

Original Article

Effect of histamine H₁ receptor antagonists on antigen-induced increase of cough sensitivity in guinea pigs

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ABSTRACT

Eosinophilic airway inflammation and increased cough sensitivity without bronchial hyperresponsiveness are the pathologic and physiologic features of bronchodilator-resistant non-productive cough-associated global atopic tendency, abbreviated herein as atopic cough. Histamine H₁ receptor antagonists are effective in relieving the cough in nearly 60% of patients with atopic cough. However, there is no direct evidence that histamine H₁ receptor antagonists can reduce cough hypersensitivity associated with eosinophilic airway inflammation. The purpose of the present study was to clarify this issue. The number of coughs caused by inhalation of increasing concentrations of capsaicin (10⁻⁸, 10⁻⁶ and 10⁻⁴ mol/L) was counted 24 h after the administration of an aerosolized antigen in actively sensitized conscious guinea pigs and then bronchoalveolar lavage (BAL) was performed. Azelastine (0.1 or 1 mg/kg) or terfenadine (0.2 or 2 mg/kg) was given intraperitoneally 60 min before capsaicin provocation 24 h after the antigen challenge in sensitized guinea pigs. In addition, azelastine (0.1 or 1 mg/kg) was administered 90 min before the capsaicin challenge in naïve guinea pigs. The cough response to capsaicin and the number of eosinophils in BAL fluid (BALF) were significantly increased after antigen challenge. Azelastine and terfenadine significantly reduced

the increased cough response after antigen challenge, while azelastine had no effect in naïve animals. In conclusion, histamine H₁ receptor antagonists reduce antigen-induced increases in cough sensitivity in sensitized guinea pigs without direct inhibition of a common cough reflex pathway.

Key words: allergic reaction, capsaicin, cough sensitivity, guinea pig, histamine H₁ receptor antagonists.

INTRODUCTION

A new clinical entity, presenting with chronic bronchodilator-resistant non-productive cough associated with global atopic tendency, abbreviated here as atopic cough, in which eosinophilic inflammation of airways and increased cough sensitivity are the respective fundamental pathologic and physiologic features has been proposed.¹⁻³ Histamine H₁ receptor antagonists are effective in treating cough in nearly of 60% patients with atopic cough.¹⁻³ Recently, three cases of atopic cough have been reported⁴⁻⁶ in which a single inhalation challenge with environmental fungal antigen extract caused non-productive cough and increased cough sensitivity to inhaled capsaicin. On the basis of these findings it is hypothesized that a single antigen challenge is able to cause an increase in cough sensitivity associated with airway eosinophilic inflammation.

Many animal experiments have been performed to assess the antitussive effect of various agents. Bolser *et al.*⁷ have reported that the antihistamines loratadine and chlorpheniramine inhibited antigen-induced cough in actively sensitized guinea pigs and capsaicin-induced cough in naïve guinea pigs. However, they did not

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examine whether the antigen-induced cough resulted from an increased cough reflex or bronchoconstriction induced by an allergic reaction. Thus, the effect of antihistamines on heightened cough sensitivity induced by allergic reactions has never been reported.

Although it has been shown that multiple antigen challenges increase cough responses to inhaled capsaicin in sensitized guinea pigs,⁸ the effect of a single antigen challenge on cough sensitivity has not been reported. The present study was conducted to elucidate whether airway cough response to inhaled capsaicin is increased after a single antigen challenge in actively sensitized guinea pigs and whether histamine H₁ receptor antagonists inhibit the antigen-induced increase in the cough response.

METHODS

Animals

Male albino Hartley guinea pigs, weighing 200–250 g, were obtained from Sankyo Laboratory Service (Toyama, Japan). They were quarantined in the Animal Research Center of Kanazawa University for 1 week before the study. All animal procedures in the present study complied with the standards set out in the Guideline for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University.

Sensitization of animals

Guinea pigs were actively sensitized by the method reported by Muraki *et al.*⁹ Guinea pigs were administered 2.0 mg, i.p., ovalbumin (OA) and 100 mg, i.p., aluminum hydroxide (Al(OH)₃) 2 days after intraperitoneal administration of 30 mg/kg cyclophosphamide. Boosting was performed by the intraperitoneal administration of 0.01 mg OA and 100 mg Al(OH)₃ 3 weeks later.

