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Therapeutic Effects of a Long-Acting Cholinergic Receptor Blocker, Tiotropium Bromide, on Asthma

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Statistical Analysis C
Data Interpretation D
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Background: The aim of this study was to evaluate the therapeutic effects of tiotropium bromide on asthma.

Material/Methods: A total of 160 patients with moderate persistent asthma were randomly divided into 4 groups (n=40): the 3 control groups were given fluticasone propionate aerosol (group A), salmeterol-fluticasone propionate inhalant (group B), and tiotropium bromide inhalation powder combined with salmeterol-fluticasone propionate inhalant (group C), respectively, and the experimental group received tiotropium bromide inhalation powder combined with fluticasone propionate aerosol (group D) and salbutamol was used to relieve symptoms when necessary.

Results: After 8 weeks of treatment, the pulmonary function of group D, which was significantly better than those of group A ($P<0.05$), was similar to those of groups B and C ($P>0.05$). Group D had significantly better asthma control test scores and nighttime symptom scores than in group A ($P<0.05$), without significant differences from those of group B or group C ($P>0.05$). The number of times salbutamol was used to alleviate symptoms was significantly different ($P<0.05$) between group D and group A ($P<0.05$), as well as between group C and group D ($P<0.05$). Groups D and B had similar results ($P>0.05$). IL-13 levels in induced sputum had significant differences ($P<0.05$). The levels in group D, which were higher than those of groups A and B ($P<0.05$), were similar to those of group C ($P>0.05$).

Conclusions: Tiotropium bromide combined with fluticasone propionate improved the respiratory function and quality of life, and is a new therapy for moderate, persistent asthma.

MeSH Keywords: **Asthma • Cholinergic Antagonists • Therapeutics**

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Background

Bronchial asthma (hereafter referred to as asthma) is a chronic inflammatory respiratory disease resulting from the interactions between many types of cells and cell products. Asthma has a morbidity rate of about 5% worldwide, affecting over 300 million people. The number of cases will further increase with global economic development and accelerated urbanization process [1].

Inhaled glucocorticoid therapy is now the most effective way to treat asthma, but after inhalation of low-dose glucocorticoids the symptoms of many adult patients still cannot be completely controlled. For these patients, it is recommended to increase the amount of glucocorticoid or to combine long-acting receptor agonists [2,3]. However, according to the dose-effect curve of inhaled glucocorticoid, increasing or doubling the amount of inhalation had little benefit for the pulmonary function of patients [4]. Long-acting β -receptor agonists can well-control the symptoms of patients, but their safety has recently been cast into doubt by the US Food and Drug Administration and researchers in the field of asthma. In particular, their effects on the heart and blood vessels have attracted wide attention. Therefore, their doses should be as low as possible [5,6].

Tiotropium bromide is a new once-daily, selective bronchodilator. On the basis of inhaled glucocorticoid therapy, it can significantly improve the pulmonary function and clinical symptoms of asthmatic patients, with equivalent effects to those of long-acting β -receptor agonists [7,8]. Recently, it has been approved to treat patients with chronic obstructive pulmonary disease (COPD) [9]. Tiotropium bromide can bind all 3 M (muscarinic) receptor subtypes, while selectively inhibiting M1 and M3 subtypes [10]. It is well-established that M3 receptors in smooth muscle of the airway play a crucial role in the pathophysiology of asthma by constricting the bronchus and secreting mucus. Currently, tiotropium bromide is clinically available as either a “soft mist” or a dry powder inhaler. Its most striking advantage is a long duration of bronchodilation, for over 24 h generally. The tiotropium-M3 receptor complex has a half-life of about 35 h [11]. The effectiveness and safety of tiotropium bromide in the treatment of asthma have not been determined yet, so the clinical value still needs in-depth studies.

In this study, we treated patients with moderate persistent asthma by using fluticasone propionate aerosol, salmeterol-fluticasone propionate inhalant, tiotropium bromide inhalation powder combined with fluticasone propionate aerosol, and tiotropium bromide inhalation powder combined with salmeterol-fluticasone propionate inhalant. To compare the therapeutic effects, we then tested their pulmonary functions – first-second forced expiratory volume (FEV1), ratio of FEV1 to forced vital capacity (FEV1/FVC), and peak expiratory flow

(PEF) – and determined the asthma control test (ACT) scores, numbers of times salbutamol was used to alleviate symptoms, and IL-13 levels in induced sputum. The results further verified the clinical value of tiotropium bromide inhalation powder combined with fluticasone propionate aerosol in the treatment of moderate persistent asthma.

