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The effect of adjuvant N−acetylcysteine effervescent tablets therapy on cardiopulmonary function and airway remodeling in patients with stable COPD

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ABSTRACT

Objective: To study the effect of adjuvant N-acetylcysteine (NAC) effervescent tablets therapy on cardiopulmonary function and airway remodeling in patients with stable chronic obstructive pulmonary disease (COPD). **Methods:** Patients with stable COPD treated in Zigong Third People's Hospital and West China Hospital, Sichuan University between May 2014 and October 2016 were selected and randomly divided into two groups, NAC group received N-acetylcysteine effervescent tablets combined with routine treatment, and control group received routine treatment. Before treatment as well as 2 weeks and 4 weeks after treatment, oxidative stress indexes and airway remodeling indexes in serum as well as inflammatory response indexes in peripheral blood were determined. **Results:** MDA, PC, 8-OHdG, MMP2, MMP3 and MMP9 contents in serum as well as NLRP3, ASC, p38MAPK and TREM-1 mRNA expression levels in peripheral blood mononuclear cells of both groups of patients after treatment were significantly lower than those before treatment while TAC levels as well as TIMP1 and TIMP2 contents in serum were significantly higher than those before treatment, and MDA, PC, 8-OHdG, MMP2, MMP3 and MMP9 contents in serum a well as NLRP3, ASC, p38MAPK and TREM-1 mRNA expression levels in peripheral blood mononuclear cells of NAC group after treatment were significantly lower than those of control group while TAC levels as well as TIMP1 and TIMP2 contents in serum were significantly higher than those of control group. **Conclusion:** Adjuvant NAC effervescent tablets treatment of stable COPD can improve the effect of oxidative stress and inflammatory response on cardiopulmonary function, and inhibit the airway remodeling caused by protease activation.

1. Introduction

Chronic obstructive pulmonary disease (COPD) is the chronic airway disease characterized by not completely reversible airway limitation, airway smooth muscle remodeling under the influence of oxidative stress, inflammation, protease activation and other factors is a key link in the progressive development of airway limitation. In clinical practice, the key of COPD treatment is to delay the development of airway remodeling, improve airway limitation and prevent the happening of the disease exacerbations. N-acetylcysteine (NAC) is a new drug in the treatment of respiratory chronic diseases, has a definite effect on promoting sputum excretion, and also resists oxidative stress and inhibits protease activity[1,2], and it has been identified in COPD animal models that it can reduce airway oxidative stress reaction and inhibit airway remodeling[3]. At present, it is not yet clear about the influence of N-acetylcysteine on oxidative stress reaction and airway remodeling in patients with COPD. In the following study, the effect of adjuvant N-acetylcysteine effervescent tablets therapy on cardiopulmonary function and airway remodeling in patients with stable COPD was analyzed.

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2. Clinical information and research methods

2.1 Patients' clinical information

A total of 114 patients with stable COPD treated in Zigong Third People's Hospital and West China Hospital, Sichuan University between May 2014 and October 2016 were selected, all patients were in line with the diagnosis of stable COPD and with lung function II-III grade, and the patients associated with bronchiectasis and bronchial asthma and the patients with a history of COPD exacerbations were ruled out. Random number table was used to divide the 114 patients into two groups, 57 cases in each group. NAC group received N-acetylcysteine effervescent tablets combined with routine drug treatment, including 38 male cases and 19 female cases that were 62-77 years old; control group accepted routine drug treatment, 39 male cases and 18 female cases that were 61-78 years old. The two groups of patients were not significantly different in general data (P>0.05).

2.2 COPD treatment

Both groups of patients received β 2 agonists combined with glucocorticoid inhalation therapy, and the methods were as follows: salmeterol-fluticasone inhalation, 50 μg/time, 2 times/d. NAC group of patients, on the basis of β 2 agonists combined with glucocorticoid inhalation therapy, received N-acetylcysteine effervescent tablets adjuvant therapy, and the methods were as follows: N-acetylcysteine effervescent tablets 600 mg, dissolved in 100 mL warm water and then taken, 2 times/d.

