



Continued inhalation of lidocaine suppresses antigen-induced airway hyperreactivity and airway inflammation in ovalbumin-sensitized guinea pigs

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Abstract

It is unclear whether inhaled lidocaine is effective against airway hyperreactivity and inflammation in asthma. The aim of this study was to investigate the effects of inhaled lidocaine on airway hyperreactivity and inflammation. Airway reactivity to inhaled histamine, cellular composition of bronchoalveolar lavage (BAL) fluid, plasma substance P (SP), and isolated lung tissue were evaluated in ovalbumin (OVA)-sensitized guinea pigs 7 days after OVA challenge. The effects of inhaled lidocaine on this model were also evaluated. Treatment with lidocaine was administered in two fashions: as single inhalation or inhalation bid for 7 consecutive days, for comparison with a saline-inhaled control group. Airway hyperreactivity to histamine, increase in number of total cells and increased proportion of eosinophils in BAL fluid, and marked eosinophil infiltration in airway walls were noted even 7 days after OVA challenge in the control group. Plasma SP level was also significantly increased. Although treatment with single lidocaine inhalation did not affect airway hyperreactivity, continued inhalation (bid for 7 days) attenuated airway hyperreactivity. Continued, but not single, inhalation of lidocaine also suppressed infiltration of eosinophils in BAL fluid and in airway walls. In addition, plasma SP levels were significantly reduced by continued but not by single inhalation. It appears possible that lidocaine when inhaled suppresses eosinophilic inflammation of the airway and SP-induced neurogenic inflammation, leading to alleviation of airway hyperreactivity.

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1. Introduction

Lidocaine is frequently used as a local anesthetic and anti-arrhythmic agent, and recent preliminary data have shown that lidocaine is beneficial for severe asthmatics. Some studies have reported that intravenous administration [1,2] or inhalation [3–5] of lidocaine is effective against bronchial asthma, though the usefulness of this agent for asthma is still controversial. In addition, inhalation of local anesthetic aerosol has been shown to be effective in markedly blocking vagal afferent fiber activity

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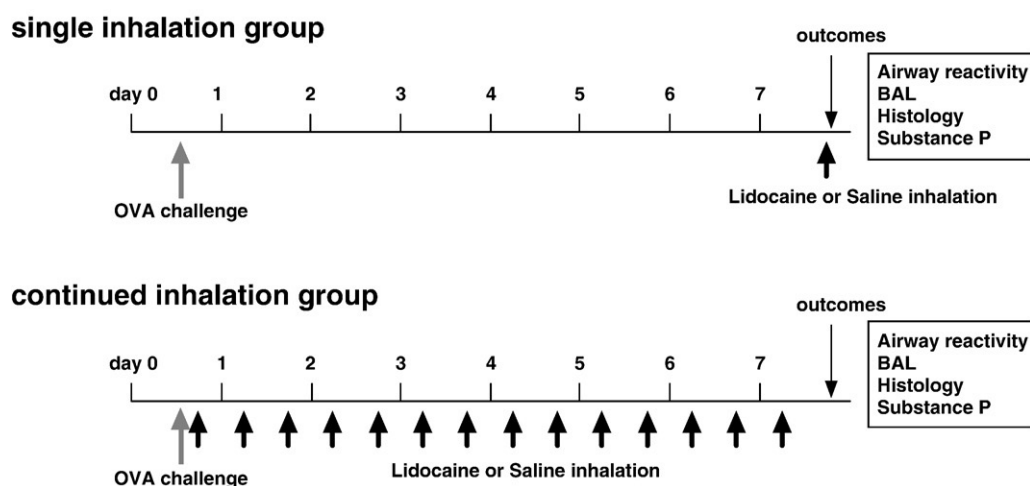


Figure 1 Experimental protocols after sensitization.

in animals and humans [6,7]. These recent findings suggest the possibility that local anesthesia with lidocaine in the airways improve airway hyperreactivity by inhibiting neurogenic inflammation, and thus can play a role in the treatment of asthma. Some *in vitro* studies have reported that lidocaine exhibits anti-inflammatory effects by blocking the activity of inflammatory cells such as eosinophils [8,9] and lymphocytes [10]. However, the *in vivo* effects of lidocaine on airway inflammation remain unclear. In this connection, several reports have cast doubt on the clinical efficacy of single intravenous administration [11,12] and inhalation of local anesthetics [13–15] in treating asthma.

