

Attenuating effect of H^+K^+ ATPase inhibitors on airway cough hypersensitivity induced by allergic airway inflammation in guinea-pigs

Y. Oribe, M. Fujimura, T. Kita, N. Katayama, M. Nishitsuji, J. Hara, S. Myou and S. Nakao

Respiratory Medicine, Cellular Transplantation Biology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan

Summary

Background Gastrooesophageal reflux (GER) is a frequent cause of chronic cough. Several investigators have indicated that inhibitors of H^+K^+ ATPase (proton pump inhibitors; PPIs) could relieve coughing via inhibition of acid reflux. However, we considered that PPIs might directly inhibit increased cough reflex sensitivity.

Objective The present study was designed to examine whether PPIs directly inhibit antigen-induced increase in cough reflex sensitivity and to elucidate the mechanism.

Methods Actively sensitized guinea-pigs were challenged with aerosol antigen (ovalbumin, OVA) and cough reflex sensitivity to inhaled capsaicin was measured 24 h later. The PPIs (omeprazole and rabeprazole) or the histamine H_2 blocker cimetidine were administered intraperitoneally 1 h before OVA challenge and before measuring cough reflex sensitivity, then bronchoalveolar lavage fluid (BALF) was immediately collected. The pH of the fluid obtained by bronchial washing was determined after examining the effect of rabeprazole on the cough response to capsaicin.

Results The number of coughs elicited by capsaicin was significantly increased 24 h after challenge with OVA compared with saline, indicating antigen-induced increase in cough reflex sensitivity. Both PPIs dose dependently and significantly inhibited antigen-induced cough hypersensitivity. Omeprazole did not influence the antigen-induced increase in the total number of cells or ratio (%) of eosinophils in BALF. Cimetidine did not affect the antigen-induced cough hypersensitivity or cellular components of BALF. The pH of the bronchial washing fluid was significantly decreased in antigen-challenged animals. Rabeprazole did not affect the antigen-induced decrease in the pH of bronchial washing fluid.

Conclusion These findings show that PPIs, but not histamine H_2 blockers, can directly decrease antigen-induced cough reflex hypersensitivity, while the mechanism remains unclear.

Keywords airway allergy, capsaicin, cough sensitivity, guinea-pig, pH, proton pump inhibitors

Submitted 5 January 2004; revised 6 September 2004; accepted 23 November 2004

Introduction

Atopic cough, cough variant asthma, eosinophilic bronchitis without asthma and the cough induced by gastrooesophageal reflux (GER) are frequent causes of chronic cough [1–3]. Among these, GER-induced cough is one of the most common causes of recurrent cough in the United States and in Europe [3], whereas it is rare in Japan [1, 4].

Two theories have been proposed to explain the pathogenesis of chronic cough caused by GER, namely microaspiration to the airways (reflux theory) and vagally mediated distal oesophageal-tracheobronchial reflexes (reflex theory) [5, 6]. Upper gastrointestinal X-rays, oesophagoscopy,

manometry, and/or prolonged oesophageal pH monitoring can evaluate GER. The last of these is presently the most sensitive and specific strategy available for diagnosing GER-induced cough even when other clinical GER symptoms such as heartburn, chest pain and persistent dysphagia are silent and/or other laboratory parameters are normal [5, 7]. However, this procedure is relatively invasive and expensive, and cannot always be implemented. Thus, GER-induced cough is often evaluated solely based on the effectiveness of H^+K^+ ATPase inhibitors (proton pump inhibitors; PPIs), which are believed to improve cough caused by GER via inhibiting acid reflux. However, the possibility that PPIs can directly affect airway cough reflex sensitivity has not been evaluated.

The present study examined whether PPIs (omeprazole and rabeprazole) could inhibit the increase in cough reflex sensitivity induced by allergic eosinophilic airway inflammation in guinea-pigs.

