ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5): 475 - 480
http://www.chinaphar.com

Effect of theophylline on airway inflammation in asthma

YANG Jiong[1], TU Hai-Yan, LI Qing-Quan (*Wuhan University People's Hospital, Wuhan 430060, China*)

KEY WORDS asthma; theophylline; eosinophils; inflammation

ABSTRACT

AIM: To investigate whether low dose theophylline has an anti-inflammatory effect in asthma. **METHODS**: Ninteen asthmatic subjects were given 200 mg sustained-release theophylline preparation twice daily for 4 weeks. The mean serum concentration of theophylline was 7.9 mg/L. The percentage of eosinophils, $EG2^+$ eosinophils, and the level of eosinophil cationic protein (ECP) in sputum pre- and post-administration were detected by Wright's stain, immunocytochemistry, and immuno-CAP system, and the symptom scores and lung function were evaluated as well. The above indices for 10 healthy subjects were regarded as control. **RESULTS**: Before using theophylline, sputum eosinophils, $EG2^+$ eosinophils, and ECP in asthmatic group were much higher than those of healthy group. After four weeks' administration, there were great decreases in percentage of total eosinophils ($40\% \pm 17\%$ *vs* $29\% \pm 11\%$, $P < 0.01$), activated ($EG2^+$) eosinophils ($28\% \pm 9\%$ *vs* $10\% \pm 8\%$, $P < 0.01$) and in the concentration of sputum ECP [(373 ± 206) *vs* (220 ± 132) $\mu g/L$, $P < 0.01$]. The symptom scores decreased greatly (7.1 ± 1.2 *vs* 5.4 ± 1.6, $P < 0.01$). There was a marked increased in forced expiratory volume one second ($FEV_{1.0}$) after administration (2.2 ± 0.6 *vs* 2.4 ± 0.5, $P < 0.01$). The $FEV_{1.0}\%$ of asthmatic subjects after administration increased ($60\% \pm 13\%$ *vs* $65\% \pm 13\%$, $P < 0.01$), too. **CONCLUSION**: These results indicated that low dose theophylline had inhibitory action on airway inflammation in asthma with noticeable improvement of the patients' symptoms and lung function.

[1] Correspondence to Prof YANG Jiong.
Phn 86-27-8804-1919, ext 2215 (O), 86-27-8384-5587 (H).
Fax 86-27-8807-3047. E-mail WLSCC@public.wh.hb.cn
Received 2000-06-12 Accepted 2001-02-26

INTRODUCTION

Theophylline has been used for the management of asthma for more than 50 years and remains a widely prescribed anti-asthmatic agent worldwide. Whilst theophylline has long been recognized as a drug capable of relaxing airway smooth muscle, and recent clinical and experimental data have suggested that it may also possess an anti-inflammatory activity[1], including inhibition of the late bronchial response to allergen inhalation, the release of certain pro-inflammatory cytokines, the action of some cells involved in the airway inflammation of asthma, and reducing microvascular leakage and hyperresponsiveness. Furthermore, some studies in human beings suggest that low-dose theophylline may have anti-inflammatory effects. Wards and co-workers[2] demonstrated that a 5-week treatment with oral sustained-release theophylline (serum concentration 7.8 mg/L) practically abolished the late asthmatic reaction to inhaled allergen in stable mild asthmatic subjects. Similar results were found by Sullivan and colleagues[3], who found that low serum theophylline concentration (6.6 mg/L) reduced the numbers of total and $EG2^+$ (activated) eosinophils in bronchial biopsies obtained from theophylline-treated patients as compared to placebo-treated patients 24 h after the allergen challenge. After two months of treatment with theophylline, the slope of the methacholine dose-response curve could be greatly reduced[4], but there was no marked lowering of the number or activation status of eosinophils and lymphocytes in peripheral blood. To further confirm whether low dose theophylline has an anti-inflammatory effect on asthmatic airways and to evaluate the clinical efficacy, we observed the eosinophil cationic protein (ECP), eosinophils, $EG2^+$ eosinophils in induced sputum, lung function, and symptom scores in patients with bronchial asthma by administering a sustained-release theophylline preparation.

