Anti-inflammatory effect of methoxyphenamine compound in rat model of chronic obstructive pulmonary disease

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ABSTRACT

AIM: To evaluate the anti-inflammatory effect of methoxyphenamine compound (MC) on chronic obstructive pulmonary disease (COPD) in rats by measurement of proinflammatory cytokines, total and differential white cell counts (WCC) of bronchroalveolar lavage fluid (BALF). **METHODS**: Adult rat model of COPD (COPD group) was induced by intratracheal instillation of lipopolysaccharides and exposure to cigarette smoke. Treatment groups received different dosage of MC (3, 9, and 27 mg daily, MC group) or prednisone (0.25 mg daily, P group) respectively. Tumor necrosis factor alpha (TNF-α), interleukin 1beta (IL-1β), interleukin-6 (IL-6), transforming growth factor β (TGF-β) of BALF were determined by ELISA. Total and differential WCC were performed after Giemsa staining. **RESULTS**: The levels of TNF-α, IL-1β, IL-6, TGF-β, total and differential WCC in BALF of MC groups were significantly decreased than that of COPD group (P<0.01), and there was no significant difference among MC groups. There was no significant decrease in the levels of TNF-α, IL-1β, and count of alveolar macrophages in P group compared to those of COPD group. More significant decrease in total WCC and neutrophils was found in P than in COPD group (P<0.01). **CONCLUSION**: MC has anti-inflammmatory effect in the rats with COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a common, costly, and preventable disease that has implications for global health. It is the fourth leading cause of death[1]. COPD is characterized by progressive airflow limitation mainly encompasses chronic bronchitis and emphysema. It is a chronic inflammatory process in the lung. Studies have revealed that numerous proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), interleukin 1beta (IL-1β), transforming growth factor β (TGF-β), and interleukin-8 (IL-8) play a pivotal role in the chronic airway inflammation and structural remodeling. Therefore, anti-inflammation should be considered in the treatment of COPD. Methoxyphenamine compound (MC), a compound which functions well as bronchodilator, is frequently used in alleviating the bronchial asthma and the symptoms of COPD including cough, sputum, and asthma. However, there are few studies of its anti-inflammatory effect on COPD. In this study, we aim to investigate the anti-inflamma-

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tory effect of MC on airway inflammation of COPD via examining the cytokines level, such as TNF-α, IL-6, IL-1β, and TGF-β as well as total and differential white cell counts (WCC) in BALF of the established rat model of COPD.

MATERIALS AND METHODS

Reagents Rat TNF-α, IL-6, IL-1β, and TGF-β ELISA kits were purchased from Biosource Co (Camariuo, CA). Lipopolysaccharides (LPS) were obtained from Sigma Chemical Co (Cat No110K4046, St Louis, MO, USA). MC is a preparation of 25 mg aminophylline, 12.5 mg methoxyphenamine hydrochloride, 7 mg noscapine and 2 mg chlorpheniramine maleate, provided by Sankyo Company (Tokyo, Japan). Each tablet of MC contains 100 mg powder and mixes with distilled water resulting in suspension for oral treatment. Prednisone was purchased from Xinyi Pharmaceutical Co (Shanghai, China)

Animals and groups Forty eight male Wistar rats weighing (200±20) g were provided by the Experimental Animal Center, Fudan University Medical School. All studies were performed with the approval of experimental animal committee of the university. The animals were randomly divided into six groups (n=8): Control: Sham treated rats were instilled intratracheally with LPS-free sterile 0.9 % NaCl; (2) COPD: 350 μg/200 μL of LPS was administrated by intratracheal instillation on d 1 and d 14; the rats were then exposed to ten cigarettes for 2 h per day from d 2 to d 13 and from d 15 to d 28[2], (3) MC groups: some COPD rats were orally administrated with 3, 9, or 27 mg MC per day from d 2 (corresponding to the first day of cigarette smoking) to d 13 and from d 15 to day 28, and designated as MC1, MC2 and MC3 group, respectively; P group: some COPD rats were orally administrated with 0.25 mg prednisone per animal per day for the same duration as in the MC groups.