Material and Methods

Main reagents and apparatus

The Master Scope spirometer was purchased from Jaeger (Germany). Salbutamol sulfate aerosol (spray), salmeterol-fluticasone propionate inhalant, and fluticasone propionate aerosol (spray) were from GlaxoSmithKline (UK). Tiotropium bromide inhalation powder was obtained from Boehringer Ingelheim (Germany). IL-13 ELISA kits were provided by Boster Biological Technology Co., Ltd. (China).

Study grouping

This study was approved by the Ethics Committee of our hospital (Ethics Approval No. ZHSU-20151209), and written consent was obtained from all patients.

Inclusion criteria: 1) in accordance with the diagnostic criteria of the Global Initiative for Asthma Guidelines (2009); 2) aged >18 years old; 3) with $60\% \leq \text{FEV}_1 < 80\%$ of estimated value or $60\% \leq \text{FEV}_1 < 80\%$ of personal best value, and FEV1 or PEF variability of <30%; 4) with correct and timely use of inhalers and inhalants, as well as compliance with pulmonary function examinations; 5) without systemic use of glucocorticoids within the last month, and without inhalation or oral administration of glucocorticoids, LABA, short-acting β receptor agonists, anticholinergic agents, antihistamines, leukotriene antagonists, or theophyllines within the last 48 h.

Exclusion criteria: 1) critical diseases besides asthma; 2) severe respiration failure, heart failure, renal failure, or hepatic failure; 3) myocardial infarction or severe arrhythmia within the last 6 months; 4) pregnant or lactating women; 5) use of receptor antagonists; 6) lung diseases in addition to asthma or pulmonary lobectomy; 7) contraindications to salmeterol or tiotropium bromide.

We selected 160 eligible patients with moderate persistent asthma and randomly divided them into 4 groups (n=40). There were 3 control groups who were given fluticasone propionate aerosol (Flixotide, group A), salmeterol-fluticasone propionate inhalant (Seretide, group B), and tiotropium bromide inhalation powder combined with salmeterol-fluticasone propionate inhalant (Spiriva-Seretide, group C). The experimental group received

Table 1. Baseline clinical data, pulmonary functions, ACT scores, nighttime symptom scores, as well as IL-13 levels and proportions of inflammatory cells in induced sputum before treatment.

Group	Case No.	Gender Male/female	Age (year)	Disease course (year)	Height (cm)	Body weight (kg)	FVC (L)	FEV1 (L)	FEV1/FVC (%)	PEF (L/S)
A	40	20/20	35.30±9.17	35.30±9.17	159.02±6.74	60.80±5.84	3.52±0.36	2.13±0.44	60.20±10.49	5.41±1.33
B	40	24/16	36.60±8.61	12.00±15.35	162.81±6.92	64.90±10.30	3.37±0.69	1.83±0.47	53.11±7.72	4.64±1.03
C	40	20/20	40.30±8.52	14.00±11.13	161.67±8.04	65.70±10.11	3.43±0.48	1.95±0.48	57.26±8.19	5.20±1.07
D	40	16/24	36.40±9.45	13.62±10.44	150.50±7.21	57.87±8.69	4.03±0.68	2.08±0.38	51.75±7.04	5.24±1.21
P			4.736	122.780	30.957	13.397	0.062	0.432	0.124	0.505

Group	Case No.	Gender Male/female	ACT score	Nighttime symptom score	IL-13 level in induced sputum	Neutrophil (%)	Eosinophil (%)	Macrophage (%)	Lymphocyte (%)
A	40	20/20	14.30±2.31	2.20±0.63	60.1±20.5	67.1±4.6	2.7±0.6	27.1±2.2	2.2±1.8
B	40	24/16	12.60±2.20	2.10±0.74	63.4±23.7	66.4±5.5	2.8±0.4	28.1±3.1	2.3±2.0
C	40	20/20	13.50±2.24	2.50±0.63	62.9±34.7	67.3±4.8	2.7±0.8	27.2±8.1	2.2±1.7
D	40	16/24	14.40±2.41	3.00±1.15	62.4±29.7	66.6±0.68	2.8±0.7	28.1±7.4	2.2±1.1
P			0.309	0.079	2.121	0.172	0.33	0.302	0.250

tiotropium bromide inhalation powder combined with fluticasone propionate aerosol (Spiriva-Flixotide, group D). Group A was given Flixotide, 250 µg/time, twice/day; group B was given Seretide (50/250 µg), 1 inhalation/time, twice/day; group C received Seretide (50/250 µg), 1 inhalation/time, twice/day and Spiriva, 18 µg/time, once/day; and group D received Flixotide, 250 µg/time, twice/day and Spiriva, 18 µg/time, once/day. The 4 groups were treated for 8 consecutive weeks.

