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Effects of diammonium glycyrrhizinate on serum inflammatory factors and peripheral blood T lymphocytes in patients with stable chronic obstructive pulmonary disease

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Abstract

Objective: To evaluate the influence of diammonium glycyrrhizinate on serum inflammatory factors and peripheral blood T lymphocytes in patients with chronic obstructive pulmonary disease (COPD) in the stable phase.

Methods: A total of 244 cases admitted from January 2015 to June 2019 were divided into control and observation groups (n=122) randomly. The control group received Western medicine therapy and the observation group was further given diammonium glycyrrhizinate capsules for three months. Their therapeutic effects, pulmonary function indices, serum inflammatory factors and peripheral blood T lymphocytes were compared.

Results: After treatment, the overall effective rates of observation and control groups were 90.68% and 75.00%, respectively ($\chi 2=10.146$, $P=0.001$). FEV1, FVC and FEV1/FVC of both groups significantly increased, especially in observation group ($P<0.05$). Serum CRP, TNF-α and IL-6 levels significantly decreased, particularly in observation group ($P<0.05$). The two groups had significantly different CD8+, CD4+ and CD3+ cell counts as well as CD4+/CD8+ cell ratio ($P<0.05$). The incidence rates of adverse drug reactions in control and observation groups were 6.56% and 9.68%, respectively ($P>0.05$).

Conclusion: For patients with stable COPD, routine treatment plus diammonium glycyrrhizinate can enhance therapeutic effects, improve pulmonary function, alleviate inflammation and boost immune function while hardly inducing adverse drug reactions.

Keywords: Diammonium glycyrrhizinate; chronic obstructive pulmonary disease; inflammatory factor; immune function

1. Introduction

Chronic obstructive pulmonary disease (COPD), as a common respiratory disease, is typified by incomplete reversible airflow limitation, with high morbidity rate, easy recurrence and complex pathogenesis (Jarnicki et al., 2016). COPD patients suffer from chronic inflammation of lung parenchyma or pulmonary vessels and trachea mediated by various inflammatory factors. At this time, the immune regulatory function of T lymphocytes plays important roles in the onset, progression and outcome of COPD (Forsslund et al., 2014). Recently,34 the systemic inflammatory response and immune dysfunction of COPD patients have been closely related to extrapulmonary manifestations such as cardiovascular disease and osteoporosis in addition to pulmonary lesions (Badawy, 2017). Clinically,

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Western medicine treats COPD mainly by combating asthma and inflammation, but the therapeutic effects are still unsatisfactory occasionally. Diammonium glycyrrhizinate, as a monomeric compound, is an effective component extracted from Chinese herbal medicine licorice, with anti-inflammatory, anti-oxidative and immunomodulatory effects as well as mild clinical adverse reactions. It is mostly used to treat chronic hepatitis (Kao, Wu, & Yen, 2014). We herein aimed to assess the influence of diammonium glycyrrhizinate on the serum inflammatory factors and peripheral blood T lymphocytes in patients with COPD in the stable phase. This study provides valuable evidence for clinical medication.

2. Materials and Methods

Subjects

This study has been approved by the ethics committee of Qilu Hospital of Shandong University. All patients have signed informed consents before enrollment. Patients with stable COPD admitted to Qilu Hospital of Shandong University from January 2015 to June 2019 were included. Inclusion criteria: 1) In accordance with the diagnostic criteria stipulated in the Guidelines for COPD Diagnosis and Treatment (revised version in 2013) (Society of Respiratory Diseases, Chinese Medical Association; 2) with percentage of forced expiratory volume in 1 second to forced vital capacity (FVC) (FEV1%) of 30%~80%; 3) aged between 40 and 75 years old; 4) without participation in other clinical studies within 3 months. Exclusion criteria: 1) Use of glucocorticoids four weeks before enrollment; 2) complication with tuberculosis, bronchial asthma, pulmonary interstitial fibrosis or lung cancer; 3) with drug allergies; 4) with immunodeficiency or history of respiratory failure; 5) with serious heart, liver or kidney dysfunction; 6) pregnant women or those preparing for pregnancy and lactating women.

The enrolled patients were divided into observation and control groups (n=122) randomly. Five cases did not complete this study, including two cases in the observation group (1 case failed to cooperate due to work reasons; 1 case withdrew due to unsatisfactory efficacy) and three cases in the control group (1 case needed long-term inpatient treatment in other hospitals because of accident; 2 cases withdrew because of unsatisfactory efficacy). A total of 234 patients completed this study. The observation group had 118 patients comprising 40 females and 78 males with the average age of (63.57 ± 5.04) years old. The control group had 116 patients consisting of 42 females and 74 males with an average age of (62.62 ± 5.13) years old. The two groups had comparable baseline clinical data (P>0.05).