In the present study, the effects of inhaled lidocaine on airway hyperreactivity were first evaluated in ovalbumin (OVA)-sensitized guinea pigs, with comparison between single and continued inhalation groups. Subsequently, the anti-inflammatory effects of lidocaine were evaluated by measuring the effects of lidocaine on infiltration of inflammatory cells in bronchoalveolar lavage (BAL) fluid, plasma levels of substance P (SP), and airway inflammation in isolated lung tissues.

2. Materials and methods

2.1. Animals and sensitization

Hartley male guinea pigs were sensitized with OVA (Sigma, St. Louis, MO) to obtain a model of asthma [16]. In brief, 4-week-old guinea pigs weighing 250 to 350 g were first intraperitoneally administered 30 mg/kg of cyclophosphamide (Shionogi & Co., Ltd. Osaka, Japan), then OVA 1 mg together with aluminum hydroxide [Al(OH)₃] (Wako Pure Chemical Industries, Ltd. Osaka, Japan) 100 mg 2 days later, and OVA 10 µg together with Al(OH)₃ 100 mg as a booster 3 weeks after sensitization with OVA. Subsequent experiments were carried out 4 weeks after administration of the booster.

Animal care and experiments were performed in accordance with guidelines for the care and use of laboratory animals of the Institutional Animal Experimentation Committee of Kinki University School of Medicine.

2.2. Challenge with OVA and treatment with lidocaine

The sensitized guinea pigs were challenged with 1 mg/ml of OVA delivered by inhalation with an ultrasonic nebulizer (Devilbis Model 646, Shizume Medical, Tokyo, Japan) for 1 min at 20:00 on day 0. The

animals were then randomly assigned to groups with single or continued inhalation of lidocaine (Fig. 1). The inhalation solution was prepared as 4% lidocaine (lidocaine hydrochloride: AstraZeneca, Osaka, Japan), which was inhaled for 6 min using an ultrasonic nebulizer in a cage for inhalation (with a capacity of 10 l). Animals in the single inhalation group inhaled lidocaine immediately before the airway reactivity experiments on day 7 after challenge with OVA. Animals in the continued inhalation group inhaled lidocaine once immediately after OVA challenge and twice daily (at 8:00 and 20:00) from the following day (day 1), 14 times in total. The experiments described below were carried out 12 h after the final inhalation of lidocaine. The control group was exposed to saline as a placebo. All animals were fasted for 1 h before and after inhalation of lidocaine solution or saline, without access to water.

2.3. Airway reactivity experiments

Airway reaction to inhaled histamine was measured in anesthetized guinea pigs under artificial respiration, with tidal air flow (10 ml/kg, 60 strokes/min) using a respirator for small animals (Harvard rodent ventilator Model 683: Harvard Apparatus, Holliston, MA) [17,18]. In brief, guinea pigs were intraperitoneally injected with pentobarbital sodium 40 mg/kg (Abbot Laboratories, Abbot Park, IL) for tracheostomy and intubation. The peak pressure at airway opening (peak Pao) was measured using a differential pressure transducer (TP-603T, Nihon Kohden, Tokyo, Japan). Then a small drug reservoir (8 ml) was applied to the built-in ultrasonic nebulizer (NE-10: Omron, Kyoto, Japan), and saline and histamine solutions (at concentrations of 4.9 µg/ml × 2ⁿ up to 159 µg/ml) were sequentially inhaled for 30 s each at intervals of 3 min to measure peak Pao.

2.4. Capsaicin-induced cough experiments

The method of Liu and Myou et al. [19,20] was partially modified to measure capsaicin inhalation-induced cough. Briefly, sensitized guinea pigs not challenged with OVA inhaled 4% lidocaine solution for 6 min, as described above in Section 2.2., and 20 min later, saline and capsaicin solutions (10⁻⁸, 10⁻⁶, 10⁻⁴ mol) for 2 min each using an ultrasonic nebulizer. The frequency of coughs was counted for periods of 2 min each during and after inhalation, for 4 min in total.

