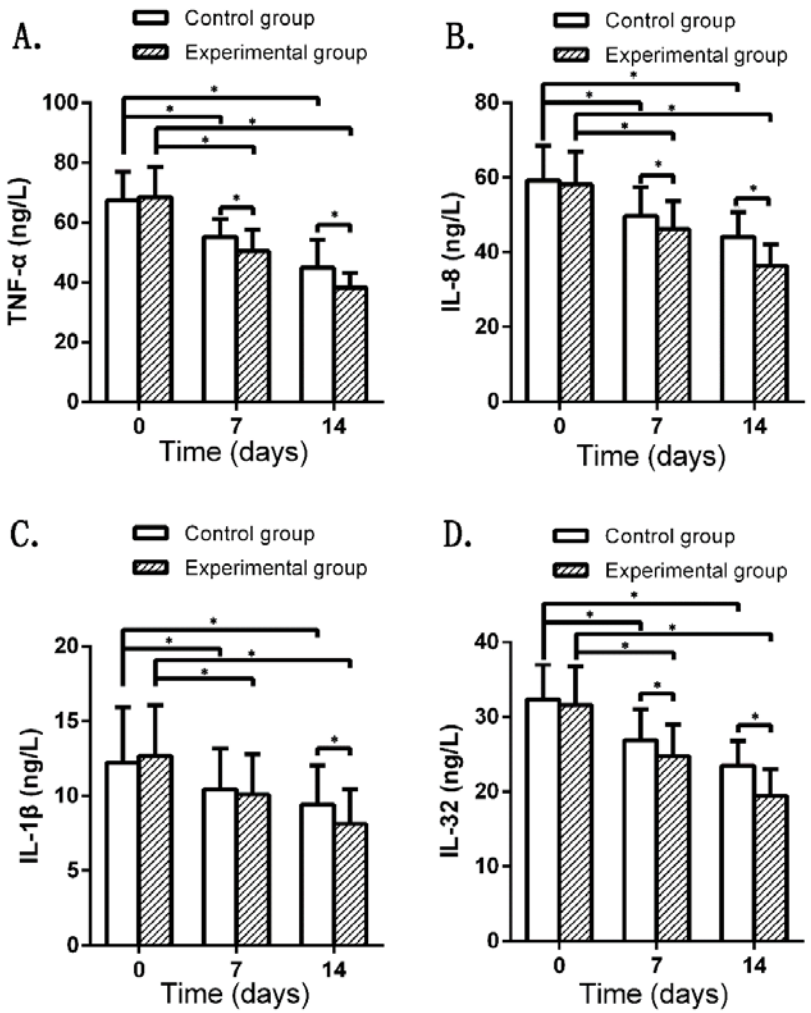

Fig. 1. TNF-α, IL-8, IL-1β, and IL-32 levels were significantly lower in the experimental group than in the control group after 7 and 14 days post-treatment. The blood samples were detected by homologousELISAkits.A. TNF-α; B. IL-8; C. IL-1β; D. IL-32.TNF: tumor necrosis factor, IL: interleukin, **P*< 0.05.

3.3. Comparison of inflammatory cytokines between the experimental and control groups

The TNF-α, IL-8, IL-1β, and IL-32 levels were significantly lower 7 and 14 days after treatment than the levels before treatment in both the experimental and control groups (all $P < 0.05$). The TNF-α, IL-8, IL-1β, and IL-32 levels were significantly lower in the experimental group than in the control group 7 and 14 days post-treatment (all $P < 0.05$) (Figure 1).

3.4. Comparison of NK, Treg, and B cell and Th/Tc ratio changes between the experimental and control groups

The NK and B cell and Th/Tc ratios were significantly higher but the Treg cell ratio was significantly lower 14 days after treatment in both the experimental and control groups (all $P < 0.05$). The Th/Tc ratio was significantly higher in the experimental group than in the control group 7 days after treatment ($P < 0.05$). The NK cell ratio was significantly higher but the Treg cell ratio was significantly lower in the experimental group than in the control group at 14 days ($P < 0.05$). No significant difference in the B cell ratio was detected between the experimental and control groups at 14 days ($P > 0.05$) (Figure 2).