Antigen challenge

Actively sensitized conscious guinea pigs were placed in a dual chamber plethysmograph (head chamber volume 1520 mL; model PMUA + SAR; Buxco Electronics, Sharon, CT, USA). Animals were challenged with a 10 mg/mL OA solution (head chamber only; 60 s; 0.08 mL/min output). The aerosol was generated by a Devilbiss 646 nebulizer (Devilbiss Co., Somerset, PA, USA) operated by compressed air at 7.57 L/min (Minipon 54B-588; Origin Medical Industry Co. Ltd, Tokyo, Japan).

Measurement of cough response

Each conscious guinea pig was placed in a transparent plastic box consisting of a head chamber (volume 1600 mL) isolated from a body chamber and pressure in the body chamber was recorded. Coughs were detected as a transient change in the pressure (a rapid inspiration followed by rapid expiration). To disregard motion- and sneezing-related changes in pressure, movements of guinea pigs were visually monitored. Coughs were counted by a trained observer and were recognized by characteristic animal posture and pressure transducer recordings. Coughs were readily distinguished from sneezes.

Capsaicin (30.5 mg) was dissolved in Tween 80 (1 mL) and ethanol (1 mL) and then dissolved in physiologic saline (8 mL) to make a stock solution of 10⁻² mol/L, which was stored at -20°C. This solution was diluted with physiological saline to make solutions of 10⁻⁸, 10⁻⁶ and 10⁻⁴ mol/L. Each conscious guinea pig was exposed to progressively increasing concentrations of the capsaicin solution as an aerosol. Solutions were inhaled for 2 min every 8 min from a Devilbiss 646 nebulizer (Devilbiss Co.) operated by compressed air at 1.6 L/min (Iwaki Air Pump AP-115AN; Iwaki Co. Ltd, Tokyo, Japan). The nebulizer output was 0.037 mL/min. The number of coughs was counted during a 2 min inhalation of each capsaicin solution and during a subsequent 1 min observation period.

Increased cough response after antigen challenge

Progressively increasing concentrations of capsaicin solution were inhaled by actively sensitized guinea pigs ($n = 9$) 24 h after a single challenge with an aerosolized OA solution or by naïve guinea pigs ($n = 9$). The number of coughs caused by each capsaicin solution was counted and recorded.

Bronchoalveolar lavage (BAL) was performed after completion of the capsaicin provocation to confirm antigen-induced airway eosinophilic inflammation. Each guinea pig was anesthetized with an intraperitoneal injection of 75 mg/kg sodium pentobarbital and the trachea was cannulated with a polyethylene tube (2.5 mm o.d.; 2.1 mm i.d.). The guinea pig was then artificially ventilated by a small animal respiratory pump (model 1680; Harvard Apparatus Co. Inc., South Natick, MA, USA) adjusted to a tidal volume of 10 mL/kg at a rate of 60 strokes/min. The lower airways were lavaged via the

tracheal cannula using two 10 mL aliquots of physiologic saline solution at 37°C. The fluid was recovered by gentle aspiration with a disposable syringe. The BAL fluid (BALF) was immediately centrifuged at 70 g for 8 min. After discarding the supernatant, cells were washed twice in Hank's solution and resuspended in 1 mL Hank's solution. They were then counted manually in a Burker Chamber (Eruma Hanbai Kabushiki Gaisha, Tokyo, Japan). Cyto-centrifuged preparations (Cytospin 2; Shandon Southern Products Ltd, Cheshire, UK) were stained with May-Giemsa and a differential cell count was performed on 500 cells according to the standard morphologic criteria.

Effect of histamine H₁ receptor antagonists on cough response to capsaicin

Two different histamine H₁ receptor antagonists, azelastine chloride and terfenadine, were used.

Azelastine, at doses of 0.1 ($n = 6$) or 1.0 mg/kg ($n = 6$), its vehicle (saline; $n = 6$), terfenadine, at doses of 0.2 ($n = 6$) or 2.0 mg/kg ($n = 6$), or its vehicle (saline; $n = 6$) was administered intraperitoneally 60 min before the measurement of cough response to capsaicin in sensitized guinea pigs. In addition, the effect of azelastine was examined in naïve animals in the same manner ($n = 6$ for each group).