Treatment Methods

The control group was subjected to conventional treatment for COPD such as smoking cessation, oxygen support, inhalation of salmeterol xinafoate powders (Seretide, GSK Pharma, France) and oral administration of ambroxol (Mucosolvan, Shanghai Boehringer Ingelheim Pharmaceutical Co., Ltd., China). On this basis, the observation group was treated with diammonium glycyrrhizinate capsules (Ganlixin, Chia Tai Tianqing Pharmaceutical Group Co., Ltd., Lianyungang, China) three times daily, 150 mg each time. The two groups were both treated for three months during which no other traditional Chinese medicine preparations were taken.

Evaluation of Therapeutic Effects

According to a previous literature (Tan et al., 2017), therapeutic effects were classified into marked effectiveness, effectiveness and ineffectiveness. 1) Marked effectiveness: Significant alleviation of cough, expectoration, gasping, breath shortness and other clinical symptoms, and disappearance of rale; 2) effectiveness: relief of the above clinical symptoms and rale, with intermittent attacks needing continuous drug treatment; 3) ineffectiveness: unobvious alleviation of the above clinical symptoms and rale. Overall effective rate = (markedly effective case number + effective case number)/total case number × 100%. The incidence rates of adverse reactions during treatment were recorded.

Detection of Pulmonary Function Indices

FVC, FEV1 and FEV1/FVC were tested by AS-507 pulmonary function detector (Minato Medical, Japan) before and after treatment.

Detection of Inflammatory Factors

In the early morning of one day before treatment and the first day 3 months after treatment, cubital venous blood was collected after 12 h of fasting and centrifuged at 4°C. TNF-α, CRP and IL-6 levels in the resulting serum were detected by ELISA using corresponding kits (GB, USA) strictly according to instructions.

Detection of T Lymphocytes

On pretreatment and posttreatment 1st days, 100 μL of peripheral venous blood was collected, incubated with 20 μL of CD4+/CD8+/CD3+ FITC/PE/PC5 and CD3-/CD16+56+ FITC/PE trichrome-marked antibodies at room temperature for 15 min in dark, lysed with Immunoprep Reagent

System lysis buffer for 10 min, and finally detected by Epics XL flow cytometer (Beckman Coulter, USA).

Statistical Analysis

SPSS16.0 software was utilized for statistical analysis. The continuous variables were represented as mean ± standard deviation. The paired t test was performed to compare the data of each group before and after treatment, and intergroup comparisons were conducted with the independent t test. The categorical data were expressed as percentage. Therapeutic effects were compared by using the χ2 test, and the Fisher's exact test was carried out to compare adverse reactions. Difference was determined as statistically significant when P<0.05.

3. Results

Clinical Therapeutic Effects

In observation and control groups, 118 and 116 cases completed this study, respectively. After treatment, the overall effective rates of observation and control groups were 90.68% and 75.00%, respectively (χ2=10.146, P=0.001) (Table 1).

Pulmonary Function Indices

Before treatment, the pulmonary function indices of the two groups were not significantly different (P>0.05). Both groups, especially the observation group, had significantly elevated FVC, FEV1 and FEV1/FVC after treatment (P<0.05) (Table 2).

Serum Inflammatory Factors

Before treatment, the two groups had similar serum inflammatory factor levels (P>0.05). The CRP, TNF-α and IL-6 levels of both groups, particularly the observation group, significantly dropped after treatment (P<0.05) (Table 3).

T Lymphocytes

Before treatment, the two groups had similar peripheral blood T lymphocytes (P>0.05). The observation group underwent significant increase of CD4+ and CD3+ cell counts as well as CD4+/CD8+ cell ratio but significant decrease of CD8+ cell count (P<0.05) after treatment. The CD4+ and CD3+ cell counts of the control group increased significantly (P<0.05), whereas CD8+ cell count or CD4+/CD8+ cell ratio hardly changed (P>0.05). The two groups had significantly different CD8+, CD4+ and CD3+ cell counts together with CD4+/CD8+ cell ratio (P<0.05) (Table 4).