2.5. Determination of lidocaine concentration in serum

Blood was collected by cardiac injection 20 min after single inhalation of 4% lidocaine and 12 h after the final inhalation of lidocaine bid for 7

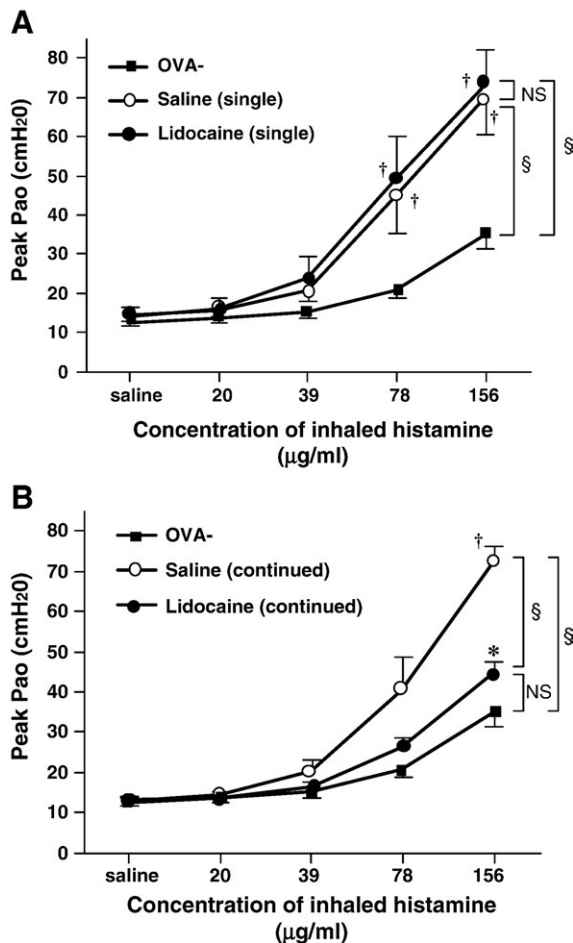


Figure 2 Effects of inhaled lidocaine on airway reactivity to histamine. A. Single inhalation of lidocaine did not affect airway hyperreactivity to histamine in guinea pigs with OVA challenge ($n=5$ per group; OVA-, sensitized guinea pigs without OVA challenge; †, $p<0.05$ vs. OVA-; NS, no significant difference; §, $p<0.05$ by two-way ANOVA). B. Continued inhalation of lidocaine bid for 7 consecutive days significantly suppressed airway reactivity to histamine in guinea pigs with OVA challenge. ($n=5$ per group; OVA-, sensitized guinea pigs without OVA challenge; †, $p<0.05$ vs. OVA-; *, $p<0.05$ vs. continued inhalation of saline; NS, no significant difference; §, $p<0.05$ by two-way ANOVA).

consecutive days. Serum concentration of lidocaine was determined by fluorescence polarization immunoassay (FPIA) [21].

2.6. Inflammatory cell count in BAL fluid

One week after exposure to OVA, BAL fluid was obtained to evaluate intra-airway inflammation. BAL was repeated 4 times with 5 ml/kg of saline by inserting a sonde just above the bifurcation of the trachea. The BAL fluid obtained was stained with 0.2% trypan blue solution (Wako Pure Chemical Industries, Ltd. Osaka, Japan) to determine total cell count. In addition, cell differential counts were performed using Diff Quik Stain (International Reagents Co., Kobe, Japan).

2.7. Histopathological evaluation of eosinophils

One week after exposure to OVA, the lungs were removed. The lungs were distended with 10% buffered formalin via the trachea (10 cm H₂O), and then immersed in fixative. After dehydration and embedding in

paraffin, histological sections (5 µm in thickness) were stained with hematoxylin–eosin or Giemsa. We evaluated eosinophil infiltrates around the airway (between the bronchial epithelium and adventitia) with a Flovel Image Filing System, Olympus Life Science Edition, Ver. 1.10 (Flovel Co., Tachikawa, Japan), by which cell counts and airway wall area could be calculated. A total of 10 fields per lung were randomly selected and analyzed in each group.

2.8. Determination of SP in plasma

In each animal in each group, one week after exposure to antigen, 5 ml of blood was drawn into a tube containing EDTA (1 mg/ml) and aprotinin (500 KIU/ml), and centrifuged. The plasma obtained was stored at -80°C until measurement. Plasma level of SP was measured using a Substance P RIA Kit (Peninsula Laboratories Inc. San Carlos, CA) with guinea pig antiserum.