Chemicals

The following chemicals were used: OA (Sigma Chemical Co., St Louis, MO, USA), Al(OH)₃ (Wako Pure Chemical Industries, Osaka, Japan), sodium pentobarbital (Abbott Laboratories, North Chicago, IL, USA), capsaicin (Sigma Chemical Co.), dimethyl sulfoxide (DMSO; Wako Pure Chemical Industries), ethanol (Sigma Chemical Co.), azelastine (4-(*P*-chlorobenzyl)-2-[*N*-methyl-perhydro-aze-pinyl-4]-1-(2H)-phthalazinone hydrochloride; Eisai Co. Ltd, Tokyo, Japan), terfenadine ((±)-α-(*P*-*tert*-butylphenyl)-4-(hydroxydiphenyl-methyl)-1-piperidine-butnol; Nippon Heochst Marion Roussel Co. Ltd, Tokyo, Japan) and physiological saline (Otsuka Pharmaceutical Co. Ltd, Osaka, Japan).

Statistical analysis

Data are shown as the mean ± SEM. Statistical differences were determined by parametric analysis of variance (ANOVA) between groups. The relationship between the percentage and number of BAL cells and the number of

coughs caused by inhaled capsaicin was analyzed using simple regression analysis. Furthermore, multiple regression analysis was performed to assess contributions of BAL cell components to the cough response. Significance was based on a 95% confidence level ($P < 0.05$).

RESULTS

The number of coughs induced by 10⁻⁶ and 10⁻⁴ mol/L capsaicin 24 h after antigen challenge in sensitized guinea pigs was significantly greater than that in naïve animals (Fig. 1), suggesting an increase in the cough response after allergic reaction. Total cells and eosinophils in BALF were significantly increased 24 h after antigen challenge in sensitized animals compared with naïve animals (Fig. 2), showing antigen-induced airway eosinophil accumulation.

Percentages of eosinophils and macrophages in BALF were significantly correlated with the number of coughs induced by 10⁻⁶ and 10⁻⁴ mol/L capsaicin (Table 1), while there was a significant correlation between the absolute number of macrophages, lymphocytes, neutrophils and eosinophils and 10⁻⁴ mol/L capsaicin-induced coughs (Table 1). Accordingly, a multiple regression analysis was performed with cough number caused by 10⁻⁴ mol/L capsaicin as the dependent variable and the percentage

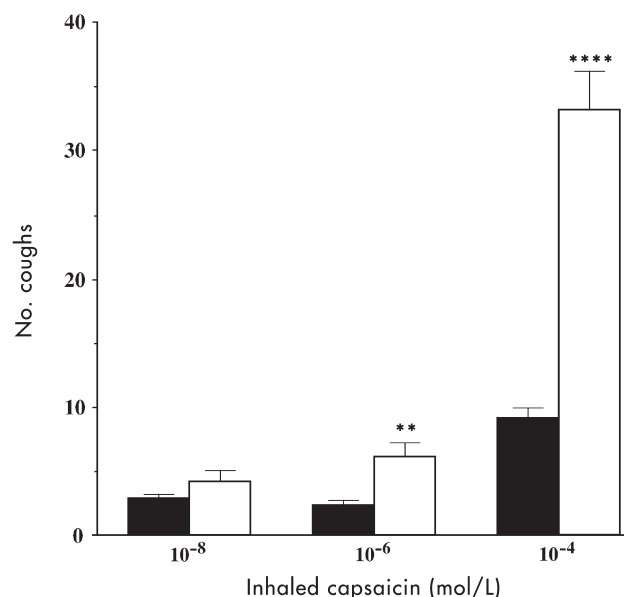


Fig. 1 Cough response to inhaled capsaicin 24 h after an antigen challenge in actively sensitized guinea pigs (□) and non-sensitized (naïve) animals (■). Each column represents the mean ± SEM ($n = 9$). ** $P < 0.01$, **** $P < 0.0001$ compared with naïve guinea pigs.