Correspondence: Yoshitaka Oribe, Respiratory Medicine, Cellular Transplantation Biology, Kanazawa University Graduate School of Medicine, 13-1 Takara-machi, Kanazawa 920, Japan. E-mail: oribe@s8.dion.ne.jp

Methods

Animal sensitization

Male albino guinea-pigs (Hartley strain) obtained from Sankyo Laboratory Service (Toyama, Japan) were quarantined in the Animal Research Center of Kanazawa University for 1 week before the study. Guinea-pigs weighing 200–250 g were actively sensitized using a modification of the method described by Muraki et al. [8]. Briefly, guinea-pigs received an intraperitoneal injection of cyclophosphamide (30 mg/kg). Two days later, the animals were immunized with 2.0 mg ovalbumin (OVA) and 100 mg aluminium hydroxide [$Al(OH)_3$]. A booster injection of 10 µg OVA together with 100 mg of $Al(OH)_3$ was administered 3 weeks after the primary immunization.

Antigen challenge

Three weeks after the booster injection, the guinea-pigs received an intraperitoneal injection of procaterol hydrochloride (0.1 mg) to prevent antigen-induced lethal respiratory distress. Thirty minutes later, the animals were challenged for 90 s by nasal exposure to aerosolized OVA (10 mg/mL in saline) generated by a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA, USA) operated by compressed air at 5 L/min and 0.08 mL/min output.

Measurement of cough reflex sensitivity

We measured cough reflex sensitivity as described in our previous studies [9, 10]. Capsaicin (30.5 mg) was dissolved in Tween 80 (1 mL) followed by ethanol (1 mL) and physiological saline (8 mL) to prepare a 10^{-2} M stock solution that was stored at -20°C . This solution was further diluted to 10^{-6} and 10^{-4} M with physiological saline. Conscious guinea-pigs were placed in airtight transparent plastic boxes consisting of isolated head (volume 1600 mL) and body chambers. Pressure in the body chamber was recorded. Coughs were detected as a transient specific change in the pressure (a rapid inspiration followed by a rapid expiration). We visually monitored movements of the animals to disregard motion- and sneezing-related changes in pressure. Capsaicin-induced coughing was measured 24 h after antigen challenge. The animals inhaled capsaicin for 2 min delivered every 8 min by the nebulizer operated by compressed air at 1.6 L/min (Iwaki Air Pump AP-115AN, Iwaki Co. Ltd, Tokyo, Japan) with an output of 0.037 mL/min. We counted the number of coughs during 3 min period that consisted of 2 min capsaicin inhalation and subsequent 1 min observation periods.

Analysis of bronchoalveolar lavage cells

After analysing cough reflex sensitivity to capsaicin, the animals were anaesthetized using an intraperitoneal injection of sodium pentobarbitone (75 mg/kg) and placed in the supine position. Sterile saline (10 mL) was infused twice at 37°C through a polyethylene cannula (outside diameter, 2.5 mm; inside diameter, 2.1 mm) placed in the trachea and manually recovered by gentle aspiration with a disposable

syringe. The recovered bronchoalveolar lavage fluid (BALF) was immediately separated by centrifugation at 70 g for 8 min.

The supernatant was discarded and cell pellets were resuspended in 10 mL of RPMI-1640 (Grand Island Biological Co., Grand Island, NY, USA) and we determined the number of cells after cytocentrifugation (Cytospin 2, Shandon Southern Products Ltd, Cheshire, UK). Cells (per 300) were differentially counted after May Giemsa staining.

Effect of proton pump inhibitors (omeprazole, rabeprazole) and cimetidine on cough response to capsaicin

Omeprazole (0.2 or 2.0 mg/kg) dissolved in a mixture of aluminum hydroxide and polyethyleneglycol (6:4 ratio), rabeprazole (0.2 or 2.0 mg/kg) or cimetidine (1.0 or 10 mg/kg) dissolved in saline, or their respective vehicles were administered intraperitoneally 1 h before OVA challenge and capsaicin inhalation. We also examined the effect of omeprazole in naïve guinea-pigs under the same conditions. Negative (NC) and positive (PC) control groups received intraperitoneal vehicle and either inhaled saline or OVA, respectively.