METHODS

Patients From April 1999 to January 2000, 19 adult asthmatic patients (8 males, 11 females) being treated in the Department of Respiratory Disease of the First Affiliated Hospital of Hubei Medical University were

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

enrolled in this study. The average age of the patients was 36 a. The mean baseline forced expiratory volume one second ($FEV_{1.0}$) as a percentage of predicted normal ($FEV_{1.0}$%) was 68.05 %. All patients demonstrated a more than 15 % improvement in $FEV_{1.0}$ following inhaling 200 μg of salbutamol. For the diagnosis of asthma the criteria in the Global Initiative for Asthma (GINA)[5] was adopted. Six of the patients presented mild persistent asthma, six patients moderate, and seven patients severe asthma. All patients had not taken theophylline, corticosteroids, or leukotriene antagonists within 6 weeks before initiation of this study. Ten healthy non-asthmatic and non-allergic subjects (medical students and hospital personnel) with a mean age of 30 a were also studied. None of the subjects (asthmatic patients or healthy individuals) had ever smoked cigarettes or had a history of cardio-pulmonary disease other than asthma. The healthy subjects had no atopic constitution.

Study protocol All the patients were administered sustained-release theophylline tablet {each tablet contained anhydrous theophylline [1,3-dimethyl-3,7-dihydro-1*H*-purine-2, 6-dione] 100 mg, Guangzhou Xinghua Pharmaceuticals Ltd} at a dose of 400 mg/d (*po* 200 mg per time, bid) for 4 weeks. In the morning on the fourth day, the serum concentration of theophylline was detected before breakfast using ACS:180 automated chemiluminescence system, USA, with a sensitivity of 0.3 mg/L – 40 mg/L. The patients kept daily records of their symptoms and the dose of used sulbutamol throughout the study. Salbutamol aerosol {(*RS*)-2-(1, 1-dimethyl) ethylamino-1-[4-hydroxy-3-(hydroxymethyl)-phenyl] ethanol} inhalation, 100 μg per dose (Glaxo Wellcome, Chongqing, China) was permitted during the treatment if it was required.

The asthma symptoms and inhaled β_2-agonist usage were recorded throughout the study period. Before and at the end of the study, the measurement of respiratory function and induction of sputum with hypertonic saline were performed.

Clinical assessment The assessment of symptomatic score was referred to the scoring key for asthma symptoms as given by Marks *et al*[6]. Spirometric function was measured on a V_{max} 229 spirometer (United States Sensor-Medics). $FEV_{1.0}$ and forced vital capacity (FVC) were recorded. The $FEV_{1.0}$ was also expressed as a percentage of the predicted values.

Sputum test Sputum was induced by inhalation of increasing concentration of hypertonic saline (3 %, 4 %, and 5 %) according to a method described previously[7, 8]. By doing this, sputum floating in saliva (white plug) can be easily distinguished. The selected sputum was placed in a graduated tube. The protocol for sputum was processed according to the method of Pavord *et al*[9] with slight modification. The sputum sample was homogenized with four volumes of 0.1 % dithiothreitol (Sigma Chemical Co) and kept in a bath at 37 ℃ for 20 min. Then three volumes of PBS (0.1 mol/L, pH 7.4) was added in and rocked for 5 min. The suspension was filtered through 48-μm nylon gauze to remove cell debris and mucus, then centrifuged at 900 × *g* for 10 min. The supernatant fluid was aspirated and stored in an Eppendorf tube at – 70 ℃ for ECP assay.

The pellet was resuspended in a volume of PBS, 200 – 600 μL depending on the macroscopic size, and a total cell count of leukocytes was obtained in a modified Neubauer hemocytometer. Cell suspension 200 μL adjusted to 1.0×10^9/L was placed into four cups of the modified cytocentrifuge (modified with LD4-2 centrifuge) and two cytospins were prepared at 225 × *g* for 1.5 min. Two slides were air-dired and stained by Wright's stain; and two others were fixed in acetone for 10 min and were then stored at – 70 ℃ for $EG2^+$ eosinophils assay.