2.9. Statistical analysis

All values are the mean $\pm$ SEM. Data were evaluated by Student's *t*-test. In the experiments on airway reactivity to histamine and capsaicin-induced cough, the interaction between dose and response curves was also evaluated by two-factor analysis of variance (ANOVA). In all statistical analyses, p values <0.05 were considered significant.

3. Results

3.1. Effects of inhaled lidocaine on airway hyperreactivity to histamine

The Pao prior to initiation of histamine-induced airway reactivity experiments did not differ significantly between any two of the saline single inhalation group, the lidocaine single inhalation group, the continued saline inhalation group, and the continued lidocaine inhalation group (13.44 ± 0.86 , 14.37 ± 1.89 , 13.59 ± 0.81 , and 12.01 ± 0.89 cm H₂O, respectively; $n=5$ per group). Airway constrictive reactivity to histamine inhalation was markedly increased in this guinea pig model even one week after challenge with OVA, compared to that in animals not challenged with OVA (Fig. 2A and B). In this study, treatment with single lidocaine inhalation did not suppress airway hyperreactivity to histamine, compared with the control group treated with saline inhalation (Fig. 2A). However, continued inhalation of lidocaine bid for 7 consecutive days significantly suppressed airway hyperreactivity at a concentration of 156 µg/ml (Fig. 2B). On the other hand, neither single nor continued inhalation of lidocaine affected airway reactivity in animals without OVA challenge (data not shown).

3.2. Effects of inhaled lidocaine on capsaicin-induced cough

The effects of inhaled lidocaine on capsaicin-induced cough were evaluated to confirm the local anesthetic effect of single inhalation of lidocaine (Fig. 3). The number of coughs induced by capsaicin inhalation was markedly reduced by lidocaine inhalation ($n=3$ per group), confirming that lidocaine acted as a local airway anesthetic.

3.3. Serum concentration of lidocaine

The serum concentration of lidocaine was 0.95 ± 0.13 µg/ml 20 min after single inhalation of 4% lidocaine solution ($n=4$). In addition, the lidocaine concentration 12 h after the final

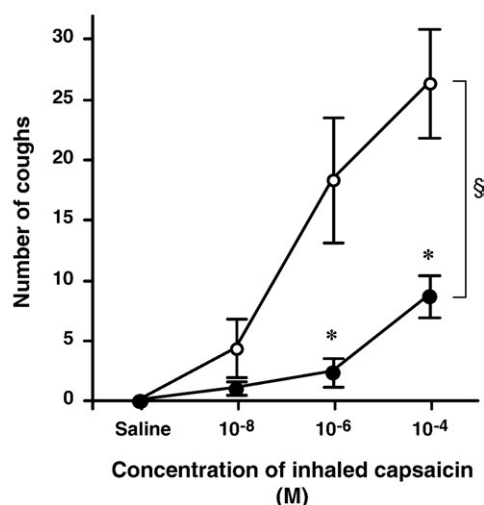


Figure 3 Effects of inhaled lidocaine on capsaicin-induced cough. The frequency of capsaicin-induced cough was significantly reduced after inhalation of 4% lidocaine. ($n=3$ per group; open circles, saline inhalation group; closed circles, lidocaine inhalation group; *, $p<0.05$; \$, $p<0.05$ by two-way ANOVA).

lidocaine inhalation bid for 7 consecutive days was below 0.1 $\mu\text{g}/\text{ml}$, the lower limit of measurement, in all of the 3 animals.

3.4. Inflammatory cells in BAL fluid

Intra-airway infiltration of inflammatory cells, particularly eosinophils, was markedly increased even one week after OVA challenge; both total number of cells and the proportion of eosinophils in BAL fluid were significantly increased in the control group, compared to the group without OVA challenge. Although single inhalation of lidocaine did not affect intra-airway inflammatory cells, continued inhalation decreased the total number of cells as well as the proportion of eosinophils (Fig. 4).