Effects of proton pump inhibitor (rabeprazole) on pH of bronchial washing fluid

We examined the effect of rabeprazole on the cough response to capsaicin and then performed bronchial washing. Animals were anaesthetized with an intraperitoneal injection of sodium pentobarbitone (75 mg/kg) and placed in the supine position. Thereafter, 5 mL of sterile saline at 37°C was infused through a polyethylene tracheal cannula and then the fluid was immediately recovered by gentle aspiration as described above. The pH of the recovered fluid was immediately measured (Denver Instrument Model 215 pH meter, Asahi Technoglass Corporation, Tokyo, Japan).

Immunohistochemistry to detect H^+K^+ ATPase

We resected the trachea and stomach from guinea-pigs under deep anaesthesia and examined whether H^+K^+ ATPase is present in tracheal mucosa using the monoclonal antibody D031-3H (Medical & Biological Laboratories Co., Ltd, Nagoya, Japan) that reacts with the α -subunit of mouse H^+K^+ ATPase. The positive control was stained gastric mucosa.

The trachea and stomach were immediately fixed in 4% paraformaldehyde overnight, embedded in paraffin and examined by immunohistochemistry as follows. Deparaffinized sections were microwave-heated in citrate buffer (pH 6.0) for 20 min. Endogenous peroxidase was blocked, and then the sections were incubated in normal goat serum for 20 min, followed by D031-3H overnight at 4°C . The sections were incubated for 1 h at room temperature with goat anti-mouse immunoglobulins conjugated with peroxidase labelled polymer (Envision+, Dako, Glostrup, Denmark). The chromogen was 3,3'-diaminobenzidine tetrahydrochloride (DAB) and the sections were lightly counterstained with haematoxylin.

The primary antibody in negative controls was replaced with similarly diluted non-immunized mouse serum.

Statistical analysis

All data are shown as means $\pm$ standard error of the mean (SEM). Student's unpaired *t*-test determined statistical differences (StatView: SAS Institute, Cary, NC, USA). We established statistical significance at a *P*-value below 0.05.

Results

Effects of omeprazole on cough reflex sensitivity and bronchoalveolar lavage fluid cells

The number of coughs induced by 1.0×10^{-4} M capsaicin was significantly ($P < 0.01$) increased in the group challenged with antigen (PC group) compared with saline (NC group). Omeprazole significantly and dose dependently inhibited the