2.3 Serum oxidative stress and airway remodeling index detection methods

Before treatment as well as 2 weeks and 4 weeks after treatment, 3 ml of cubital venous blood was collected from two groups of patients respectively and centrifuged to get serum, thiobarbituric acid chromatometry kits were used to detect the content of MDA, enzyme-linked immunosorbent assay kit kits were used to detect PC, 8-OHdG, MMP2, MMP3, MMP9, TIMP1 and TIMP2 content, and phenanthroline colorimetry kits were used to detect TAC levels.

2.4 Peripheral blood inflammation index detection methods

Before treatment as well as 2 weeks and 4 weeks after treatment, 3 mL of cubital venous blood was collected from two groups of patients respectively, added in lymphocyte separation medium and centrifuged to get mononuclear cells, the RNA extraction kits and cDNA synthesis kits were used to separate the total RNA in peripheral blood mononuclear cells and synthesize cDNA, and the fluorescence quantitative PCR reaction was performed to determine NLRP3, ASC, p38MAPK and TREM-1 mRNA expression.

2.5 Statistical methods

SPSS 19.0 software was used to input and statistically process data, measurement data between two groups was analyzed by t test to clarify the differences and P<0.05 was the standard of statistical significance in differences.

3. Results

3.1 Serum oxidative stress-related product contents

Before treatment as well as 2 weeks and 4 weeks after treatment, analysis of serum oxidative stress-related products MDA (μmol/L), PC (μg/L) and 8-OHdG (ng/L) contents as well as TAC (kU/L) levels between two groups of patients was as follows: (1) serum MDA, PC and 8-OHdG contents as well as TAC levels were not significantly different between two groups of patients before treatment (P>0.05); (2) serum MDA, PC and 8-OHdG contents of both groups of patients after treatment were significantly lower than those before treatment while TAC levels were significantly higher than those before treatment (P<0.05); (3) serum MDA, PC and 8-OHdG contents of NAC group after treatment were significantly lower than those of control group while TAC levels were significantly higher than those of control group (P<0.05).

Table 1.

Serum oxidative stress-related product contents before and after treatment.

Groups	n	Time	MDA	PC	8-OHdG	TAC
NAC group	57	Before treatment	3.88±0.52	4.79±0.83	375.74±51.26	10.38±1.85
		2 weeks after treatment	2.21±0.42*&	2.66±0.47*&	210.47±28.96*&	15.68±2.15*&
		4 weeks after treatment	1.78±0.29*&	1.89±0.28*&	164.68±22.15*&	19.33±2.74*&
Control group	57	Before treatment	3.94±0.58	4.74±0.67	380.11±47.69	10.12±1.25
		2 weeks after treatment	2.98±0.45*	3.34±0.52*	302.15±41.29*	12.26±1.85*
		4 weeks after treatment	2.33±0.37*	2.52±0.34*	262.37±33.68*	14.68±2.02*

*: comparison within group before and after treatment, P<0.05; &: comparison between two groups at the same point in time after treatment, P<0.05.

3.2 Peripheral blood inflammation-related molecule expression

Before treatment as well as 2 weeks and 4 weeks after treatment, analysis of inflammation-related molecules NLRP3, ASC, p38MAPK and TREM-1 mRNA expression in peripheral blood mononuclear cells between two groups of patients was as follows: (1) NLRP3, ASC, p38MAPK and TREM-1 mRNA expression in peripheral blood mononuclear cells were not significantly different between two groups of patients before treatment (P>0.05); (2) NLRP3, ASC, p38MAPK and TREM-1 mRNA expression levels in peripheral blood mononuclear cells of both groups of patients after treatment were significantly lower than those before treatment (P<0.05); (3) NLRP3, ASC, p38MAPK and TREM-1 mRNA expression levels in peripheral blood mononuclear cells of NAC group after treatment were significantly lower than those of control group (P<0.05).

3.3 Serum airway remodeling-related protease and the inhibitor contents

Before treatment as well as 2 weeks and 4 weeks after treatment, analysis of serum airway remodeling-related proteases MMP2 (μg/L), MMP3 (μg/L) and MMP9 (ng/L) as well as their inhibitors TIMP1 (ng/L) and TIMP2 (ng/L) contents between two groups of patients was as follows: (1) serum MMP2, MMP3, MMP9, TIMP1 and TIMP2 contents were not significantly different between two groups of patients before treatment (P>0.05); (2) serum MMP2, MMP3 and MMP9 contents of both groups of patients after treatment were significantly lower than those before treatment while TIMP1 and TIMP2 contents were significantly higher than those before treatment (P<0.05); (3) serum MMP2, MMP3 and MMP9 contents of NAC group after treatment were significantly lower than those of control group while TIMP1 and TIMP2 contents were significantly higher than those of control group (P<0.05).

