

Effects of phosphodiesterase 4 inhibitor on cough response in guinea pigs sensitized and challenged with ovalbumin

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Background There is currently considerable interest in the potential value of selective inhibitors of cyclic nucleotide phosphodiesterase 4 in the treatment of asthma. However, whether they influence eosinophilic airway inflammation-associated cough remains unclear. The objective of this study was to investigate the effects of selective phosphodiesterase 4 inhibitor SB207499 on cough response and airway inflammation in guinea pigs sensitized and challenged with ovalbumin.

Methods Forty sensitized guinea pigs were randomly divided into four groups: control (n = 10), challenge (n = 10), SB207499 (n = 10) and aminophylline (n = 10), then challenged with aerosol of 1% ovalbumin or saline. Two hours later, animals were intraperitoneally injected with either saline, 25 mg/kg of SB207499 or aminophylline. At the 24th hour, the injection was repeated with 2.5 mg/kg and 25 mg/kg SB207499 or aminophylline, then cough response to inhaled capsaicin and airway responsiveness to methacholine inducing a 150% of the peak airway pressure to the baseline (PC₁₅₀) was measured. Finally, total cell number and differentials in bronchoalveolar lavage fluid were analysed.

Results The cough frequency per 3 minutes and PC₁₅₀ in the challenge group were (22 ± 4) times/3 minutes and (198 ± 54) μg/ml, which were significantly different from (6 ± 2) times/3 minutes and (691 ± 81) μg/ml in the control group (P < 0.05, respectively). The injection of 25 mg/kg SB207499 significantly inhibited the increased cough response and airway hyperresponsiveness, the cough frequency and PC₁₅₀ in guinea pigs were (13 ± 2) times/3 minutes and (680 ± 81) μg/ml (P < 0.05), which differed significantly from (18 ± 2) times/3 minutes and (400 ± 86) μg/ml after the administration of the same dose of aminophylline (P < 0.05). The inhibition of SB207499 on cough response was dose-dependent. Similarly, SB207499 decreased the total cell number and percentage of eosinophils in bronchoalveolar lavage fluid to (2.1 ± 0.5) × 10⁶/ml and (20 ± 5)% respectively, which were significantly different from (3.2 ± 0.5) × 10⁶/ml and (29 ± 5)% in the aminophylline group (P < 0.05, respectively) or (4.2 ± 0.7) × 10⁶/ml and (35 ± 4)% in the challenge group (P < 0.05, respectively).

Conclusion Phosphodiesterase 4 inhibitor may be more useful than aminophylline for cough associated with eosinophilic airway inflammation via inhibiting airway inflammation and airway hyperresponsiveness.

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Cough is the sole presenting symptom in cough variant asthma and eosinophilic bronchitis, and also a common manifestation in most cases of typical asthma. However, cough variant asthma and eosinophilic bronchitis, well known as significant causes of chronic cough, are characterized by lack of wheezing commonly found in typical asthma. But both are histologically characterized by eosinophilic airway inflammation similar to that seen in typical asthma.^{1,2} Since it is generally accepted that cough is generated by the

stimulation of cough receptors in airway due to inflammation and bronchoconstriction, bronchodilators and corticosteroids are widely used as anti-tussive agents

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during these situations.

There is currently considerable interest in the potential value of selective inhibitors of cyclic nucleotide phosphodiesterase 4 (PDE₄) in the treatment of asthma. PDE₄ inhibitors have many actions, such as eliminating antigen- and mediator-induced eosinophil infiltration into airway,^{3,4} suppressing ovalbumin (OVA)-induced bronchoconstriction and airway hyperresponsiveness in guinea pigs,^{5,6} and significantly attenuating the late response to allergen challenge but not airway hyperresponsiveness in asthmatic patients.⁷ However, whether PDE₄ inhibitors can influence eosinophilic airway inflammation-associated cough remains unclear. To clarify the question, we investigated the effects of SB207499, a selective PDE₄ inhibitor, on cough response in guinea pigs sensitized and challenged with OVA by repeated administration.

METHODS

Main Reagents

The main reagents included OVA and methacholine (Sigma, USA), Tween 80 (Amresco, USA), capsaicin and aluminium hydroxide (Wako Pure Chemical Ind. Ltd., Japan), *c*-4-cyano-4-(3-cyclopentyloxy-4-methoxy-phenyl)-*r*-1-cyclohexanecarboxylic acid (SB207499, Smith Kline Beecham Pharmaceuticals, USA), amino-phylline (Shanghai Xinyi Pharmaceuticals, China). Capsaicin (30.5 mg) was dissolved in 1 ml of Tween 80 and 1 ml of ethanol, then mixed with 8 ml of physiological saline to make the stock solution of 10⁻² mol/L, which was diluted with physiological saline into 10⁻⁴ mol/L. Methacholine was dissolved in physiological saline to make the solution of 25 - 1600 µg/ml.

