

Effects of inhaled aminophylline on airway constriction and inflammation in ovalbumin-sensitized guinea pigs

Masato Muraki¹, Shota Wada¹, Takeshi Ohno¹, Souichirou Hanada¹, Hirochiyo Sawaguchi¹, Takashi Iwanaga², Hiroaki Kume², and Yuji Tohda²

¹Department of Respiratory Medicine and Allergology, Nara Hospital, Kinki University Faculty of Medicine, Ikoma, Japan and ²Department of Respiratory Medicine and Allergology, Kinki University School of Medicine, Osakasayama, Japan

Abstract

Background: The systemic administration of theophylline is useful for asthma treatment. However its narrow therapeutic range makes it difficult to use. Little is known about its potential in inhalation therapy, particularly repeated inhalation.

Objective: The purpose of this study is to investigate the therapeutic usefulness of inhaled aminophylline in an asthma model.

Methods: The effects of pretreatment with inhaled aminophylline (25 mg/mL for 30 min/dose) on airway response and inflammation after an ovalbumin (OVA) challenge and airway hypersensitivity to acetylcholine (ACh) were evaluated using guinea pigs sensitized with OVA. **Results:** Aminophylline relaxed the ACh-induced contraction of tracheal smooth muscle *in vitro* in a concentration-dependent manner. Pretreatment with single-dose aminophylline inhalation suppressed OVA-induced airway constriction to the same extent as the intraperitoneal pretreatment with high-dose aminophylline (10–20 mg/kg). However, pretreatment with single-dose aminophylline inhalation did not suppress eosinophil infiltration into airways (neither bronchoalveolar lavage [BAL] fluid nor lung tissue) and did not suppress airway hyperreactivity to ACh, 24 h after OVA challenge. Repeated inhalation of aminophylline (twice daily for 7 days) suppressed the infiltration of eosinophils and suppressed airway hypersensitivity to ACh. In addition, high concentrations of aminophylline inhibited production of oxygen radicals by BAL cells.

Conclusion: Single-dose inhalation treatment with aminophylline has transient but relatively strong bronchodilating effects due to delivery of high doses into local airways. Repeated inhalation treatment suppressed airway inflammation and hypersensitivity induced by allergens. Therefore, inhaled aminophylline may be useful for asthma treatment.

Keywords

Airway hyperreactivity, airway inflammation, asthma, inhalation, theophylline

History

Received 25 July 2013

Revised 15 September 2013

Accepted 15 September 2013

Introduction

Theophylline and related xanthines have been used in the treatment of airway diseases for over 50 years and remain commonly prescribed worldwide for the treatment of bronchial asthma (Persson, 1986). However, data on the relative efficacy of theophylline as a long-term controller are lacking (Global Initiative for Asthma, 2012). Furthermore, adverse effects of theophylline, particularly at higher doses (10 mg/kg body weight/day or more), are significant and reduce its usefulness (Global Initiative for Asthma, 2012). Although systemic administration of high-dose theophylline may have a stronger bronchodilating effect, its narrow therapeutic range makes it difficult to use.

In fact, we have experienced two cases of theophylline overdose. Both patients had severe, uncontrolled asthma who experienced persistent wheezing despite taking high-dose

inhaled corticosteroids, long-acting inhaled β_2 -agonists, a leukotriene modifier, oral theophylline and frequent courses of short-term systemic glucocorticoids. When one of these patients took an additional dose of theophylline (blood theophylline concentration, 59.8 $\mu\text{g/mL}$), her refractory chronic wheezing completely disappeared but was accompanied by severe adverse effects including nausea, vomiting, fatigue, palpitations and tachycardia. In the other case, the patient's blood concentration of theophylline became excessive (28 $\mu\text{g/mL}$) owing to the influence of concomitant quinolone. Her peak expiratory flow was over 20% above the usual level but was accompanied by nausea and fatigue.