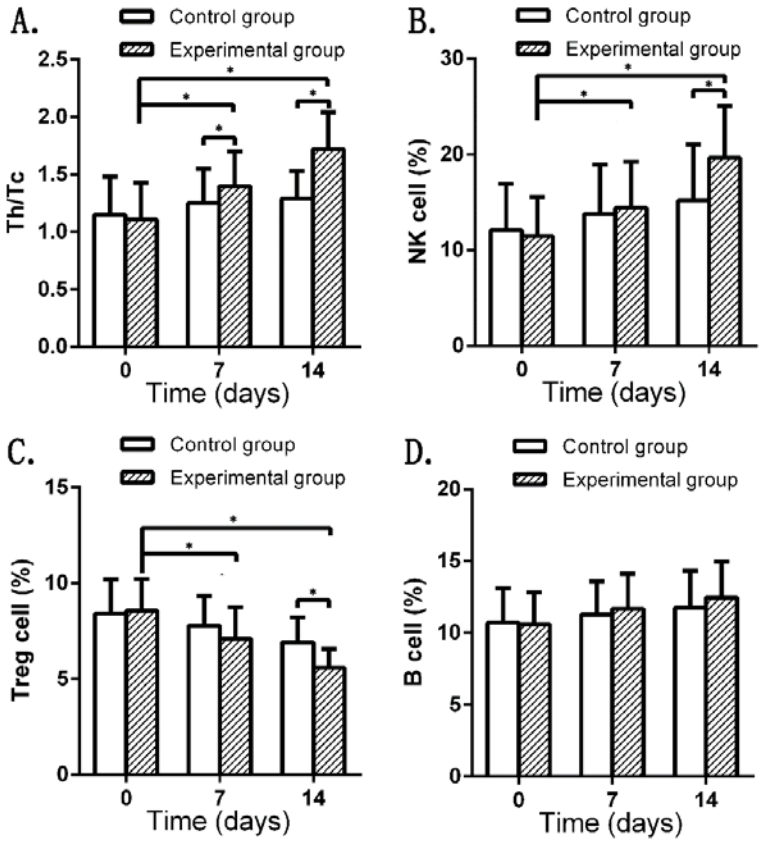

Fig. 2. Comparison of NK, Treg, and B cells and Th/Tc ratio between the experimental and control groups according to treatment time. Th/Tc ratio and NK cell ratio were significantly higher but the Treg cell ratio was significantly lower in the experimental group than in the control group after 14 days post-treatment. A. Th/Tc ratio; B. NK cell ratio; C. Treg cell ratio; D. B cell.NK: natural killer cell, Treg: regulatory T cell, Th: T helper cell, Tc: cytotoxic T cell; *$P < 0.05$.

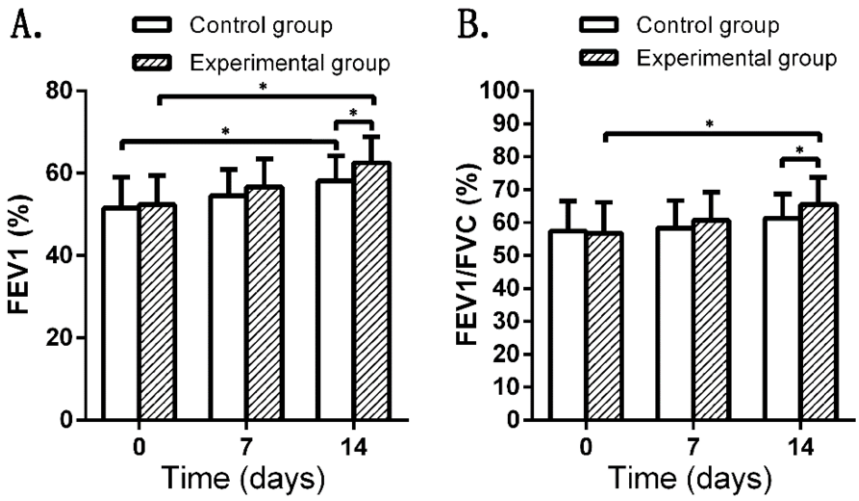

Fig. 3. FEV1 and FEV1/FVC were significantly higher in the experimental group than in the control group after 14 days post-treatment. A. FEV1; B. FEV1/FVC.FEV1: forced expiratory volume in 1 second, FVC: forced vital capacity, *P < 0.05.

3.5. Alterations in FEV1 and FEV1/FVC between the experimental and control groups

FEV1 and FEV1/FVC were significantly higher 7 and 14 days after treatment than before treatment in both the experimental and control groups ($P < 0.05$). FEV1 and FEV1/FVC were significantly higher in the experimental group than in the control group at 14 days post-treatment ($P < 0.05$) (Figure 3).

3.6. Adverse reactions

In the experimental group, two patients developed mild diarrhea and one developed abdominal distension; both conditions resolved after symptomatic treatment. No adverse reactions were observed in the control group.