The concentration of ECP (μg/L) in the thawed supernatant was determined by a sensitive radioimmunoassay (RIA, Pharmacia *Diagnostics* AB, *Uppsala*, *Sweden*); $EG2^+$ eosinophils were determined by immunocytochemistry after staining with $EG2^+$ eosinophils monoclonal antibody (Pharmacia, Uppsala, Sweden). At least 500 cells were counted from the Wright's stained slides for the differential cell count. The sputum measurements were performed blindly to the clinical characteristics.

Statistical analysis The paired *t*-test and independent group *t*-test were used when appropriate. Data are expressed as $\bar{x} \pm s$ and a probability value of $P < 0.05$ was considered significant.

RESULTS

The percentage of eosinophils and lymphocytes in sputum of asthmatic patients before theophylline treatment were much higher than those of healthy, but the percentage of macrophage was lower than that of healthy; no $EG2^+$ eosinophils were found in the sputum of healthy controls (Tab 1). The level of ECP in sputum of asthmatic subjects before 4 weeks' treatment was much higher than that in the healthy controls [(373 ± 206) *vs* (44 ±

Tab 1. Cell counts (%) in induced sputum of healthy subjects ($n = 10$), pre-, and post-treatment asthmatics ($n = 19$). $\bar{x} \pm s$. $^cP < 0.01$ *vs* healthy values. $^eP < 0.05$, $^fP < 0.01$ *vs* theophylline pre-treatment values.

	Eosinophils	Macrophage	Lymphocyte	Neutrophil	EG2$^+$ eosinophil
Healthy	1.2 ± 0.7	75 ± 4	1.5 ± 0.6	22 ± 3	0
Pre-treatment	40 ± 17^c	21 ± 7^c	7 ± 4^e	34 ± 17	28 ± 9^c
Post-treatment	29 ± 11^f	26 ± 9^e	7 ± 4	39 ± 16	10 ± 8^f

23) μg/L, $P < 0.01$]. After 4 weeks' treatment with theophylline (the mean serum theophylline concentration was 7.9 mg/L), the proportion of eosinophils and EG2$^+$ eosinophils in sputum of asthmatic patients decreased. The ECP level of sputum also decreased [(373 ± 206) *vs* (220 ± 132) μg/L, $P < 0.01$, Fig 1].

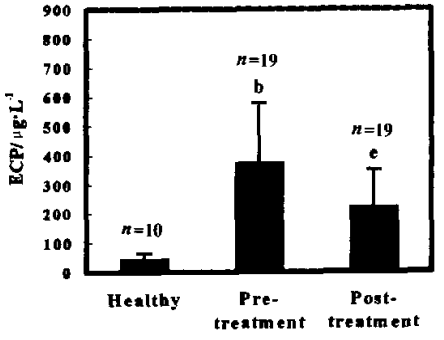

Fig 1. Eosinophil cationic protein (ECP) level in induced sputum. $\bar{x} \pm s$. $^bP < 0.05$ *vs* healthy values. $^eP < 0.05$ *vs* theophylline pre-treatment values.

After 4 weeks' treatment, the symptom scores of asthma group decreased (7.1 ± 1.2 *vs* 5.4 ± 1.6, $P < 0.01$, Fig 2). The $FEV_{1.0}$% ($FEV_{1.0}$/FVC) also observed to be improved (Fig 3).

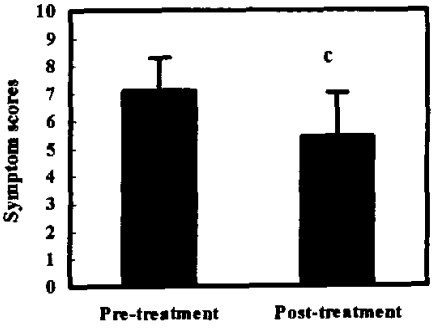

Fig 2. Asthmatic symptom scores before and after theophylline treatment. $n = 19$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* theophylline pre-treatment values.

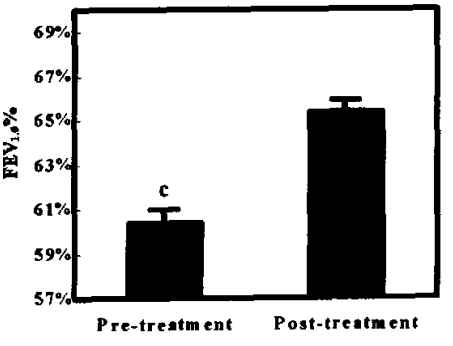

Fig 3. The mean percentage of forced expiratory volume one second of predicted normal ($FEV_{1.0}$%) for asthmatics after four weeks of theophylline pre- and post-administration. $n = 19$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* theophylline post-treatment values.