OVA sensitization and challenge

Forty male guinea pigs weighing 200 - 250 g, obtained from Shanghai Experimental Animal Centre of Chinese Academy of Sciences, were randomly divided into four groups: control (n = 10), challenge (n = 10), SB207499 (n = 10) and aminophylline (n = 10). OVA sensitization and challenge were performed according to the method described by Muraki et al.⁸ Briefly, guinea pigs were intraperitoneally administered with 2 mg OVA emulsified in 100 mg aluminium hydroxide two days after an intraperitoneal injection of 30 mg/kg cyclophosphamide. Three weeks later, boosting was carried out by intraperitoneal injection of suspension containing 0.01 mg OVA and 100 mg aluminium

hydroxide. Six weeks after the primary sensitization, guinea pigs were placed in a plastic box and challenged with aerosol of 1% OVA for 90 seconds under spontaneous breathing. The animals in the control group were aerosolized with physiological saline. The aerosol was generated with a nebulizer (Model 646, Devilbiss Co., USA) at an output of 37 µl/min. The guinea pigs in SB207499 and aminophylline groups were intraperitoneally administered with 25 mg/kg SB207499 or aminophylline two hours later, repeated with 2.5 mg/kg and 25 mg/kg SB207499 or aminophylline respectively at the 24th hour. In the same procedure, the animals in the control and challenge groups were injected with 1 ml of physiological saline.

Measurement of cough response to capsaicin

Cough response to capsaicin was measured following the previously described protocols with some modifications.^{9,10} Before and half hour after each repeated injection of 2.5 mg/kg or 25 mg/kg SB207499 and aminophylline, guinea pigs were placed in a self-designed airtight dual plastic box consisting of a head part separated from a body part. The head part of the box was connected to a Devilbiss 646 nebulizer with the same output as described above, and pressure in the body part was monitored with a pressure transducer (TP-603T, Nihon Koden Kogyo Company, Japan). Cough was judged when a sudden high positive pressure was followed by rapid small negative pressure. To differentiate the motion- and sneezing-related changes in the pressure from cough, movements of the guinea pigs were visually monitored and excluded from calculation. After establishing the baseline of cough response to the inhalation of saline aerosol, each conscious guinea pig was exposed to an aerosolized solution of 10⁻⁴ mol/L capsaicin for 2 minutes. The number of cough was counted from the initiation of a 2 minute inhalation of capsaicin solution to a subsequent 1 minute observation time.

Measurement of airway responsiveness to methacholine

Six hours after the measurement of cough response to capsaicin, guinea pigs were anaesthetized with an intraperitoneal administration of 75 mg/kg sodium pentobarbitone and were placed in a supine position. After the trachea were cannulated with a 16-gauge blunt metal needle, the animals were artificially ventilated by using an animal ventilator (Model DH-140B, Experimental Apparatus Factory of Zhejiang Medical University, China) adjusted to deliver a tidal volume of 10 ml/kg at a rate of 60 strokes/min. Changes in peak airway pressure of the tracheal tube were measured with

a pressure transducer (TP-603T, Nihon Koden Kogyo Company, Japan). Five minutes after the airway pressure became stable, a succession of ascending concentration of methacholine in the range of 25-1600 $\mu\text{g/ml}$ was inhaled for 1 minute by using an ultrasonic nebulizer, the change in airway pressure was recorded for 5 minutes. The nebulizer (Model WA18-II, Tianjing Wan'an Ultrasonic Electronic Science & Technology Company, China) generated aerosol at a rate of 1 ml/min with a diameter of the particles in 1 - 5 μm . The methacholine concentration inducing a 150 % of the peak airway pressure to the baseline (PC_{150}) was used as an indicator of airway responsiveness in guinea pigs.¹¹

Collection of bronchoalveolar lavage fluid and differential cell count

After the assessment of airway responsiveness to methacholine, animals were euthanized with an overdose of pentobarbital. Bronchoalveolar lavage fluid (BALF) was collected by injecting a 5ml aliquot of physiologic saline at 37 °C into the lungs twice and withdrawing with a gentle suction via the intratracheal needle. When the total cell number was determined, cytocentrifuged preparations (Cytospin 3, Shandon, UK) were stained with May-Grunwald-Giemsa. The cells were differentiated through counting 400 nucleated cells according to the standard morphologic criteria.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation (SD) and analyzed by one-way analysis of variance followed by *q* test using StatView 5.12. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Effects of SB207499 on cough response

OVA challenge induced an obvious cough response to capsaicin in guinea pigs, the cough frequency increased from (6 ± 2) times/3 minutes in the control group to (22 ± 4) times/3 minutes in the challenge group ($P < 0.05$). Aminophylline apparently attenuated the elevated cough response. The effects of aminophylline did not seem to depend on the doses used since the cough frequency was similar between 2.5 mg/kg and 25 mg/kg of aminophylline. In contrast, SB207499 in a dose-dependent manner presented a further inhibition to increased cough response compared with aminophylline. However, even 25 mg/kg of SB207499 could not decrease the cough frequency to the level in the control group ($P < 0.05$, Fig. 1).