Safety Evaluation

The observation group had 2 cases of headache, 2 cases of nausea, 1 case of feeling of oropharyngeal irritation and 1 case of arrhythmia. The control group had 2 cases of headache, 1 case of nausea and 1 case of arrhythmia. The incidence rates of adverse drug reactions in observation and control groups were 9.68% and 6.56%, respectively (P>0.05).

4. Discussion

COPD belongs to "lung distension" in traditional Chinese medicine, and can also be categorized into "dyspnea syndrome" and "phlegm and retained fluid", which is caused by the invasion of exogenous pathogenic factors on lungs which lose dispersion and descending, and intrinsic phlegm heat (Li JP, 2016). The symptom is chronic with more blood stasis which causes protracted cough and asthma, resulting in yang deficiency of lung, spleen and kidneys, loss of Qi caused by lung deficiency, lung failing to distribute fluid, phlegm and retained fluid. The symptom has an exterior symptom in the lung, but is rooted in the spleen and kidneys. Therefore, the treatment of the disease should focus on clearing away the lung-heat, relieving asthma, facilitating blood circulation to eliminate stasis and replenishing lungs and kidneys (Ni HB, 2016). Diammonium glycyrrhizinate is an active ingredient extracted and concentrated from licorice by modern extraction technology, with the advantages of less dosage and quick onset. It can quickly act on the lung, improve its blood circulation, alleviate airway spasm, and increase alveolar ventilation to relieve symptoms (Seo et al., 2017). The Compendium of Materia Medica recorded that licorice was applicable to the treatment of typhoid sore throat, lung heat sore throat, consumptive lung disease, and enduring cough for consumptive lung. It is recorded in the Pharmaceutical Characterization that licorice can strengthen the middle warmer in the grilled way, treat spleen insufficiency, chronic diarrhea, stomach deficiency, thirst, chills and fever and cough, shortness of breath, fatigue, labor loss, asthenic disease, whose sweet and warm features can improve the spleen functions (Yang, Yuan, Ma, Zhou, & Liu, 2017). This study showed that the cough, wheezing and breath shortness of the observation group were more obviously mitigated. In addition, the lung function indicators of FEV1, FVC and FEV1/FVC (%) were also significantly improved in the observation group, suggesting that the drug can effectively reduce airway resistance and increase oxygen partial pressure in patients with stable COPD. Fan et al. used the adjuvant therapy of diammonium glycyrrhizinate to treat COPD patients complicated by pulmonary tuberculosis, and found that the therapy could improve lung ventilation and clinical efficacy (Fan YH, 2018), with the results similar to this study.

The immune system and inflammatory factors essentially participate in the progression of COPD as an inflammatory reactive disease (Zhang et al., 2016),(Blumenthal et al., 2016). Smoke from cigarettes can stimulate inflammatory mediators releasing chemokines in airway epithelial cells, triggering a cascade effect that causes the body to develop a systemic inflammatory response. Therefore, all patients were required to take measures to quit smoking in this study to reduce interference (Gu, Chu, Zeng, Bao, & Liu, 2017). CRP is an acute phase protein that is a reliable indicator of inflammatory response (Qiu et al., 2018). It can respond quickly to the inflammatory response of the body with a large variation, which is easy to detect. At the same time, the factors such as the body's immune level, age and drugs have little effect on the protein, and its level changes with whether the body inflammatory state is effectively controlled (Gabriel & Salazar, 2014). IL-8 can cause the chemotaxis of inflammatory cells, especially neutrophils, and allow cells to release cathepsins and enhance oxidative stress, leading to increased inflammatory response and worsening COPD conditions (Schwameis et al., 2016). TNF-α is involved in the systemic inflammatory response, and high-level TNF-α can affect the body's exercise tolerance, and can lead to decreased oxygen partial pressure, resulting in difficulty breathing (Refahi, Pourissa, Zirak, & Hadadi, 2015). TNF-α reduction is beneficial for alleviating the progression of COPD (Howard & Vincent, 2016). In this study, after the observation group was given diammonium glycyrrhizinate, the levels of CRP, TNF-α and IL-6 were significantly reduced. Licorice can eliminate high reactivity and attenuate inflammatory cell infiltration, which explains to some extent the mechanism of action of diammonium glycyrrhizinate capsules in reducing the inflammatory state of patients with stable COPE (Amatngalim et al., 2017).