DISCUSSION

Bronchial asthma has been defined as a chronic inflammatory airway disorder in which many cells are involved, especially mast cells, eosinophils, and T-lymphocytes[5]. In addition, activated eosinophils release granule-derived proteins, cytokines, and lipid mediators, which cause bronchoconstriction[10], bronchial hyperresponsiveness[11], and increased vascular permeability[12]. The cytotoxic basic proteins and superoxide species from eosinophils contribute to epithelial damage and induce chronic changes, so eosinophils play an important role in the pathogenesis of asthma. The granule proteins include four principal cationic proteins: major basic protein (MBP), and eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase (EPO). These proteins are toxic for epithelial tissues and as ECP is more cytotoxic to respiratory epithelium than other cationic proteins, so its expression is a

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

useful marker for eosinophil activation. In this study, we have demonstrated that the administration of a sustained-release theophylline to 19 asthmatic patients, resulted in a great increase in $FEV_{1.0}$ and $FEV_{1.0}$% and a great decrease in the asthmatic symptom scores. Furthermore, evaluation of airway inflammation with hypertonic saline-induced sputum showed that there was a great decrease in the percentage of eosinophils, $EG2^+$ eosinophils, and the level of ECP. This indicates that theophylline had both a bronchodilating activity and an inhibitory effect on eosinophilic inflammation at a subtherapeutic plasma theophylline concentration. The evidence showed that low dose theophylline was beneficial in the management of asthma by partly acting as an anti-inflammatory drug.

Recently, three studies have demonstrated effects of theophylline on airway inflammation in asthma. Minoguchi and colleagues[13] demonstrated that on withdrawing theophylline from stable asthmatic patients whose condition was also controlled with a moderate dose of inhaled corticosteroids, an increase in the percentage of eosinophils and $EG2^+$ eosinphils in sputum and a decrease in morning peak expiratory flow (PEF) and $FEV_{1.0}$ was observed. However, this design had defect, because the anti-inflammatory effect of corticosteroids is strong enough to overlap the effect of theophylline, and inhaled corticosteroids is very effective at suppressing the eosinophilic inflammation in the airways of patients with asthma. The study by Horiguchi *et al*[14] had analogous design and results with our study. And their design was better as some subjects were put into a placebo group as control. In addition to that, it also demonstrated that after 8 weeks' administration of theophylline sputum and serum ECP decreased more markedly than that after 4 weeks' administration, suggesting that the longer the period of treatment, the more distinct are the anti-inflammatory effects of theophylline. However, the serum concentration ($13.9\ mg/L \pm 0.9\ mg/L$) in their study was much higher than our study. In our study, only ECP in cell and in sputum of 10 healthy subjects were detected, no patient was given placebo, so the possibility of selfimprovement in some patients can not be ruled out. Another study found that the serum and sputum ECP decreased greatly after 4 weeks' theophylline treatment, but in this study theophylline failed to improve the lung function[15]. However, all these studies hold identical views that theophylline has an anti-inflammatory effect in asthma.