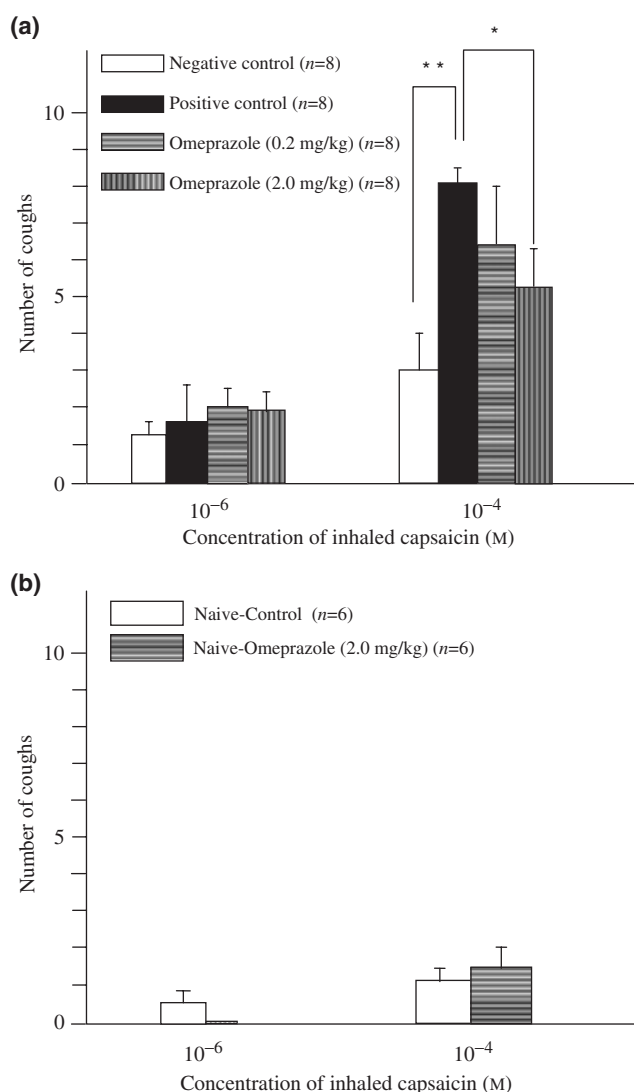


Fig. 1. Attenuating effect of omeprazole on increased cough reflex sensitivity to inhaled capsaicin 24 h after antigen challenge in sensitized guinea-pigs (a) but no influence on normal cough reflex sensitivity in non-sensitized (naive) guinea-pigs (b). ** $P < 0.01$, * $P < 0.05$ compared with positive control group. (a); $n = 8$ in each group, (b); $n = 6$ in each group. Negative control, saline inhalation in sensitized animals; positive control, antigen challenge in sensitized animals. Each bar indicates mean $\pm$ SEM.

antigen-induced increase in the number of coughs ($P < 0.05$) (Fig. 1a). The number of coughs induced by 1.0×10^{-6} M capsaicin did not significantly differ between the two groups (Fig. 1a). Omeprazole did not affect the number of capsaicin-induced coughs in naive guinea-pigs (Fig. 1b).

The total number of cells and the ratio of eosinophils in BALF from the PC group were significantly ($P < 0.05$ and $P < 0.01$, respectively) increased compared with those from the NC group and omeprazole influenced neither of these findings (Fig. 2).

Effects of cimetidine on cough reflex sensitivity and bronchoalveolar lavage fluid cells

Cimetidine did not inhibit the antigen-induced increase in the number of coughs elicited by 1.0×10^{-4} M capsaicin (Fig. 3).

The total number of cells and ratio of eosinophils were significantly ($P < 0.01$ and $P < 0.01$, respectively) increased in

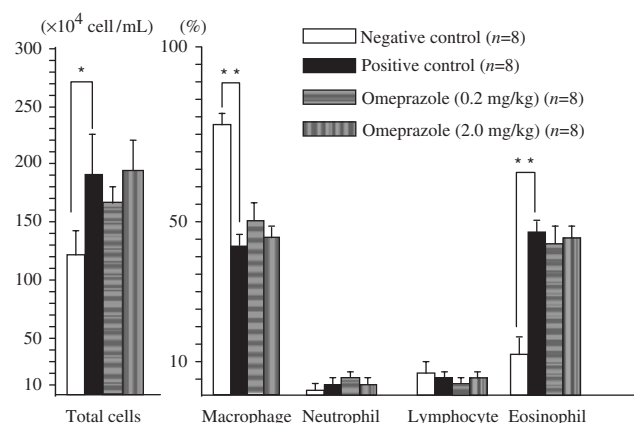


Fig. 2. No influence of omeprazole on changes in bronchoalveolar lavage cells 24 h after antigen challenge in sensitized guinea-pigs. ** $P < 0.01$, * $P < 0.05$ compared with positive control group. $n = 8$ in each group. Negative control, saline inhalation in sensitized animals; positive control, antigen challenge in sensitized animals. Each bar indicates mean $\pm$ SEM.

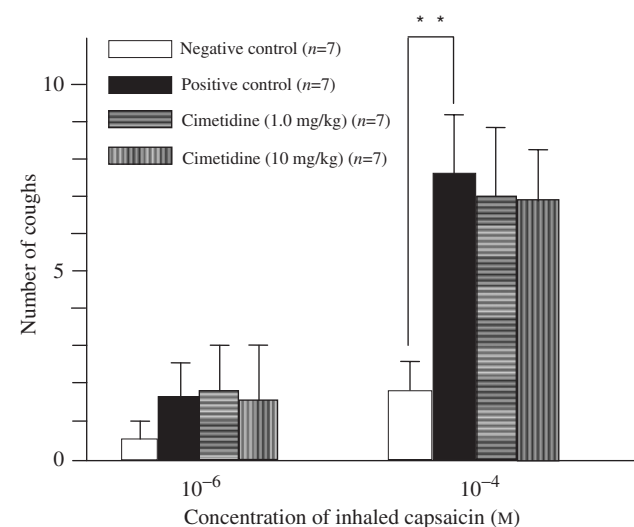


Fig. 3. No influence of cimetidine on increased cough reflex sensitivity to inhaled capsaicin 24 h after antigen challenge in sensitized guinea-pigs. ** $P < 0.01$ compared with positive control group. Negative control, saline inhalation in sensitized animals; positive control, antigen challenge in sensitized animals. $n = 7$ in each group. Each bar indicates mean $\pm$ SEM.