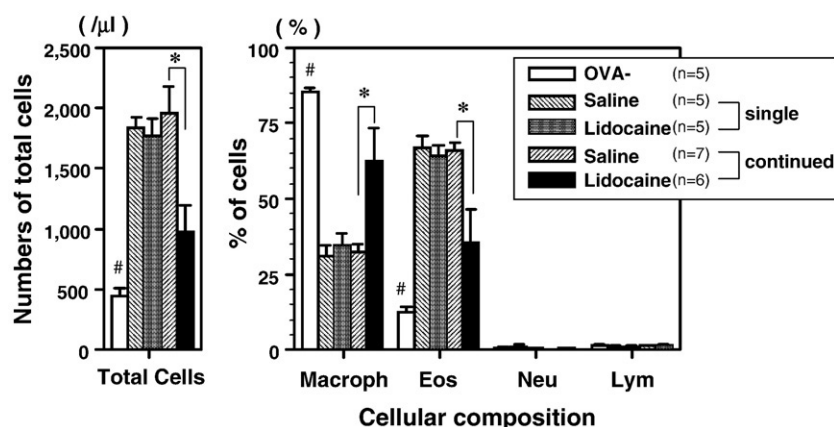


Figure 4 Effects of inhaled lidocaine on numbers of inflammatory cells in BAL fluid. Exposure to OVA significantly increased the number of total inflammatory cells in BAL fluid one week later, with relative increase in eosinophils and reduction in macrophages. Single inhalation of lidocaine affected neither total number of cells nor cellular composition. Continued inhalation of lidocaine significantly reduced total number of cells, with a relative decrease in eosinophils in BAL fluid. (OVA-, sensitized guinea pigs without OVA challenge; #, $p<0.05$ vs. single inhalation of saline or lidocaine and continued inhalation of saline; *, $p<0.05$ vs. continued inhalation of saline).

3.5. Histopathological evaluation of lungs

Histological findings and numbers of eosinophils in the airway walls are shown in Fig. 5 and Fig. 6, respectively. Marked eosinophil infiltration in the airway walls was observed 7 days after OVA challenge. Single inhalation of lidocaine did not affect infiltration of eosinophils in the airway walls. Although eosinophilic infiltration in the airway walls was also observed after continued inhalation of lidocaine, numbers of eosinophils were significantly less than in the control group treated with saline ($n=5$ per group).

3.6. Effects of inhaled lidocaine on plasma levels of SP

Plasma SP level was increased in guinea pigs challenged with OVA, compared to guinea pigs without OVA challenge. Plasma SP level was not significantly reduced after single inhalation of lidocaine, but was significantly reduced after continued inhalation of it (Fig. 7).

4. Discussion

Since asthma is defined as a chronic inflammatory disorder of the airways, anti-inflammatory agents are preferred for management of it. Corticosteroids are used as anti-inflammatory therapeutic agents, and inhaled steroids are first-line treatment for asthma. However, many asthma patients cannot be controlled with monotherapy with inhaled corticosteroids, and occasionally patients cannot be controlled even with systemic corticosteroids. Inflammation persists in severely symptomatic asthmatics despite treatment with high-dose corticosteroids, a finding which may be attributable to the severity of disease, corticosteroid treatment itself, or other factors yet to be identified [22]. Lidocaine is likely to be useful for treatment of asthma as adjuvant therapy, and a controlled study recently found that inhalation of lidocaine is effective in patients with asthma [23], although the reason for this has yet to be determined.

The cough reflex is probably elicited via airway C-fibers, and lidocaine inhibits transmission in sensory nerves and blocks the coughing induced by inhalation of capsaicin [24].