Although the precise mechanisms that account for the decrease in eosinophils in induced sputum after the treatment of theophylline is not clear, several factors that influence the eosinophil differentiation, activation, and chemotaxis may be involved. Recent studies have provided that $CD4^+$ T-cell, especially TH_2 type T-cells, play an important role in the allergic inflammation by producing IL-4 and IL-5. IL-5 from mast cells and T lymphocytes promotes eosinophil recruitment, activation, and survival, perpetuating eosinophilic inflammation in asthma. IL-4 can increase adhesion molecule expression on the endothelium. This endothelial event contributes to the recruitment of inflammatory cells, particularly eosinophils, to the airways. In addition, IL-4 plays a crucial role in the development of TH_2 lymphocytes. It has been reported that theophylline reduced cytokine release from T lymphocytes *in vitro*[16,17]. After treating asthmatic patients with low dose theophylline for 6 weeks, the number of TH_2-like cells in bronchial biopsies decreased greatly, suggesting that theophylline had a direct inhibitory effect on cytokine production by T cells in asthmatic airways[18]. Kidney and colleagues[19] reported that theophylline withdrawal was associated with an increase in the number of activated T cells in the airway and a decrease in the number of activated T cells in the peripheral blood. The mean steady-state serum theophylline concentration prior to withdrawal was 8.6 mg/L, such a concentration is below the conventional therapeutic range. These results suggest that theophylline may directly inhibit both the chemotaxis of T-cells from the peripheral blood into the airway and their cytokine production as a consequence and lead to a decreased number of esosinophils in the asthmatic airways. This may in part explain the mechanisms leading to decreased percentage of eosinophils and $EG2^+$ eosinophils in sputum after administration of theophylline. Recently, Ohta and co-workers[20] reported that theophylline had an inhibitory effect on IL-5-induced eosinophil survival via apoptosis *in vitro*, suggesting that theophylline may be involved in the apoptosis of inflammatory cells.

It was also demonstrated that the administration of theophylline resulted in a marked increase in $FEV_{1.0}$ and $FEV_{1.0}$% and marked decrease in asthmatic symptom scores. Such effects of the drug were mainly based on the improvement of airflow obstruction other than relaxation of bronchial smooth muscle, due to its being more effective in long-term control of asthma in spite of its relatively poor bronchodilator properties[14]. Three possible links between asthmatic airway obstruction and airway inflammation have been postulated: (1) constriction of air-

way smooth muscle, (2) thickening of the airway epithelium, (3) the presence of liquids within the confines of the airway lumen. The latter two are the consequences of airway inflammation. Therefore, the persistent improvement in airway obstruction may be a result of the inhibition of the airway inflammation.

In conclusion, low dose theophylline is a useful anti-inflammatory agent for chronic inflammation of asthma acting by inhibiting the mucosal inflammatory response.