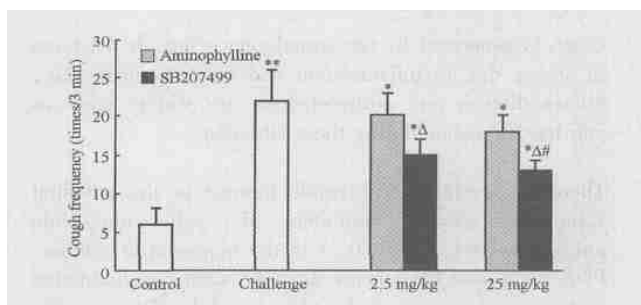


Fig. 1. The effect of SB207499 and aminophylline on cough response in guinea pigs. SB207499: a selective phosphodiesterase 4 inhibitor. * $P < 0.05$ vs the challenge group, $\Delta P < 0.05$ vs the same dosage of aminophylline group, # $P < 0.05$ vs the 2.5 mg/kg of SB207499 group, and * $P < 0.05$ vs the control group.

Changes in airway responsiveness

Exposure to OVA induced airway hyperresponsiveness in the guinea pigs of the challenge group as compared with the animals in the control group ($P < 0.05$). Aminophylline significantly inhibited the increased airway responsiveness, in terms of higher PC_{150} than that in the challenge group ($P < 0.05$). The inhibition was more pronounced with SB207499 since PC_{150} was significantly higher than that in aminophylline group ($P < 0.05$) but comparable with that in the control group ($P > 0.05$, Fig. 2).

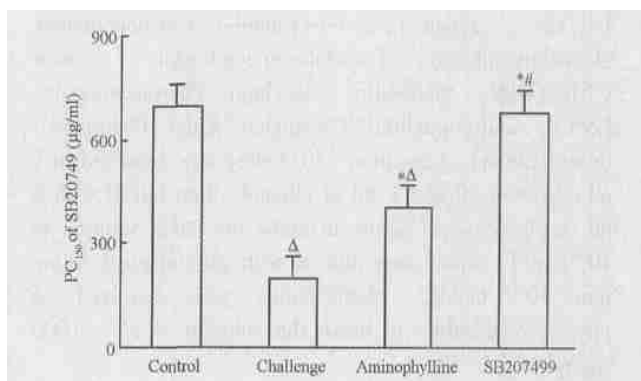


Fig. 2. The effects of SB207499 on the airway hyperresponsiveness in guinea pigs. PC_{150} : the concentration inducing a 150 % of the peak airway pressure to the baseline; and SB207499: a selective phosphodiesterase 4 inhibitor. $P < 0.05$ vs the challenge group, $\Delta P < 0.05$ vs the control group, # $P < 0.05$ vs the aminophylline group.

Changes in cell components in BALF

Challenge of guinea pigs with OVA resulted in an apparent increase in total cell number and percentage of eosinophils but a decrease in differential macrophage count in BALF. The changes were markedly reversed by repeated administration of either aminophylline or SB207499. Compared with aminophylline, SB207499

Table. Total cell number and cell components in bronchoalveolar lavage fluid from each group (n = 10, mean $\pm$ SD)

Groups	Total cell number ($\times 10^6$ /ml)	Neutrophils (%)	Macrophages (%)	Eosinophils (%)	Lymphocytes (%)
Control	1.5 $\pm$ 0.5	15 $\pm$ 5	62 $\pm$ 10	11 $\pm$ 2	12 $\pm$ 4
Challenge	4.2 $\pm$ 0.7 [#]	12 $\pm$ 5	43 $\pm$ 6 [#]	35 $\pm$ 4 [#]	10 $\pm$ 3
Aminophylline	3.2 $\pm$ 0.5 [*]	15 $\pm$ 4	47 $\pm$ 7	29 $\pm$ 5 ^{*#}	9 $\pm$ 4
SB207499	2.1 $\pm$ 0.5 ^{△#}	15 $\pm$ 3	52 $\pm$ 7 ^{△#}	20 $\pm$ 5 ^{△#}	13 $\pm$ 3

SB207499: a selective phosphodiesterase 4 inhibitor. ^{*} $P < 0.05$ vs the challenge group, [△] $P < 0.05$ vs the aminophylline group, and [#] $P < 0.05$ vs the control group.

induced a more remarkable reduction in total cell number and percentage of eosinophils in BALF (Table).