4. Discussion

Patients with COPD have immune dysfunction. Viral or bacterial infections are met with poor resistance and immune imbalance [10] characterized by decreases in the Th/Tc ratio, NK cells, and B cells [11, 12]; increases in neutrophil and Treg cell stress (For Treg cells, it could be argued that long-term inflammatory exposure appears to cause a dysfunction of Treg cell, resulting in a negative feedback to augment further Treg cell influx) [10, 13-15]; and increases in various inflammatory cytokines such as TNF-α, IL-8, IL-1β, and IL-32 [16-20]. TNF-αand IL-1β are major inducers of the inflammatory immune response. As inflammatory cytokines, TNF-α and IL-1β can activate nuclear factor-κB and the mitogen-activated protein kinase signaling pathway in airway epithelial cells, macrophages, and lung tissue cells. Adherence and infiltration of lymphocytes, monocytes, and neutrophils result in lung injury under conditions of inflammation [21, 22] and induce serial inflammatory cytokine expression. IL-32 is mainly expressed in alveolar macrophages and alveolar walls. COPD markedly upregulates IL-32 expression. IL-32 induces the production of TNF-α, IL-1β, and IL-8 by nuclear factor-κB, p38 mitogen-activated protein kinase, and the caspase-1 and caspase-3

signal transduction pathways. IL-1β may increase to 10 times its baseline level [23-25]. IL-8 is a key cytokine and chemokine that can activate neutrophils. It is mainly synthesized and released by mononuclear macrophages induced by IL-1 and TNF-α [26, 27]. IL-8 induces neutrophils to release elastase, which can injure lung cell walls, and to release a large number of oxygen free radicals that injure lung tissue and directly lead to bronchial smooth muscle spasm [28, 29]. These processes result in airway obstruction and progression of inflammation. In addition, the release of neutrophil elastase induces IL-8 gene expression in airway epithelial cells, leading to a vicious circle of chronic airway inflammation and sustained damage [30]. These disordered immune cells interact with inflammatory factors and form an internal network that can promote sustained progression of COPD.

The main components of milkvetch root are astragalus polysaccharide, more than 40 saponins, more than 30 flavones, 25 amino acids, and various trace elements [31, 32]. Milkvetch root has a variety of biological effects: it is a good immunomodulator [33-35], regulates T-lymphocyte function, inhibits nuclear factor-κB activation, increases the secretory immunoglobulin A content in respiratory mucosa, controls humoral immunity, reduces inflammatory edema of the bronchial mucosa, and controls clinical symptoms in patients with COPD. Milkvetch root suppresses the production of oxygen free radicals, prevents their interaction with membrane lipids to generate lipid peroxides, reduces damage to the biomembrane [36], and suppresses platelet adhesion.

In this study, the serum TNF-α, IL-8, IL-1β, and IL-32 levels significantly decreased after 14 days of antispasmodic, expectorant, antiasthmatic, and anti-infection treatment in patients with acute exacerbation of COPD in both the experimental and control groups (all $P < 0.01$). However, the NK cells, B cells, and Th/Tc ratio significantly increased (all $P < 0.05$) and the Treg cells significantly decreased ($P < 0.05$). These results suggest that the strategy used in this study has definite advantages and is associated with apparent clinical improvement in both FEV1 and FEV1/FVC. These results also demonstrated that the serum TNF-α, IL-8, IL-1β, and IL-32 levels were significantly lower but that the Th/Tc ratio and NK cells were significantly higher in the experimental group (administration of milkvetch root) than in the control group 14 days after treatment (all $P < 0.01$). Moreover, Treg cells were significantly lower in the experimental group than in the control group at 14 days ($P < 0.01$). The above-described results indicated that milkvetch root affected the humoral immunity of patients with acute exacerbation of COPD through its various active ingredients Milkvetch evidently contributed to proliferation and activation of Th cells and NK cells and to the return of Treg cells, weakened the cascade of inflammatory cells and inflammatory cytokines, enhanced protection against infection, and achieved better therapeutic outcomes. The high FEV1 and FEV1/FVC in the experimental group also illustrated this point. Milkvetch root used in this study has various active ingredients, and that ingredients improved the immune function of patients with acute exacerbation of COPD in this study are unknown. This will be an essential component of future studies of the ingredients of milkvetch root.

In conclusion, the application of milkvetch root to antispasmodic, expectorant, antiasthmatic, and anti-infection treatment helps to balance humoral and cellular immunity and improves pulmonary function in patients with acute exacerbation of COPD.

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