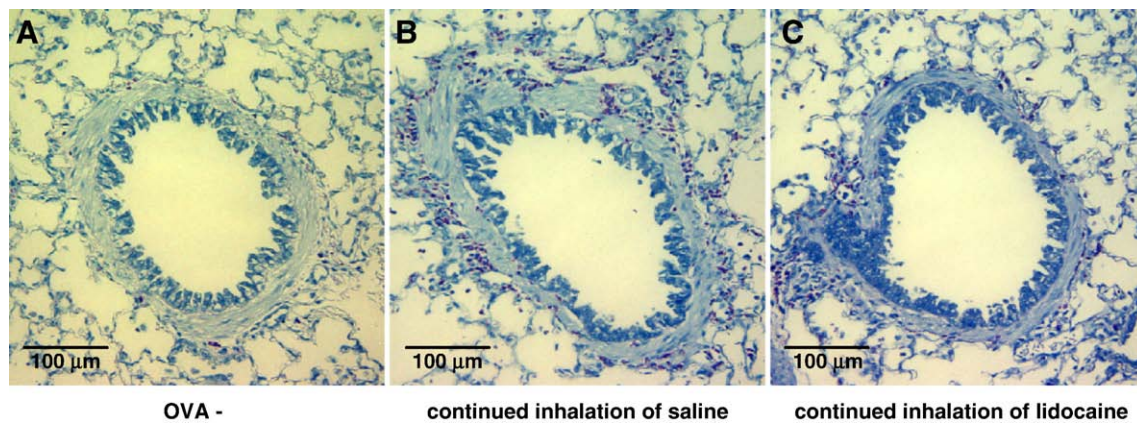


Figure 5 Histopathological findings. Eosinophils were present in small numbers in airway walls in sensitized guinea pigs without OVA challenge (A). Marked eosinophil infiltration in airway walls was observed in the control group treated with saline 7 days after OVA challenge (B). The eosinophil infiltration in the group treated with lidocaine bid for 7 consecutive days was mild, and less than that in the control group (C).

Since airway constriction in patients with asthma is believed to be partially mediated by the vagus nerves, local anesthetics may partially block airway constriction. In the present study, single inhalation of lidocaine appeared to produce a local anesthetic effect in the airways and thereby reduced capsaicin-induced cough. However, single inhalation of lidocaine did not affect airway hyperreactivity to histamine, suggesting that this local anesthetic did not itself attenuate airway hyperreactivity.

In this model, eosinophilic airway inflammation and airway hyperreactivity exist even one week after antigen challenge. We reached no definite conclusion as to whether eosinophils are responsible for airway hyperreactivity. However, it seems possible that eosinophils are one factor aggravating airway reactivity. Although single inhalation of lidocaine did not affect airway hyperreactivity, it was unclear how deep the inhaled lidocaine reached submucosally to suppress inflammation over a brief period of time. In the present study, the major reactions involved in histamine-induced airway constriction were related to submucosal reactions, especially smooth muscle constriction. Although it is possible that lidocaine did not reach deep into the airway smooth muscle after single inhalation, airway hyperreactivity was not suppressed significantly by inhaled lidocaine bid for 3 days (6 inhalations in total, with airway hyperreactivity tested immediately after the final inhalation) after challenge with OVA in a preliminary study (data not shown). This consecutive 3-day inhalation of lidocaine was also unable to significantly suppress eosinophilic airway inflammation. These findings suggest that airway inflammation including eosinophilic infiltration and SP release cannot be suppressed by short-term inhalation of lidocaine but can be suppressed by prolonged inhalation for one week or longer, and that prolonged inhalation leads to improvement of airway hyperreactivity. In addition, airway hyperreactivity was suppressed 12 h after the final inhalation of lidocaine following continued inhalation. It also appeared that lidocaine might have suppressed airway hyperreactivity via a mechanism not involving its local anesthetic effect.

Lidocaine is known to induce remission of experimental enema colitis and granulocyte migration in rats, in addition to its intrinsic effects as an anesthetic agent [25]. Lidocaine

is also known to inhibit eosinophil-activating cytokines and thereby shorten the time of survival of eosinophils [8], and to suppress eosinophilic superoxide production [9]. Lidocaine also inhibited migration of eosinophils *in vitro* [26]. Furthermore, since lidocaine can function as an immunomodulator of T cells, it has the potential for use as an anti-inflammatory agent [10]. Lidocaine has thus been observed to have various types of anti-inflammatory effects. The suppression of eosinophilic infiltration in the airway by lidocaine inhalation observed in the present study may have been due to suppression of eosinophil activation, induction of apoptosis of eosinophils, or anti-inflammatory effects via immunoregulatory effects on T cells. These anti-inflammatory effects may attenuate airway hyperreactivity.