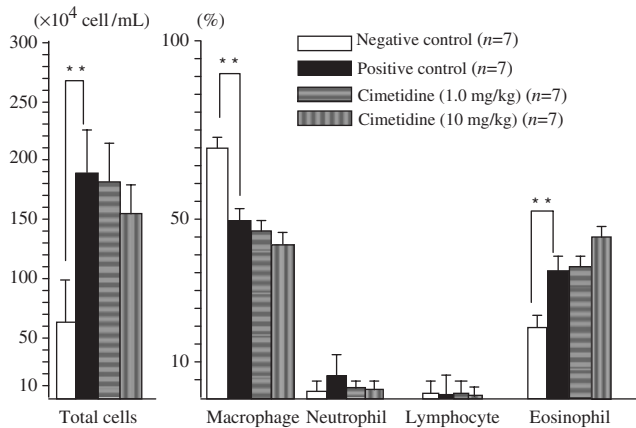


Fig. 4. No influence of cimetidine on changes in bronchoalveolar lavage cells 24 h after antigen challenge in sensitized guinea-pigs. $**P < 0.01$ compared with positive control group. $n = 7$ in each group. Negative control, saline inhalation in sensitized animals; positive control, antigen challenge in sensitized animals. Each bar indicates mean $\pm$ SEM.

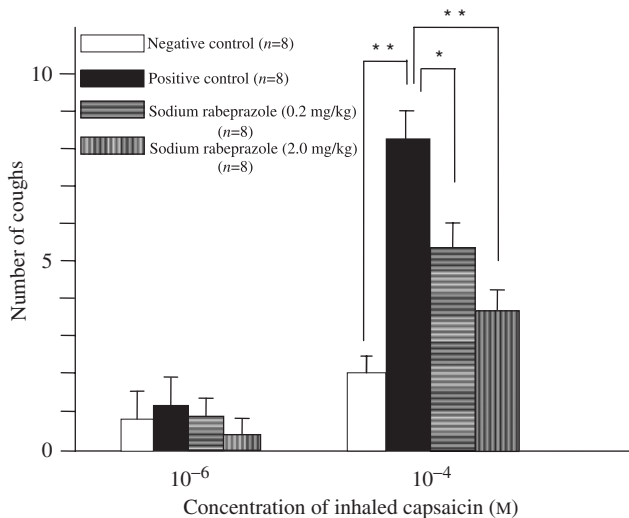


Fig. 5. Attenuating effect of rabeprazole on increased cough reflex sensitivity to inhaled capsaicin 24 h after antigen challenge in sensitized guinea-pigs. $**P < 0.01$, $*P < 0.05$ positive control group. $n = 8$ in each group. Negative control, saline inhalation in sensitized animals; positive control, antigen challenge in sensitized animals. Each bar indicates mean $\pm$ SEM.

BALF from the PC group, compared with the NC group, and cimetidine did not influence these values (Fig. 4).

Effects of rabeprazole on cough reflex sensitivity and pH of bronchial washing fluid

Rabeprazole significantly (2 mg/kg; $P < 0.01$, 0.2 mg/kg; $P < 0.05$) inhibited the antigen-induced increase in number of coughs elicited by 1.0×10^{-4} M capsaicin (Fig. 5).

We recovered an average of 3.2 mL of bronchial washing fluid, the pH of which was significantly decreased in the PC group, compared with the NC group ($P < 0.01$), and rabeprazole did not affect the antigen-induced decrease in the pH (Fig. 6).