Bronchoalveolar lavage and cell counts On d 29, all the rats were anesthetized with ketamine (100 mg/kg) and sacrificed by bleeding from abdominal aorta. Right primary bronchus was ligated and the left bronchus was cannulated. Bronchoalveolar lavage (BAL) was performed by flushing the airways with 5 mL saline through the tracheal cannula three times. BAL fluid (BALF) was pooled and centrifuged at 1500 rpm for 5 min. The supernatant was harvested for cytokine analysis and the pellet was smeared onto slides for cell classification and counting in BALF. After the cell smear was stained with Wright-Giemsa, WCC, neutrophile granulocyte and alveolar macrophage (AM) were measured by counting 200 cells under light microscopy.

Cytokines analysis in BALF The levels of TNF-α, TGF-β, IL-1β, or IL-6 were measured by ELISA according to manufacturers' instruction, and corrected by total protein (TP) in BALF using Bradford biophotometer method.

Statistical analysis Data are expressed as mean standard deviation (SD). Student t-test was used for comparison between the control and COPD group; Dunnett test was used for comparison between COPD and MC-treated groups; one-way ANOVA was used for comparison among MC groups using Sudent-Newman-Keuls test (Statistical Package for the Social Sciences, version 10.0 for Windows; SPSS Inc, Chicago, IL). A P value <0.05 denotes the presence of a significant statistical difference.

RESULTS

WCC in rat BALF of each group is shown in Tab 1. Compared with the control, total numbers of leukocyte and neutrophile granulocyte in COPD group were significantly increased (P<0.01). Compared with COPD group, the total number of leukocyte, neutrophile granulocyte and AM differential count were both decreased in each MC group (P<0.01). However the difference among the MC groups was not significant. Compared with COPD group, the total numbers of leukocyte and the neutrophile granulocyte differential count were both decreased in P group (P<0.01), but AM count was not decreased (P>0.05).

The cytokine levels in rat BALF of each group are shown in Tab 2. Compared with the control, levels of

Tab 1. Leukocyte count and classification in rat BALF of each group. n=8. Mean±SD. ^cP<0.01 *vs* control group. ^fP<0.01 *vs* COPD group.

Group	10^{-6}×Leukocyte count/L^{-1}	Cell classification/%	
		Neutrophile granulocytes	Alveolar macrophages
Control	217±38	7.4±1.2	63±4
COPD	883 ±70^c	29±3^c	42±5
MC1	632 ±49^f	22.4±2.7^f	32±3^f
MC2	684± 56^f	20.8±3.0^f	33±4^f
MC3	610±132^f	19.1±2.7^f	35±9^f
P	713±43^f	20±4^f	47±7

Tab 2. Cytokine levels (pg/mg of total proteins) in rat BALF of each group. *n*=8. Mean±SD. [c]*P*<0.01 *vs* control group. [f]*P*<0.01 *vs* COPD group.

Group	TNF-α	TGF-β	IL-1β	IL-6
Control	44±11	88±18	36±8	49±10
COPD	95±21[c]	197±36[c]	72±15[c]	95±26[c]
MC1	57±10[f]	111±22[f]	42±8[f]	56±14[f]
MC2	54±14[f]	107±12[f]	51±12[f]	60±4[f]
MC3	55±19[f]	104±5[f]	53±9[f]	58±15[f]
P	78±16	132±29[f]	67±17	68±19[f]

TNF-α, TGF-β, IL-1β, or IL-6 were significantly increased in the COPD (P<0.01), however, the cytokine levels were significantly decreased in each MC group (P<0.01), compared to the COPD. TGF-β and IL-6 levels were decreased in P group compared with COPD group.

DISCUSSION

Cigarette smoking and tracheobronchial infection are the predisposing factors in COPD. Although the exact pathologic mechanism is unclear, the infiltration and activation of neutrophils and AM, the predominant inflammatory cells in airways, as well as the release of inflammatory cytokines, are believed to play a central role in the pathophysiology of COPD. Smoking can stimulate AM and airway and alveolar epithelial cells to produce proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8 in the lungs[3], and recruit white blood cells (WBC) from peripheral blood into the lungs. The activated WBC produce elastase, oxidants, IL-8, leukotriene, *ect,* which contribute to the progressive fibrosis, airway obstruction, and destruction of the lung parenchyma. TGF-β, as an airway structural remodeling factor, can stimulate hyperplasia of fibroblasts and hypertrophy of airway smooth muscle.