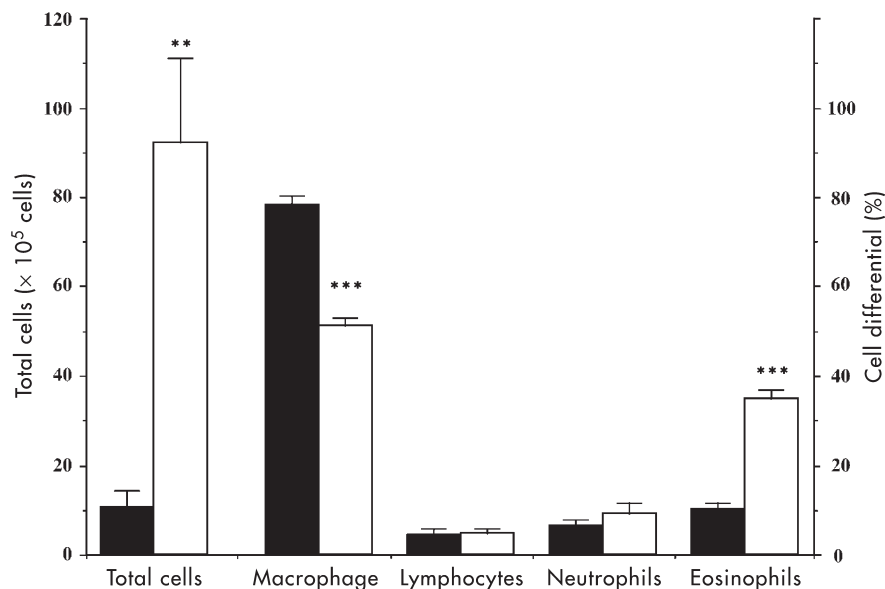


Fig. 2 Bronchoalveolar lavage cell findings 24 h after an antigen challenge in actively sensitized guinea pigs (□) and nonsensitized (naïve) animals (■). Each column represents the mean ± SEM (*n* = 9). ***P* < 0.01, ****P* < 0.001 compared with naïve guinea pigs.

Table 1 Relationship between number of coughs induced by inhaled capsaicin and cell differentials in bronchoalveolar lavage fluid

		No. coughs							
		Concentration of inhaled capsaicin (mol/L)							
		10 ⁻⁸		10 ⁻⁶		10 ⁻⁴			
		<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>		
Total cells	(cells/mL)	0.325	0.1881	0.487	0.0406	0.834	< 0.0001		
Macrophages	(%)	-0.256	0.3048	-0.610	0.0072	-0.882	< 0.0001		
	(cells/mL)	0.336	0.1731	0.451	0.0603	0.793	< 0.0001		
Lymphocytes	(%)	0.269	0.2801	0.289	0.2445	0.224	0.3726		
	(cells/mL)	0.076	0.7644	0.384	0.1158	0.636	0.0045		
Neutrophils	(%)	0.408	0.0930	0.241	0.3350	0.050	0.8453		
	(cells/mL)	0.075	0.7659	0.511	0.0302	0.782	< 0.0001		
Eosinophils	(%)	0.392	0.1080	0.557	0.0163	0.899	< 0.0001		
	(cells/mL)	0.392	0.1079	0.487	0.0406	0.831	< 0.0001		

Simple regression analysis was performed in two groups: sensitized and antigen-challenged guinea pigs (*n* = 9) and naïve animals (*n* = 9). *r* = regression coefficient.

or number of BAL cells as independent variables. This showed a significant contribution of eosinophil and neutrophil numbers, but not macrophage or lymphocyte numbers, to the cough response. The overall adjusted coefficient of multiple regression for prediction of capsaicin cough response by the cell numbers was 0.819 (*P* ≤ 0.0001) and the partial regression coefficient for eosinophils and neutrophils was 0.556 (*P* ≤ 0.0001) and 0.398 (*P* ≤ 0.0001), respectively. The same analysis for the percentage of BAL cells showed that the overall coefficient was 0.892 (*P* ≤ 0.0001) and only eosinophil percentage significantly contributed to the cough response (partial regression coefficient 0.831; *P* ≤ 0.0001).

Both azelastine and terfenadine dose-dependently reduced the number of coughs elicited by each concentration of capsaicin 24 h after antigen challenge in sensitized guinea pigs (Figs 3,4), but azelastine had no effect in naïve guinea pigs (Fig. 5).

DISCUSSION

In the present study, capsaicin was used to elicit coughing. Capsaicin is the active ingredient of red pepper and has been presumed to produce cough mainly by stimulating C-fiber endings.¹⁰⁻¹² However, because capsaicin also stimulates some rapidly adapting receptors with myelinated fibres, this view has been questioned.^{13,14} In the