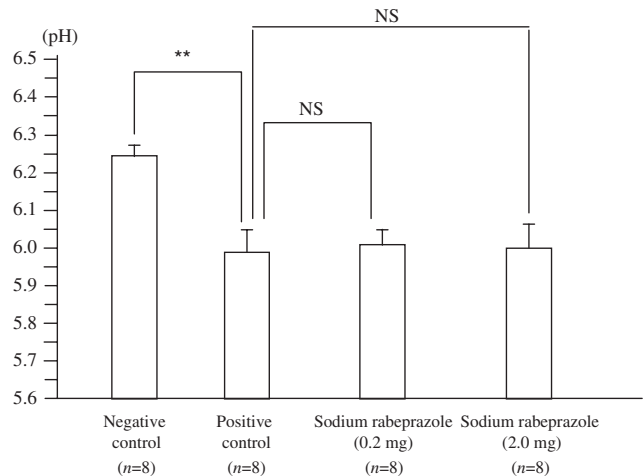


Fig. 6. No influence of rabeprazole on decreased pH of bronchial washing fluid 24 h after antigen challenge in sensitized guinea-pigs. $**P < 0.01$ positive control group. $n = 8$ in each group. Negative control, saline inhalation in sensitized animals; positive control, antigen challenge in sensitized animals. Each bar indicates mean $\pm$ SEM.

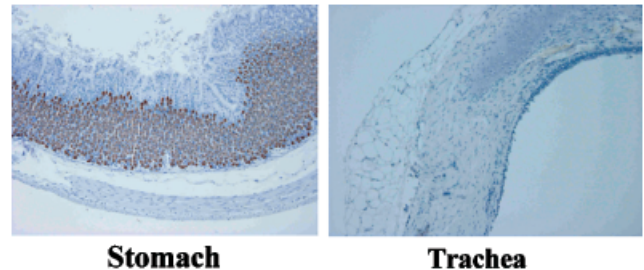


Fig. 7. Immunohistochemistry to detect gastric $H^+K^+ATPase$ in tracheal mucosa using D031-3H monoclonal antibody. Positive control consisted of stained gastric mucosa.

Immunohistochemistry to detect $H^+K^+ATPase$

D031-3H obviously stained the gastric, but not the tracheal mucosa (Fig. 7), indicating that the tracheal wall does not contain gastric $H^+K^+ATPase$.

Discussion

The present study showed that omeprazole inhibited the increase in cough reflex sensitivity to inhaled capsaicin that developed 24 h after antigen challenge in actively sensitized guinea-pigs. In contrast, omeprazole did not alter either the antigen-induced eosinophilic airway inflammation assessed by BALF cells from sensitized animals or normal cough sensitivity in non-sensitized (naïve) animals. Rabeprazole also reduced the antigen-induced increase in cough reflex sensitivity, but did not affect the antigen-induced decrease in pH of bronchial washing fluid. Cimetidine did not affect antigen-induced cough reflex hypersensitivity or eosinophil accumulation in BALF. Immunohistochemistry revealed the absence of gastric $H^+K^+ATPase$ in the tracheal walls of guinea-pigs.

Fass et al. [11] have reported that PPIs improve the digestive symptoms of GER within 1 week in over 80% of patients. On the other hand, Irwin et al. [3] found that medical therapy for 169–179 days is required to relieve GER-

induced cough. In addition, the frequency of GER-induced cough differs among countries, races and institutions. We therefore considered that PPIs might directly affect airway cough reflex sensitivity. The present study showed that PPIs can inhibit an antigen-induced increase in cough reflex sensitivity. So we are questioning whether the inhibition of gastric acid secretion is the true or sole mechanism by which PPIs inhibit cough. However, PPIs did not affect the increase in eosinophils in BALF or the decrease in pH of bronchial washing fluid 24 h after antigen challenge. Thus, the mechanism(s) underlying the inhibitory effect of PPIs on antigen-induced cough reflex hypersensitivity remains unclear.

McGarvey et al. [12] have reported that the ratio of eosinophils in BALF is increased in some patients with GER-induced cough, indicating that GER induces eosinophilic airway inflammation. However, such patients might have had an extant eosinophilic airway disorder such as atopic cough [1] or eosinophilic bronchitis without asthma [2] in addition to GER. In fact, although most commonly because of a single source (82%), chronic cough can also have dual causes (18%) [13, 14]. The present study suggests that the inhibitory effect of PPIs on cough reflex hypersensitivity associated with eosinophilic airway inflammation contributes to their effects in GER-induced cough complicated with eosinophilic airway inflammation.