T cells play key roles in cellular immunity and tumor resistance. CD3+ cells belong to mature lymphocytes with the highest cell activity during immunization (Dziewulska, Stenzel, Śmiałek, Tykałowski, & Koncicki, 2018). As the main marker of T helper cells, CD4+ cells participate in activating B cells, macrophages, natural killer cells and cytotoxic T cells (Fouladi, Masjedi, Ghasemi, Hakemi, & Eskandari, 2018). CD8+ cells, also known as T suppressor or cytotoxic T cells, inhibit the formation of antibodies and immune response mainly through suppressing the functions of B and CD4+ cells, as a negative regulation. In the T cell subset, CD8+ and CD4+ cells regulate the pivot, and the changes in their counts and ratio correspond to the immune function of human body (Aipire et al., 2017). The level of T lymphocyte subsets is related to the immune function of COPD patients (Durham, Caramori, Chung, & Adcock, 2016). Herein, the observation group had higher CD8+, CD4+ and CD3+ cell counts as well as CD4+/CD8+ cell ratio than those of the control group after treatment, suggesting that diammonium glycyrrhizinate capsules can boost the immune function, which may also be a crucial part of reducing inflammation. Moreover, the incidence rates of adverse reactions in the two groups were not significantly different, indicating that the concurrent treatment of diammonium glycyrrhizinate capsules in patients with stable COPD is safe and feasible.

5. Conclusions

In summary, for patients with stable COPD, routine treatment in combination with administration of diammonium glycyrrhizinate can enhance therapeutic effects, improve pulmonary function, alleviate inflammation and boost immune function, without elevating the incidence rate of adverse drug reactions. Hence, this method is worthy of clinical application.

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Table 1. **Clinical therapeutic effects (n, %)**

Group	Clinical therapeutic effects			Overall effective rate
	Markedly effective	Effective	Ineffective	
Observation (n=118)	72 (61.02)	35 (29.66)	11 (9.32)	107 (90.68)
Control (n=116)	60 (51.72)	27 (23.283)	29 (25.00)	87 (75.00)
χ2				10.146
P				0.001

Table 2. **Pulmonary function indices before and after treatment (x ± s)**

Group	Time	FEV1	FVC	FEV1/FVC (%)
Observation (n=118)	Before treatment	1.51±0.24	2.25±0.32	45.96±4.23
	After treatment	2.02±0.41*	2.97±0.43*	55.41±4.61*
t		8.298	11.265	10.265
P		<0.001	<0.001	<0.001
Control (n=116)	Before treatment	1.52±0.24	2.26±0.33	45.91±4.23
	After treatment	1.69±0.38	2.43±0.43	49.32±4.64
t		4.328	3.276	4.372
P		<0.001	0.007	<0.001

*Compared with control group, P<0.05 (FEV1, t=6.383, P<0.001; FVC, t=9.605, P<0.001; FEV1/FVC, t=10.071, P<0.001).

Table 3. **Serum inflammatory factor levels before and after treatment**

Group	Time	CRP (ng/ml)	IL-6 (pg/mL)	TNF-α (pg/mL)
Observation (n=118)	Before treatment	54.48±4.31	32.14±4.76	51.16±4.31
	After treatment	18.08±2.24*	16.21±3.10*	26.79±2.46*
t		45.767	13.234	31.287
P		<0.001	<0.001	<0.001
Control (n=116)	Before treatment	55.42±4.11	32.26±4.15	51.25±4.41
	After treatment	25.65±2.12	24.76±3.15	34.34±2.54
t		28.119	7.039	19.287
P		<0.001	<0.001	<0.001

*Compared with control group, P<0.05 (CRP, t=26.542, P<0.001; IL-6, t=20.926, P<0.001; TNF-α, t=23.098, P<0.001).

Table 4. **T lymphocytes before and after treatment (%, x ± s)**

Group	Time	CD3+	CD4+	CD8+	CD4+/CD8+
Observation (n=118)	Before treatment	54.32±6.35	32.25±6.64	32.96±7.132	1.14±0.
	After treatment	71.67±9.33*	40.42±7.51*	26.56±5.242*	1.85±0.
t		16.218	8.655	7.931	13.204
P		<0.001	<0.001	<0.001	<0.001
Control (n=116)	Before treatment	54.27±9.125	31.81±6.16	32.92±7.528	1.13±0.
	After treatment	59.34±8.21	35.65±7.22	31.35±7.839	1.27±0.
t		4.584	4.161	1.276	2.271
P		<0.001	<0.001	0.084	0.036

*Compared with control group, P<0.05 (CD3+, t=10.727, P<0.001; CD4+, t=4.876, P<0.001; CD8+, t=5.523, P<0.001; CD4+/CD8+, t=10.942, P<0.001).