REFERENCES

1 Barnes PJ, Pauwels RA. Theophylline in the management of asthma: time for reappraisal? Eur Respir J 1994; 7: 579 - 91.
2 Ward AJ, McKenniff M, Evans JM, Page CP, Costello JF. Theophylline — an immunomodulatory role in asthma? Am Rev Respir Dis 1993; 147: 518 - 23.
3 Sullivan P, Bekir S, Jaffar Z, Page C, Jeffery P, Costello JF. Anti-inflammatory effects of low-dose oral theophylline in atopic asthma. Lancet 1994; 343: 1006 - 8.
4 Page CP, Cotter T, Kilfeather S, Sullivan P, Spina D, Costello JF. Effect of chronic theophylline treatment on the methacholine dose-response curve in allergic asthmatic subjects. Eur Respir J 1998; 12: 24 - 9.
5 Lenfant C, Khaltaev N. Global initiative for asthma: global strategy for asthma management and prevention. NHLB/WHO workshop report (based on a 1993 Mar meeting). National Institutes of Health, National Heart Lung and Blood Institute; 1995 Jan. Publication № 95-3659.
6 Marks GB, Dunn SM, Woolcock AJ. An evaluation of an asthma quality of life questionnaire as a measure of change in adults with asthma. J Clin Epidemiol 1993, 10: 1103 - 11.
7 Pin I, Gibson PG, Kolendowicz R, Girgis-Gabardo A, Denburg JA. Use of induced sputum cell counts to investigate airway inflammation in asthma. Thorax 1992, 1: 25 - 9.
8 Pizzichini E, Pizzichini MM, Efthimiadis AE, Hargreave FE, Dolovich J. Measurement of inflammatory indices in induced sputum: effects of selection of the sputum to minimize salivary contamination. Eur Respir J 1996; 9: 1174 - 80.
9 Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. Thorax 1997; 52: 498 - 501.
10 Piacentini GL, Kaliner MA. The potential roles of leukotrienes in bronchial asthma. Am Rev Respir Dis 1991; 143: S96 - S99.
11 Henderson WR. Eicosanoids and platelet activating factor in allergic respiratory diseases. Am Rev Respir Dis 1991; 143: S86 - S90.
12 Evans TW, Rogers DF, Aursudkij B, Chung KF, Barnes PJ. Regional and time dependent effects of inflammatory mediators on airway microvascular permeability in the guinea pig. Clin Sci 1986; 76: 479 - 85.
13 Minoguchi K, Kohno Y, Oda N, Wada K, Miyamoto M, Yokoe T, *et al*. Effect of theophylline withdrawal on airway inflammation in asthma. Clin Exp Allergy 1998; 28 Suppl 3: 57 - 63.
14 Horiguchi T, Tachikawa S, Kasahara J, Doi M, Shiga M, Miyazaki J, *et al*. Suppression of airway inflammation by theophylline in adult bronchial asthma. Respiration 1999; 66: 124 - 7.
15 Tohda Y, Muraki M, Iwanaga T, Kubo H, Fukuoka M, Nakajima S. The effect of theophylline on blood and sputum eosinophils and ECP in patients with bronchial asthma. Int J Immunopharmacol 1998; 20: 173 - 81.
16 Scordamaglia A, Ciprandi G, Ruffoni S, Garia M, Paolieri F, Venuti D, *et al*. Theophylline and the immune response: *in vitro* and *in vivo* effects. Clin Immunol Immunopathol 1988; 48: 238 - 46.
17 Prieur AM, Granger GA. The effect of agents which modulate levels of the cyclic nucleotides on human lymphotoxin secretion and activity *in vitro*. Transplantation 1975; 20: 331 - 7.
18 Finnerty JP, Lee C, Wilson S, Madden J, Djukanovic R, Holgate ST. Effects of theophylline on inflammatory cells and cytokines in asthmatic subjects: a placebo-controlled parallel group study. Eur Respir J 1996; 9: 1672 - 7.
19 Kidney JC, Dominguez M, Taylor PM, Rose M, Chung KF, Barnes PJ. Immunomodulation by theophylline in asthma: demonstration by withdrawal of therapy. Am J Respir Crit Care Med 1995; 151: 1907 - 14.
20 Ohta K, Sawamoto S, Nakajima M, Kubota S, Tanaka Y, Miyasaka T, *et al*. The prolonged survival of human eosinophils with interleukin-5 and its inhibition by theophylline via apoptosis. Clin Exp Allergy 1996; 26 Suppl 2: 10 - 5.

茶碱对哮喘气道炎症的作用

杨 炯[1], 涂海燕, 李清泉 (武汉大学人民医院, 武汉 430060, 中国)

关键词 哮喘; 茶碱; 嗜酸细胞; 炎症

目的: 研究小剂量茶碱对哮喘气道炎症的作用. **方法**: 19名哮喘患者用茶碱缓释剂(200 mg, bid, 平均血浆茶碱浓度 7.9 mg/L)治疗 4 周, 分别用瑞氏染色、免疫组织化学及荧光免疫法检测治疗前后高渗盐水诱导痰中嗜酸细胞(Eos)、激活的 Eos ($EG2^+$ Eos)和嗜酸细胞阳离子蛋白(ECP)的变化, 并观察治疗前后症状积分和肺功能的变化. **结果**: 用茶碱治疗前, 患者痰中 Eos、$EG2^+$ Eos 和 ECP 比健康人明显增加; 用茶碱治疗 4 周后, 哮喘患者诱导痰中 Eos 百分数下降(40 % ± 17 % *vs* 29 % ± 11 %, $P < 0.01$), $EG2^+$ Eos 百分数显著下降(28 % ± 9 % *vs* 10

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 May; 22 (5)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

% ± 8 %, $P < 0.01$), 痰 ECP 明显下降[(373 ± 206) *vs* (220 ± 132) μg/L, $P < 0.01$]; 症状明显好转(7.1 ± 1.2 *vs* 5.4 ± 1.6, $P < 0.01$); 肺功能明显改善, $FEV_{1.0}$增加(2.2 ± 0.6 *vs* 2.4 ± 0.5, $P < 0.01$), $FEV_{1.0}$%也增加(60 % ± 13 % *vs* 65 % ± 13 %, $P < 0.01$). **结论**: 小剂量茶碱对哮喘气道炎症有明显抑制作用, 同时使患者症状和肺功能明显改善.

(责任编辑 朱倩蓉)