The pH of bronchial washing fluid obtained from antigen-challenged animals were significantly lower than saline-challenged animals ($P < 0.01$ Fig. 6). Hunt et al. [15] have reported that the extracellular pH or pH of breathing condensate is substantially lower in asthmatics compared with normal individuals. They also reported that glutaminase activation may be relevant both to the regulation of airway pH and to the pathophysiology of acute asthmatic airway inflammation [16]. Reduced airway pH may cause airway epithelial dysfunction, eosinophil necrosis, decreased ciliary motility, abnormal airway mucous production, and nitric oxide generation [16–18]. Furthermore, airway pH reduction is caused to release neuropeptides, such as substance P and neurokinin A that induce bronchoconstriction, coughing and bronchial hyper-responsiveness [19]. The cause of the reduced pH of bronchial washing fluids in antigen challenged animals remains unclear. But abnormal airway mucus production or releasing of neuropeptides may contribute.

Protons and an increased temperature can activate afferent nerves by opening various ion channels including acid sensing channels and capsaicin receptors, which are in fact vanilloid receptors (VR1) [20–22]. Therefore, protons might contribute to inflammation-induced changes in afferent nerve activity (cough sensitivity) in the airway [22]. We surmised that PPIs directly affect the airway wall and reduce inflammation-induced cough reflex hypersensitivity by suppressing the production of protons in the presence of $H^+K^+ATPase$ in the airway tract. However, our results showed that gastric $H^+K^+ATPase$ is absent in the tracheal wall of guinea-pigs. In addition to $H^+K^+ATPase$, vacuolar $ATPase$ (V- $ATPase$) is also involved in proton transport across membranes to regulate pH. This enzyme is an oligomeric complex that is located in several intracellular organelles and PPIs inhibit this enzyme in the turtle bladder and in osteoclasts [23–25]. Thus, PPI-induced suppression of V- $ATPase$ activity might reduce

the antigen-induced increase in cough reflex sensitivity by inhibiting the decrease in the subcellular pH of afferent nerves.

Further studies are required to elucidate the mechanism through which PPIs inhibit antigen-induced cough reflex hypersensitivity. Measuring the intracellular pH and identifying other pharmacological activities of PPIs might clarify the mechanism.

In conclusion, this is the first report to suggest that PPIs can directly inhibit cough reflex hypersensitivity induced by airway allergies. Further studies are required to elucidate the mechanism through which PPIs inhibit antigen-induced cough reflex hypersensitivity. Measuring the intracellular pH and identifying other pharmacological activities of PPIs might clarify the mechanism.

Acknowledgement

This study was supported in part by a grant-in-aid for Scientific Research from the Japanese Ministry of Education, Science and Culture 14570546).

References

- 1 Fujimura M, Ogawa H, Nishizawa Y, Nishi K. Comparison of atopic cough with cough variant asthma: is atopic cough a precursor of asthma? *Thorax* 2003; 58:14–8.
- 2 Gibson PG, Fujimura M, Niimi A. Eosinophilic bronchitis: clinical manifestations and implications for treatment. *Thorax* 2002; 57:178–82.
- 3 Irwin RS, Boulet LP, Cloutier MM et al. Managing cough as a defense mechanism and as a symptom: a consensus panel report of the American College of Chest Physicians. *Chest* 1998; 114 (Suppl.):133S–81S.
- 4 Nishi K, Amemiya T, Mizuguchi M, Ooka T, Fujimura M, Matsuda T. A case of chronic persistent cough caused by gastroesophageal reflux. *Nihon Kyobu Shikkan Gakkai Zasshi* 1995; 33:652–9.
- 5 Irwin RS, French CL, Curley FJ et al. Chronic cough due to gastroesophageal reflux: clinical, diagnostic, and pathogenetic aspects. *Chest* 1993; 104:1511–7.
- 6 Ing AJ, Ngu MC, Breslin AB. Pathogenesis of chronic persistent cough associated with gastroesophageal reflux. *Am J Respir Crit Care Med* 1994; 149:160–7.
- 7 Poe RH, Kallay MC. Chronic cough and gastroesophageal reflux disease: experience with specific therapy for diagnosis and treatment. *Chest* 2003; 659–60.
- 8 Muraki M, Tohda Y, Sugihara R. The effect of TYB-2285 on dual phase bronchoconstriction and airway hypersensitivity in guinea pigs actively sensitized with ovalbumin. *J Pharm Pharmacol* 1994; 46:883–6.
- 9 Liu Q, Fujimura M, Tachibana H, Myou S, Kasahara K, Yasui M. Characterization of increased cough sensitivity after antigen challenge in guinea pigs. *Clin Exp Allergy* 2001; 31:474–84.
- 10 Myou S, Fujimura M, Kurashima K et al. Effect of suplatast tosilate, a new type of anti-allergic agent, on airway cough hypersensitivity induced by airway allergy in guinea-pigs. *Clin Exp Allergy* 2001; 31:1939–44.
- 11 Fass R, Ofman JJ, Sampliner RE, Camargo L, Wendel C, Fennerty MB. The omeprazole test is as sensitive as 24-hr oesophageal pH monitoring in diagnosing gastro-oesophageal reflux disease in symptomatic patients with erosive oesophagitis. *Aliment Pharmacol Ther* 2000; 389–96.