4. Discussion

COPD is the respiratory chronic disease with the highest incidence, and in the progression of COPD, the activation of oxidative stress reaction and the inflammatory response is a key link causing airway remodeling and airway limitation, and it is also the important pathological factor affecting patients' cardiopulmonary function. The key to the treatment of patients with stable COPD is to inhibit oxidative stress and inflammatory response to delay the process of airway remodeling and avoid the happening of the disease exacerbations. β 2 agonists and glucocorticoid inhalation are the common means to treat stable COPD, they can effectively inhibit airway inflammation and promote the airway smooth muscle relaxation, but there are still some patients who will be with disease exacerbations. N-acetyl cysteine is the drug for treatment of respiratory chronic diseases in recent years, which not only dissolves sputum and promotes sputum discharge, but can also through the self anti-oxidative stress effect and protease activity-inhibiting effect to reduce oxidative stress damage in the process of COPD and delay the airway remodeling caused by protease activation, which is beneficial to the condition of stable COPD[4,5]. Existing animal studies have confirmed that N-acetyl cysteine has significant inhibitory effect on airway oxidative stress reaction and smooth muscle remodeling in COPD model rat[2,3], but there is not much research about the drug

Table 2.

Inflammation-related molecule expression in peripheral blood mononuclear cells before and after treatment.

Groups	n	Time point	NLRP3	ASC	p38MAPK	TREM-1
NAC group	57	Before treatment	1.06±0.14	1.03±0.16	0.98±0.11	1.08±0.18
		2 weeks after treatment	0.54±0.06*&	0.49±0.05*&	0.60±0.09*&	0.43±0.06*&
		4 weeks after treatment	0.32±0.08*&	0.31±0.04*&	0.38±0.05*&	0.25±0.07*&
Control group	57	Before treatment	1.03±0.15	1.07±0.13	1.02±0.15	1.04±0.19
		2 weeks after treatment	0.76±0.09*	0.78±0.10*	0.81±0.11*	0.71±0.08*
		4 weeks after treatment	0.51±0.08*	0.49±0.8*	0.59±0.07*	0.43±0.07*

*: comparison within group before and after treatment, ^{P}<0.05; &: comparison between two groups at the same point in time after treatment, P<0.05.

Table 3.

Serum airway remodeling-related protease and the inhibitor contents before and after treatment.

Groups	n	Time point	MMP2	MMP3	MMP9	TIMP1	TIMP2
NAC group	57	Before treatment	0.47±0.08	22.12±3.62	164.36±19.33	33.22±5.56	10.21±1.86
		2 weeks after treatment	0.23±0.05*&	10.38±1.87*&	89.67±10.35*&	56.34±7.86*&	25.68±4.42*&
		4 weeks after treatment	0.17±0.03*&	7.75±0.93*&	67.65±8.44*&	72.11±9.35*&	42.77±6.26*&
Control group	57	Before treatment	0.50±0.08	22.65±4.21	166.12±20.19	32.89±4.28	10.08±1.86
		2 weeks after treatment	0.39±0.06*	16.75±2.68*	134.52±17.97*	40.29±5.72*	17.68±2.74*
		4 weeks after treatment	0.28±0.05*	12.14±1.95*	102.53±16.95*	52.18±7.93*	23.21±4.58*

*: comparison within group before and after treatment, P<0.05; &: comparison between two groups at the same point in time after treatment, P<0.05.

application in the clinical treatment of COPD.