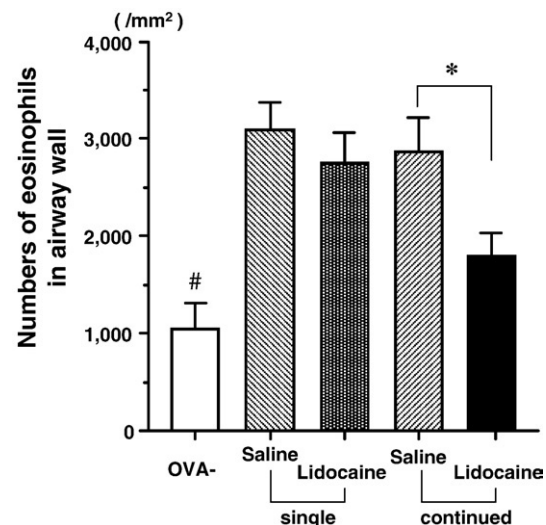


Figure 6 Numbers of eosinophils in airway walls. Eosinophils significantly infiltrated the airway walls after challenge with antigen. Although single inhalation of lidocaine did not suppress eosinophil infiltration in airway walls, continued inhalation significantly suppressed it ($n=5$ per group; OVA-, sensitized guinea pigs without OVA challenge; #, $p<0.05$ vs. single inhalation of saline or lidocaine and continued inhalation of saline; *, $p<0.05$ vs. continued inhalation of saline).

Tachykinins such as SP and neurokinin A in the nervous system of the airway are related to increase in airway secretion and vascular permeability and induction of smooth muscle contraction and coughing [27]. SP induces the release of chemical mediators from mast cells or basophils, such as leukotrienes and prostaglandins, which are likely to be involved in airway hyperreactivity [28]. SP may play an important role in airway constriction and various inflammatory changes in asthma [29]. It is thus possible that suppression of SP release affects the airway hyperreactivity induced by these mediators. Blood SP level is high in patients with asthma, and is correlated with both eosinophil count and degree of airway constriction [30]. Local anesthesia suppresses the dermal inflammatory reactions induced by SP [31,32], suggesting that lidocaine is involved not only in suppression of SP release but also in SP-induced inflammation. It is known that exposure to allergen increases SP concentration in the airway [33,34], and plasma SP level in our experimental model was high even one week after antigen challenge. The present findings suggested that local application of lidocaine suppressed intra-airway release of SP, resulting in reduction of blood level of SP. SP may be responsible for neurogenic inflammation, and thus contribute to aggravation of asthma. It appeared that lidocaine partially suppressed SP-induced inflammation in guinea pigs.

This study has several limitations. First, it did not include evaluation of the local anesthetic effect of lidocaine at 12 h after the last dose of it administered by continued inhalation. For this reason, we cannot rule out a local anesthetic effect of this drug 12 h after the last dose. Second, it was not possible in this study to perform a detailed analysis of the time course of change in plasma SP level or local airway SP level following exposure to antigen. Furthermore, no conclusion has yet been reached whether eosinophils are one of the factors responsible for airway hyperreactivity. However, our results suggest the possibility that long-term treatment with lidocaine suppresses eosinophilic airway inflammation associated with asthma and SP-induced neurogenic inflammation, leading to alleviation of airway hyperreactivity. It will be important to clarify the relationship between eosinophils and airway hyperreactivity and to elucidate the mechanism of involvement of SP in airway hyperreactivity.

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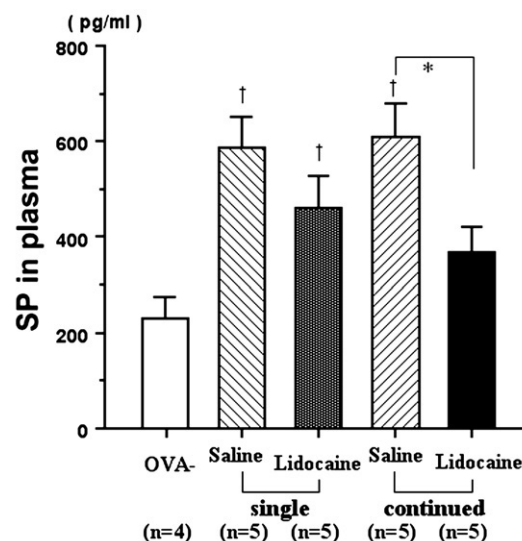


Figure 7 Effects of inhaled lidocaine on plasma SP levels. Plasma SP level was significantly increased one week after challenge with OVA. Plasma SP level was not significantly reduced after single inhalation of lidocaine, but was significantly reduced after continued inhalation of it. (OVA-, sensitized guinea pigs without OVA challenge; †, $p < 0.05$ vs. OVA-; *, $p < 0.05$ vs. continued inhalation of saline).

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