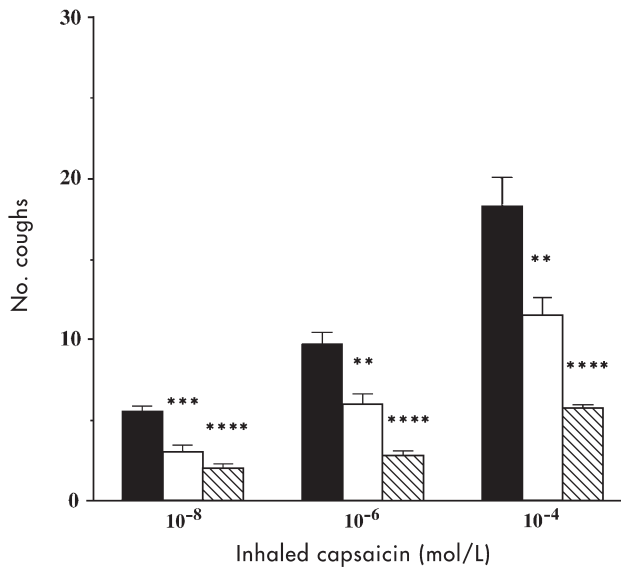


Fig. 3 Effect of azelastine on cough response to capsaicin 24 h after antigen challenge in actively sensitized guinea pigs. (□), 0.1 mg/kg azelastine; (▨), 1.0 mg/kg azelastine; (■), vehicle (saline). Each column represents the mean \pm SEM ($n = 6$). ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ compared with vehicle.

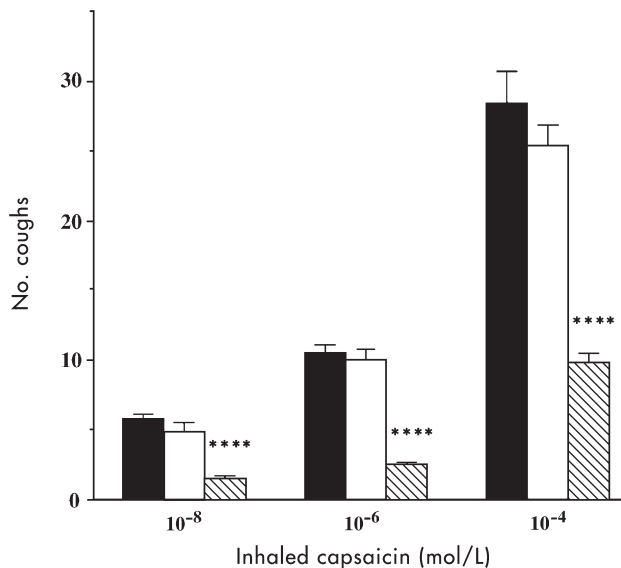


Fig. 4 Effect of terfenadine on cough response to capsaicin 24 h after antigen challenge in actively sensitized guinea pigs. (□), 0.2 mg/kg terfenadine; (▨), 2.0 mg/kg terfenadine; (■), vehicle (saline). Each column represents the mean \pm SEM ($n = 6$). **** $P < 0.0001$ compared with vehicle.

present study, capsaicin was used as a commonly used inducer of cough to examine overall cough sensitivity.

In the present study, the number of coughs induced by inhaled capsaicin was significantly increased 24 h after a

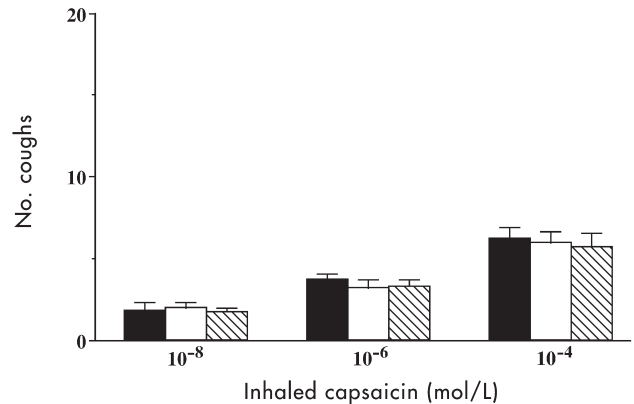


Fig. 5 Effect of azelastine on cough response to capsaicin in non-sensitized (naïve) guinea pigs. (□), 0.1 mg/kg azelastine; (▨), 1.0 mg/kg azelastine; (■), vehicle (saline). Each column represents the mean \pm SEM ($n = 6$).

single aerosolized antigen challenge in actively sensitized guinea pigs. The histamine H₁ receptor antagonists azelastine and terfenadine reduced the antigen-induced increase in cough response to inhaled capsaicin in sensitized guinea pigs, but azelastine had no effect on the cough response in naïve guinea pigs. Because it has been confirmed that the bronchodilators procaterol and atropine have no effect on antigen-induced increases in cough response (Q Liu, unpubl. data, 1998), the increase in cough response in the present study is thought to result from antigen-induced increases in cough receptor sensitivity in spite of bronchospasm.