Our study revealed that the levels of TNF-α, TGF-β, IL-1β, and IL-6 in BALF in rat COPD were significantly higher than those in the control, demonstrating these cytokines participate in the process of inflammation and remodeling in COPD model. Neutrophils in BALF in rat model of COPD were markedly elevated by 2-3 folds, suggesting that neutrophils are the main inflammatory cells in airway inflammation in COPD model. Therefore, it is suitable to select therapeutic candidates to assess efficacy using the COPD rat model[2,4].

Since inflammation play crucial roles in the pathogenesis of COPD, anti-inflammatory treatment becomes an important therapeutic method for COPD[5,6]. Our study showed that the levels of TNF-α, IL-1β, IL-6, and TGF-β as well as neutrophils and AM in BALF in COPD group treated with MC were decreased compared to those in COPD, illustrating that MC may inhibit airway inflammation in COPD via blocking various agents in inflammatory network. In MC compound, aminophylline can antagonize inflammation and regulate immunoreaction. It significantly inhibited inflammatory cell from release of IL-1β, TNF-α, LTB_4, and IL-2, and furthermore, it improved the expression of the anti-inflammatory cytokine IL-10[7]. Recently, Kazuhiro[8] indicated that low-dose theophylline exerts an anti-inflammatory effect at least in part through increasing histone deacetylases (HDACs) activity. Aminophylline can directly activate $HDAC_1$ and $HDAC_3$ to inhibit the acetylation of core histones that is necessary for inflammatory gene transcription, and thus, interrupt the expression of inflammatory genes. Besides aminophylline, chlorpheniramine maleate also has the effect of inhibiting airway inflammation. Research indicated that H_1-antihistamine might modulate eosinophils-infiltrated airway inflammation by down-regulating the activity of airway epithelial cells and decreasing the expression of adhesion molecules as well as the release of inflammatory mediators[9]. It was also reported that the addition of beta-agonist increased inhibitory effect of phosphodiesterase (PDE) inhibitor on the production of TNF-alpha and IL-1beta[10]. Hence, we proposed that the anti-inflammatory effect of MC might be the result of synergistic cooperation among the above three components, aminophylline, methoxyphenamine hydrochloride and chlorpheniramine maleate. Our study also demonstrated that there was no difference in anti-inflammatory effect between COPD groups treated with MC at three different doses, meaning only small-dose of MC is capable of presenting anti-inflammatory property. This provides the theoretical basis for the efficacy and safety of MC in its clinical application. MC in the treatment for COPD not only relieves bronchial spasm but also reduces the side-effects of aminophylline at routine dose. Moreover, it can attenuate pulmonary injury by inhibiting inflammation.

Our study also showed that prednisone did not present inhibitory effect on main proinflammatory

cytokines TNF-α and IL-1β in the rat model of COPD. Moreover, our study manifested that the total WCC and neutrophils in prednisone treated group were decreased compared to those in COPD group, while AM was not reduced, illustrating prednisone can inhibit neutrophils to alleviate airway inflammation at certain degree but not to completely block inflammation. Currently, there are some controversies about corticoid therapy in COPD. Studies have shown that corticoid cannot inhibit the expression of TNF-α and IL-8 in the sputum of the patients with COPD[11]. Recently, Culpitte and colleagues[12] reported a trend to increased resistance to glucocorticoid by AM from normal subjects to smokers to patients with COPD, while the quantity of glucocorticoid receptors on AM did not evidently change accordingly, proposing the function of glucocorticoid receptors was altered in the patients with COPD, proposing corticosteroid insensitivity of AM in the respiratory tract restricted the application of corticosteroid therapy in COPD.

In conclusion, according to our study, MC can be used for the control of chronic inflammation in COPD rats.

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