Methods

Pulmonary ventilation function was assessed before and after treatment. FVC, FEV1, FEV1/FVC, and PEF were assessed 24 h after discontinuation of using the drugs.

The ACT score was used to assess the control of symptoms before and after treatment, and to determine the therapeutic effects. In the ACT scoring system, 25 points indicates that asthma was completely controlled, 20–25 points indicates that asthma was under control, and <20 points indicates that asthma was uncontrolled.

During treatment, the number of times salbutamol used was recorded.

In the early morning, 3 g/dl hypertonic saline spray was used to induce sputum, which was then mixed with an equal amount of N-acetylcysteine and centrifuged at 2000 rpm for 20 min. The supernatant was then collected and stored at –80°C prior to use. IL-13 level in the induced sputum was detected according to the instructions of the ELISA kit.

Statistical analysis

All data were analyzed by SPSS 19.0. Categorical data are represented as mean ± standard deviation. Analysis of variance was performed. P<0.05 was considered statistically significant.

Results

Baseline clinical data, pulmonary functions, ACT scores, nighttime symptom scores, and IL-13 levels and proportions of inflammatory cells in induced sputum before treatment

Before treatment, the 4 groups had similar baseline clinical data, pulmonary functions, ACT scores, and nighttime symptom

Table 2. Pulmonary functions, ACT scores and nighttime symptom scores after 8 weeks of treatment as well as differences from those before treatment.

Group	Case No.	FVC (L) difference	FEV1 (L) difference	FEV1/FVC (%) difference	PEF (L/S) difference	ACT score difference	Nighttime symptom score difference
A	40	0.22±0.15	0.38±0.13	6.80±3.30	1.30±0.78	5.90±2.23	1.50±0.85
B	40	0.36±0.29	0.68±0.26	13.28±6.62	1.91±0.68	9.50±2.46	2.40±0.84
C	40	0.32±0.16	0.75±0.44	11.98±7.22	1.98±0.75	10.12±2.36	2.38±0.51
D	40	0.28±0.23	0.73±0.31*	13.73±6.41*	1.93±0.88*	8.90±2.92*	2.20±1.06*
P		0.377	0.029	0.040	0.013	0.004	0.048

* We compared the results of groups B–D with those of group A, $P<0.05$.

Table 3. Use of salbutamol to alleviate symptoms during treatment.

Group	Case No.	Number of times used to alleviate symptoms
A	40	3.90±1.45
B	40	2.50±0.85
C	40	1.20±1.00
D	40	2.20±0.92*#
P		0.000

* We compared the result of group D with that of group A, $P<0.05$; # we compared the result of group D with that of group C, $P<0.05$.

scores, as well as IL-13 levels and proportions of inflammatory cells in induced sputum ($P>0.05$) (Table 1).

Pulmonary functions, ACT scores, and nighttime symptom scores after treatment

The pulmonary functions, ACT scores, and nighttime symptom scores of the 4 groups after 8 weeks of treatment (Table 2) were significantly different from those before treatment ($P<0.05$), indicating that the symptoms of all patients were relieved. However, different drugs had various treatment outcomes. The pulmonary function of group D, which was significantly better than those of group A ($P<0.05$), was similar to those of groups B and C ($P>0.05$). Group D had significantly better asthma control test score and nighttime symptom score than those of group A ($P<0.05$), without significant differences from those of group B or group C ($P>0.05$) though.

Table 4. IL-13 levels in induced sputum after treatment.

Group	Case No.	IL-13 level in induced sputum
A	40	41.5±21.8
B	40	32.8±33.9
C	40	30.4±20.7
D	40	30.4±29.7*#
P		0.000

* We compared the result of group D with that of group A, $P<0.05$; # we compared the result of group D with that of group B, $P<0.05$.

Use of salbutamol to alleviate symptoms during treatment

During treatment, the number of times salbutamol was used to alleviate symptoms was significantly different ($P<0.05$) between group D and group A ($P<0.05$). Groups D and B had similar results ($P>0.05$) (Table 3). Therefore, after using different drugs, the patients had different times of acute attack and different symptom aggravation.