Antihistamines have been shown to have an antitussive effect on atopic cough,¹⁻³ cough due to upper respiratory tract stimulation by post-nasal drip associated with the common cold¹⁵ and allergic cough.¹⁶ Although cough sensitivity is heightened in atopic cough,¹⁻³ but not in cough due to post-nasal drip,¹⁷ it has not been reported whether cough sensitivity is increased or not in allergic cough. Bolser *et al.*⁷ have shown that both loratadine and chlorpheniramine inhibit antigen-induced cough in actively sensitized guinea pigs and capsaicin-induced cough in naïve guinea pigs. They have also shown that salbutamol reduces both the allergic cough in sensitized animals and the capsaicin-induced cough in naïve animals, suggesting the antigen-induced cough may result from antigen-induced bronchoconstriction. The antitussive effects of loratadine and chlorpheniramine on capsaicin-induced cough are different from the result of the present study, namely that azelastine did not affect cough induced by capsaicin in naïve guinea pigs. Chlorpheniramine is a first-generation antihistamine

having sedative and anticholinergic activity, but loratadine, azelastine and terfenadine are second-generation antihistamines (histamine H₁ receptor antagonists) with little or no sedative or anticholinergic effects. Because anticholinergics have been shown to have antitussive effects on capsaicin-induced cough in naïve guinea pigs,⁷ it is likely that the antitussive effect of chlorpheniramine may be due to its anticholinergic activity. Although the exact reason for the discrepancy of the effects on capsaicin-induced cough in naïve guinea pigs between azelastine in the present study and loratadine in the study of Bolser *et al.* is not known, the results of the present study are in agreement with results of human studies. Choudry *et al.*¹⁸ have shown that prior inhalation of histamine does not effect capsaicin-induced cough in normal humans and Studham and Fuller¹⁹ have reported that oral terfenadine has no effect on cough elicited by capsaicin in normal volunteers. The latter results have been confirmed using azelastine (Q Liu, unpubl. data, 1998). It is possible that the dose of loratadine (10 mg/kg) used that inhibited capsaicin-induced cough in naïve guinea pigs was relatively high so that the antitussive effects of loratadine may have been due to unknown pharmacologic properties of the drug, other than histamine H₁ receptor antagonism. Accordingly, it is proposed that histamine H₁ receptor antagonists have no direct effect on capsaicin-induced cough in naïve guinea pigs as well as in normal humans. Thus, the inhibitory effect of azelastine and terfenadine on the increased cough sensitivity induced by antigen challenge is likely to be due to indirect effects of the drugs on cough receptor sensitivity. Because atropine did not alter the increased cough sensitivity induced by an airway allergic reaction in the same experimental system (Q Liu, unpubl. data, 1998), it is unlikely that the inhibitory effects of azelastine and terfenadine are due to their anticholinergic activity. Intrinsic histamine released by antigen challenge may potentiate airway cough sensitivity.

In the present study, the effect of azelastine or terfenadine on BAL cell components was not examined. Chand *et al.*²⁰ have clearly shown that aeroantigen-induced bronchial eosinophilia is not inhibited by terfenadine when it is administered 4 h after antigen challenge, whereas histamine H₁ receptor antagonists have been reported to suppress allergic eosinophilic infiltration in the BAL of guinea pigs when administered prophylactically. Accordingly, it is suggested that histamine H₁ receptor antagonists inhibit antigen-induced increases in cough receptor sensitivity via antagonism of the actions of histamine, which is released by airway

allergic reactions, without direct effect on airway inflammatory cell components.

In conclusion, because there has been no direct evidence for the involvement of histamine in increased cough sensitivity in humans or animal experiments, especially that induced by airway allergic reactions or eosinophilic inflammation, the effects of the histamine H₁ receptor antagonists azelastine and terfenadine on increased cough sensitivity induced by allergic reactions in actively sensitized guinea pigs were examined. Both histamine H₁ receptor antagonists dose-dependently reduced antigen-induced increases in cough sensitivity to capsaicin in sensitized guinea pigs, whereas azelastine had no effect on the cough response in naïve animals. These findings strongly suggest the involvement of intrinsic histamine in the heightened cough sensitivity induced by allergic reactions. Namely, histamine released by allergic reactions may sensitize airway cough receptors, but aerosolized capsaicin-induced cough may not be mediated by histamine release.

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