DISCUSSION

To assess the effect of selective PDE₄ inhibitor SB207499 on the cough response associated with eosinophilic airway inflammation, we examined the changes in cough frequency induced by the inhalation of capsaicin in the guinea pigs after OVA sensitization and challenge followed by repeated intraperitoneal administration of SB207499 or aminophylline. The results demonstrated that SB207499 remarkably inhibits the increased cough response to capsaicin in the guinea pigs on a dose-dependent manner. The effects of SB207499 were more conspicuous than that of aminophylline. These observations indicate that SB207499 might be a better anti-tussive agent than aminophylline for eosinophilic airway inflammation-associated cough, at least in this animal model.

Cough is a common clinical problem in cough variant asthma and eosinophilic bronchitis. Since bronchodilators including theophylline and β_2 receptor agonists are effective against cough in cough variant asthma, bronchoconstriction is commonly believed as an important mechanism of cough associated with eosinophilic airway inflammation.¹ However, unlike cough variant asthma, eosinophilic bronchitis shows normal airway response to methacholine and histamine, in which cough fails to relieve with bronchodilators and only responds to inhaled and/or oral corticosteroids,² suggesting that airway inflammation is also responsible for cough pathogenesis. Indeed, several studies have demonstrated that eosinophilic airway inflammation is associated with increased cough reaction in guinea pigs following antigen provocation.⁹ Our previous work shows that eosinophilic airway inflammation might be a more key factor in the generation of cough compared with bronchoconstriction, but the effect of airway inflammation on cough is amplified by bronchoconstriction.¹¹ In the present study, guinea pigs exhibit much milder airway hyperresponsiveness and eosinophilic airway inflammation after repeated

administration of either SB207499 or aminophylline, in addition to the decreased cough response to capsaicin. These data suggest that the influence of SB207499 and aminophylline on cough frequency should be primarily due to both the inhibition of airway inflammation and the relaxation of airway smooth muscle.

Adenosine 3', 5'-cyclic monophosphate (cAMP) is an intracellular messenger that has important regulatory roles in the function of the cells involved in the pathophysiology of asthma. The intracellular cAMP levels represent the balance between the generation of cAMP from ATP by adenylate cyclase and its inactivation by PDEs. Presently, at least eleven PDE isoenzyme family members have been identified,¹² of which PDE₄ isoenzyme receives the increasing attention for its potential use in the treatment of asthma and chronic obstructive pulmonary diseases. It has been well established that aminophylline, a non-selective PDE inhibitor, increases cAMP level in the cells via inhibiting the activity of all PDEs. The increase in cAMP can relax airway smooth muscle, and broadly suppress the chemotaxis and activation of a variety of immune and inflammatory cells including eosinophils, lymphocytes and monocytes.¹³ PDE₄ isoenzyme is also showed to regulate the function of eosinophils and mast cells.¹⁴ Several lines of evidence have demonstrated that PDE₄ inhibitor such as rolipram and Ro 20-1724 markedly inhibit eosinophil infiltration into airway induced by antigen challenge³ or by cytokines such as platelet activating factor, interleukin-5 and interleukin-13.^{15,16} Rolipram has also been reported to reduce polymorphonuclear cell accumulation in the airway and attenuate airway inflammation and airway hyperresponsiveness in an animal model of secondary allergen challenge.^{6,17} Moreover, SB207499 has been demonstrated to prevent inflammatory mediator release from mast cells and inhibit OVA-induced contraction of guinea pig isolated tracheal strips *in vitro*, markedly reduce bronchospasm and subsequent eosinophil influx *in vivo*.¹⁸ Taken together, these findings suggest that PDE₄ inhibitors have bronchodilatory and anti-inflammatory effect in the airways. It is well known that eosinophilic airway inflammation is related to

airway hyperresponsiveness.¹⁹ Since PDE₄ isoenzyme is a predominant component of the catabolic system for cAMP in airway smooth muscle and in inflammatory and immune cells,²⁰ it is imaginable that SB207499 can suppress airway inflammation and hyperresponsiveness, then cough response to greater degree than aminophylline. It is supported by our data that the treatment of SB207499 results in less eosinophilic in BALF, higher PC₁₅₀ and lower cough frequency than aminophylline in this animal model. These observations suggest that SB207499 may be a promising agent in the management of cough associated with eosinophilic airway inflammation. Further clinical studies are needed to elucidate the usefulness of PDE₄ inhibitor in this aspect.

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