IL-13 levels in induced sputum after treatment

After treatment, the IL-13 levels in induced sputum had significant differences ($P<0.05$). The level of group D, which was higher than those of groups A and B ($P<0.05$), was similar to that of group C ($P>0.05$) (Table 4).

Proportions of inflammatory cells in induced sputum after treatment

After treatment, the proportion of neutrophils in induced sputum significantly decreased, but that of macrophages

Table 5. Proportions of inflammatory cells in induced sputum after treatment.

Group	Case No.	Neutrophil (%)	Eosinophil (%)	Macrophage (%)	Lymphocyte (%)
A	40	53.9±7.2*	2.7±0.6	41.4±5.2*	1.8±0.9
B	40	56.4±4.3*	2.3±0.8	39.1±3.1*	1.9±0.7
C	40	57.3±4.8*	2.6±0.7	38.0±8.1*	1.9±0.8
D	40	42.3±2.8*#	2.0±0.3	54.4±5.2*#	1.3±0.9

* We compared the data after treatment with those before treatment, $P<0.05$; # we compared the results of group D with those of other groups, $P<0.05$.

significantly increased ($P<0.05$). The results of group D were significantly better than those of other groups ($P<0.05$) (Table 5).

Adverse reactions

All patients completed this study. Adverse reactions were very minor, with only 1 case of hoarseness each in groups B–D and 1 case of mouth dryness each in groups B and C.

Discussion

Asthma is a complex chronic airway disease caused by multiple factors. With in-depth research on its pathogenesis, it is recognized that its essence is a chronic non-specific inflammation mediated by a variety of cells and cytokines, with the characteristics of high airway reactivity and reversible airflow restriction [12–14]. The present study shows that asthma patients have increased respiratory tract cholinergic nerve tension. The cholinergic nerve-mediated bronchospasm is a major adverse factor in asthma airflow limitation, and anticholinergic drugs and human M receptor can have a curative effect through specific combinations. There are 3 receptor subtypes in the human lung: M1, M2, and M3 receptors. M1 receptors are mainly located in the parasympathetic ganglia, and primarily promote the transport of cholinergic neurotransmitters. M2 receptors are found in the cholinergic postganglionic nerve, sympathetic nerve, and airway smooth muscle, with a major function of inhibiting the further release of acetylcholine through negative feedback regulation [15]. M3 receptors, which are mainly located in the airway smooth muscle, are the main cause of airway contraction, and they also exist in the submucosal gland, which can regulate the secretion of mucin [16]. Another study has found that the cholinergic receptor subtype has an acetylcholine affinity that changes with patient age, but the result is always $M3>M2>M1$ [17]. Tiotropium bromide and ipratropium have the same affinity with each receptor, but the dissociation speed is different. Tiotropium bromide exhibits a unique kinetic selectivity to the receptor that can block the combination of acetylcholine with M3 receptor,

reduce airway smooth muscle tension, and achieve bronchial dilation. Tiotropium bromide can be more rapidly dissociated with M2 receptors, reduce the release of acetylcholine, and alleviate the negative effects of receptor excitement on bronchial dilation. Due to the slow rate of dissociation between tiotropium bromide and M3 receptor, tiotropium bromide has become a long-term anticholinergic drug. With a one-time inhalation of tiotropium bromide, bronchial expansion can be sustained for 24 h [18], but the onset time is relatively slow. Another study found that tiotropium bromide can reverse allergen-induced airway smooth muscle remodeling, reduce the expression of smooth muscle myosin, reduce the number of smooth muscle cells, and inhibit airway smooth muscle thickening and airway hyperresponsiveness, which may be an expansion of one of the causes of bronchiectasis [19]. *In vivo* pre-clinical studies in guinea pig asthma models have shown that tiotropium bromide can alleviate airway inflammation and slow the process of reconstitution in these models [20,21]. In the present study, tiotropium bromide powder combined with fluticasone propionate aerosol (inhalation) was used to treat moderately persistent asthma patients. After 8 weeks of treatment, the results showed that the FVC, FEV1, FEV1/FVC, and PEF levels of pulmonary function after treatment were significantly higher than before treatment ($P<0.05$). The difference values of improvement in FEV1, FEV1/FVC, and PEF levels of pulmonary function were increased compared with the single therapy of fluticasone propionate aerosol (inhalation), with no significant difference from salmeterol-fluticasone propionate powder (inhalation) ($P>0.05$). The above results are consistent with the literature [7]. However, there was no significant difference in the values of improvement in FEV1, FEV1/FVC, and PEF levels between tiotropium bromide powder combined with fluticasone propionate aerosol and tiotropium bromide powder combined with salmeterol-fluticasone propionate inhalant before and after treatment ($P<0.05$), which was not consistent with previous studies [22,23]. The differing experimental outcomes may be due to different grouping baselines. Their grouping used asthma patients whose conditions were not controlled after inhalation with salmeterol-fluticasone propionate powder, while the present study enrolled moderately persistent