N-acetyl cysteine itself has antioxidant properties, which can on the one hand, uses the sulfydryl in its own molecular structure to play to the role of the reducing agent and avoid the oxidative damage to the sulfydryl in molecular structure[6], and on the other hand, can produces cysteine through metabolism to provide the substrate for GSH synthesis and enhance the antioxidant effect of GSH[7]. During the oxidative stress response activation in COPD patients, the massive generation of reactive oxygen species will cause the oxidation reaction of intracellular lipid, protein and nucleic acid composition, the product of lipid peroxidation is MDA, the oxidation reaction product of protein amino acid side chain is the PC, and the oxidation product of guanine in nucleic acid is 8-OHdG[8-10]. In order to define the N-acetylcysteine treatment effect on oxidative stress responses in patients with stable COPD, serum contents of oxidative stress product were compared before and after treatment, and the result showed serum MDA, PC and 8-OHdG contents of both groups of patients significantly decreased while TAC levels significantly increased after treatment, and serum MDA, PC and 8-OHdG contents of NAC group after treatment were significantly lower than those of control group while TAC levels were significantly higher than those of control group. It means that regular treatment can reduce the oxidative stress reaction in patients with stable COPD to a certain extent, and the combination of N-acetyl cysteine can further inhibit oxidative stress reaction, reduce the formation of oxidation products and enhance antioxidant capacity.

Oxidative stress will not only directly cause airway epithelial injury, but will also activate the NLRP3, p38MAPK, TREM-1 and other molecules in inflammatory cells to aggravate the inflammatory response. NLRP3 is a kind of pattern recognition receptor, and it can form complex with ASC to promote the secretion of inflammatory mediators IL-1 β and IL-18[11,12]; p38MAPK is an important member of the family of MAPKs, and the activated p38MAPK can promote the secretion of inflammatory mediators IL-6 and IL-8 through the downstream cascade amplification reaction[13,14]; TREM-1 is a kind of membrane receptor that recognizes pathogen-associated molecular patterns, and it can form complexes with DAP12 to activate the downstream PI3K, ERK and other pathways, and then promote the secretion of inflammatory mediators such as TNF- and IL-1 β [15]. In order to define the degree of inflammation activation caused by oxidative stress reaction in patients with stable COPD, the inflammation-related molecule expression levels in the peripheral blood mononuclear cells were analyzed in the study, and the result showed that NLRP3, ASC, p38MAPK and TREM-1 mRNA expression levels in peripheral blood mononuclear cells of both groups of patients significantly decreased after treatment, and NLRP3, ASC, p38MAPK and TREM-1 mRNA expression levels in peripheral blood mononuclear cells of NAC group after treatment were significantly lower than those of control group. This means that conventional treatment can reduce the inflammatory response in patients with stable COPD to a certain extent, and combined use of N-acetyl cysteine can further inhibit the inflammatory response activation mediated by NLRP3, p38MAPK and TREM-1.

The activation of oxidative stress responses and inflammatory response in patients with COPD is not only associated with the change of cardiopulmonary function, but can also activate a variety of proteases to cause the progress of airway remodeling. The degradation and synthesis imbalance of extracellular matrix in airway smooth muscle is an important part of the airway remodeling. The MMP2, MMP3 and MMP9 in matrix metalloproteinase family are closely related to the degradation of extracellular matrix in airway smooth muscle, and the excessively secreted MMP2, MMP3 and MMP9 can cause excessive extracellular matrix degradation, and then lead to airway remodeling[16,17]. TIMP1 and TIMP2 are the inhibitors of MMPs molecules, and they can inhibit the activity of a variety of MMPs molecules to degrade extracellular matrix[18,19]. In the study, analysis of serum contents of airway remodeling-associated proteases and their inhibitors between two groups of patients before and after treatment showed that serum MMP2, MMP3 and MMP9 contents of both groups of patients significantly decreased while TIMP1 and TIMP2 contents significantly increased after treatment, and serum MMP2, MMP3 and MMP9 contents of NAC group after treatment were significantly lower than those of control group while TIMP1 and TIMP2 contents were significantly higher than those of control group. This means that conventional treatment can inhibit the airway remodeling caused by protease activation to a certain extent, and joint use of N-acetyl cysteine can further inhibit the airway remodeling mediated by MMP2, MMP3 and MMP9.

Based on the discussion of above serum and peripheral blood indexes, it shows that the adjuvant NAC effervescent tablets therapy for stable COPD can inhibit oxidative stress reaction, reduce the formation of oxidation products, enhance the antioxidant capacity, inhibit the inflammatory response activation mediated by NLRP3, p38MAPK and TREM-1 as well as the airway remodeling mediated by MMP2, MMP3 and MMP9.

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