- 12 McGarvey LP, Forsythe P, Heaney LG, MacMahon J, Ennis M. Bronchoalveolar lavage findings in patients with chronic non-productive cough. *Eur Respir J* 1999; 13:59–65.
- 13 Irwin RS, Curley FJ, French CL. Chronic cough: the spectrum and frequency of causes, key components of the diagnostic evaluation, and outcome of special therapy. *Am Rev Respir Dis* 1990; 141: 640–7.
- 14 Ludviksdottir D, Bjornsson E, Janson C, Boman G. Habitual coughing and its associations with asthma, anxiety, and gastro-esophageal reflux. *Chest* 1996; 109:1262–8.
- 15 Hunt JF, Fang K, Malik R et al. Endogenous airway acidification. Implications for asthma pathophysiology. *Am J Respir Crit Care Med* 2000; 161:694–9.
- 16 Hunt JF, Erwin E, Palmer L, Vaughan J, Malhotra N, Platts-Mills TEA. Expression and activity of pH-regulatory glutaminase in the human airway epithelium. *Am J Respir Crit Care Med* 2002; 165:101–7.
- 17 Holma B, Lindegren M, Andersen JM. Ph effects on ciliomotility and morphology of respiratory mucosa. *Arch Environ Health* 1977; 32:216–26.
- 18 Holma B. Influence of buffer capacity and ph-dependent rheological properties of respiratory mucus on health effects due to acidic pollution. *Sci Total Environ* 1985; 41:101–23.
- 19 Ricciardolo FL, Rado V, Fabbri LM, Sterk PJ, Di Maria GU, Geppetti P. Bronchoconstriction induced by citric acid inhalation in guinea pigs. *Am J Respir Crit Care* 1999; 159: 557–62.
- 20 Tominaza M, Caterina MJ, Malmberg AB et al. The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 1998; 21:531–43.
- 21 Waldmann R, Champigny G, Lingueglia E, De Weille JR, Heurteaux C, Lazdunski M. $H(+)$ -gated cation channels. *Ann NY Acad Sci* 1999; 868:67–76.
- 22 Kollarik M, Undem BJ. Mechanisms of acid-induced activation of airway afferent nerve fibers in guinea-pigs. *J Physiol* 2002; 543: 591–600.
- 23 Mattsson JP, Vaananen K, Wallmark B, Lorentzon P. Omeprazole and bafilomycin, two proton pump inhibitors: differentiation of their effects on gastric, kidney and bone H^+ -translocating ATPases. *Biochim Biophys Acta* 1991; 1065:261–8.
- 24 Kohn OF, Mitchell PP, Steinmetz PR. Sch-28 080 inhibits bafilomycin-sensitive H^+ secretion in turtle bladder independently of luminal $[K^+]$. *Am J Physiol* 1993; 265:F174–9.
- 25 Rhoden KJ, Tallini G, Douglas JS. H^+K^+ ATPase inhibitors cause relaxation of guinea pig and human airway smooth muscle in vitro. *Pharmacol Exp Ther* 1996; 276:897–903.