asthma patients who did not receive either regular treatment or who only received initial treatment. In addition, there were also racial differences and different reactions to drugs.

The asthma control test (ACT) [24] and the nighttime symptom score [25] are simple and easy-to-use indicators for evaluating asthma control symptoms and quality of life. ACT score can enable asthma patients to control their asthma symptoms using ladder treatment, which has clinical and scientific value. This study used tiotropium bromide powder combined with fluticasone propionate aerosol in the treatment of moderate persistent asthma for 8 weeks. The results showed that ACT score and Nighttime Symptoms Score after treatment were significantly higher than those before treatment ($P < 0.05$). The ACT scores and nighttime symptom scores were significantly improved compared with the single therapy of fluticasone propionate aerosol (inhalation) ($P < 0.05$). The results were not statistically significant compared with salmeterol-fluticasone propionate powder or tiotropium saddle powder combined with salmeterol-fluticasone propionate powder (inhalation) ($P > 0.05$). The results were consistent with those reported before [23,24]. Huang et al. reported that the levels of plasma inflammatory cytokines IL-4, IL-8, IL-10, and TNF- α were correlated with the pulmonary functions of patients with asthma-COPD overlap syndrome [26]. Also, IL-13 plays an important role in the pathogenesis of asthma, which is the dominant factor in the inflammatory process of asthma and the basic factor of airway hyperresponsiveness. The effect of the tiotropium bromide powder combined with fluticasone propionate aerosol was superior to those of the fluticasone propionate aerosol group and the salmeterol-fluticasone propionate inhalant group, but was similar to that of the tiotropium bromide powder combined with salmeterol-fluticasone propionate inhalant group. The above results prove that the inhalation of tiotropium bromide powder combined with fluticasone propionate aerosol can significantly improve the clinical symptoms of patients with moderate persistent asthma. It is superior to single fluticasone propionate aerosol in terms of the improvement of clinical symptoms, and has a similar effect as the salmeterol-fluticasone propionate powder and tiotropium bromide powder combined with salmeterol-fluticasone propionate powder.

In addition to inhaled hormone anti-inflammatory effect and airway dilation, its efficacy is also associated with the fact that tiotropium bromide can block airway smooth muscle receptors and can inhibit smooth muscle thickening and airway hyper-responsiveness for smooth muscle relaxation, airway expansion, and decreased secretions, thereby reducing the clinical symptoms of asthma patients [27].

Tiotropium bromide is a new long-acting inhalable anticholinergic drug that has all the adverse effects of other inhaled anticholinergic drugs, such as thirst, rare urinary retention, increased intraocular pressure, and very rare paradoxical bronchospasm [28]. Singh et al. conducted a meta-analysis of randomized clinical trials and suggested that inhalation of anticholinergic drugs could significantly increase the risk of cardiovascular adverse events [29]. However, a large clinical study did not show that tiotropium bromide increased the risk of myocardial infarction, cardiac death, or death due to other causes [30]. For IL-13 levels and proportions of inflammatory cells in induced sputum, group D also had the best treatment outcomes.

In this study, 2 patients had thirst, which was self-mitigated without any special treatment, and there were no other adverse effects, consistent with the literature. Whether there is a cardiovascular accident needs to be confirmed by expanding the sample size and extending the research time.

Conclusions

In conclusion, this study adopted a randomized, open control trial principle. The lung function data were obtained by experienced technicians through repeated testing, which was superior and reliable. The experimental results were credible, with a certain clinical significance. Tiotropium bromide has shown good prospects for the treatment of asthma. However, since this study had small sample size and short duration, the effects of acute attack or number of hospitalization could not be evaluated, and the safety of long-term treatment is also unknown. Therefore, further in-depth studies are needed to validate the treatment outcomes and safety of tiotropium bromide.

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