Asthma treatment can be administered in different ways – inhaled, orally or parenterally (by subcutaneous, intramuscular or intravenous injection). The major advantage of inhaled therapy is that the drugs are delivered directly into the airways, resulting in faster action and higher local concentrations with significantly less risk of systemic adverse effects. Theophylline is usually not administered as an inhaled medication. The use of inhaled theophylline for asthma treatment would be expected to have higher efficacy, as it would deliver higher airway concentrations and produce

Address for correspondence: Masato Muraki, Department of Respiratory Medicine and Allergology, Nara Hospital, Kinki University Faculty of Medicine, 1248-1 Otsoda-Cho, Ikoma, Nara 630-0298, Japan. Tel: +81(0)743 77 0880. Fax: +81 (0)743 77 0890. Email: muraki@nara.med.kindai.ac.jp

fewer systemic adverse effects. However, there are no reports describing the effects of repeated inhaled administration of theophylline. The aim of this study is to investigate the potential use of theophylline as inhaled medication for: (1) the prevention of acute asthma exacerbation and (2) the control of chronic asthma, particularly as an anti-inflammatory medication. Theophylline (using aminophylline) on ovalbumin (OVA)-sensitized guinea pigs were studied with respect to the following parameters: (1) acetylcholine (ACh)-induced airway contraction *in vitro*, (2) airway constriction by OVA challenge, (3) airway hyperreactivity to ACh after OVA challenge, (4) airway inflammation after OVA challenge (evaluation of inflammatory cells in the bronchoalveolar lavage [BAL] fluid and airway tissues) and (5) reactive oxygen species (ROS) production by BAL cells.

Materials and methods

Animals and sensitization

Male Hartley guinea pigs were sensitized with OVA (Sigma, St. Louis, MO) to generate an asthma model (Muraki et al., 2011). In brief, 4-week-old guinea pigs weighing 250–350 g were intraperitoneally administered the following: (1) 30 mg/kg of cyclophosphamide (Shionogi & Co., Ltd. Osaka, Japan); (2) 2 days later, 1 mg of OVA together with 100 mg aluminum hydroxide [Al(OH)₃] (Wako Pure Chemical Industries, Ltd. Osaka, Japan) and (3) 10 µg of OVA with 100 mg Al(OH)₃ as a booster, 3 weeks after the initial sensitization with OVA. Subsequent experiments were carried out 4 weeks after booster administration.

Animal care and experiments were performed in accordance with the guidelines for the care and use of laboratory animals of the Institutional Animal Experimentation Committee of Kinki University School of Medicine and The Code of Ethics of the World Medical Association (Declaration of Helsinki), respectively.

Tracheal tissue preparation and tension records

The experiments were performed as previously described, (Tsukiyama et al., 2007) with slight modifications. In brief, guinea pigs ($n=5-7$) were deeply anesthetized by intraperitoneal injection of pentobarbital sodium (70 mg/kg), and the tracheae were isolated and immersed in a bathing solution (118 mM NaCl, 4.6 mM KCl, 1.1 mM MgSO₄, 11.1 mM glucose, 0.9 mM KH₂PO₄, 25 mM NaHCO₃, 1.8 mM CaCl₂ and 2 µM indomethacin). The tracheae were carefully cleaned of unnecessary adipose and connective tissues and cut into transverse strips of two adjacent cartilage rings (3 mm wide).

Each tracheal ring preparation was mounted vertically in an organ bath (made by the Kinki University School of Medicine) to measure the tension isometrically at a resting tension of 1 g. The bath containing the above solution was maintained at 37 °C and gassed with 95% O₂/5% CO₂. The isometric tension was measured with a force displacement transducer (RMP-6008, Nihon Kohden, Tokyo, Japan). The tracheal ring preparations were allowed to equilibrate for at least 60 min before starting the experiments. At the end of equilibration, the ring preparation was contracted with 5 µM ACh. After 60 min, the inhibitory action of aminophylline

(aminophylline hydrate; Eisai Co. Ltd., Tokyo, Japan) against the ACh-induced contraction was examined. To obtain concentration–response curves, aminophylline (range, 2.5–147 µg/mL) or saline (as control) were added to the organ bath at intervals of 20 min. At the end of the experiments, isoproterenol (10 µM; Sigma, Tokyo, Japan) was added; the percent relaxation by aminophylline was then calculated using the relaxation by isoproterenol as 100% (Gallos et al., 2008).

Treatment with inhaled or intraperitoneal aminophylline

The sensitized animals were randomly assigned to groups receiving inhaled aminophylline or inhaled saline as control. These groups were further divided into two groups: single and long-term administration (six animals per group). Other animals were treated with intraperitoneal injections of aminophylline.

Animals in the inhalation groups inhaled aminophylline (25 mg/mL) using an ultrasonic nebulizer (Devilbiss Model 646, Shizume Medical, Tokyo, Japan) for 30 min in an inhalation cage (capacity, 10 L). Animals in the single administration group were treated only once, while those in the long-term administration group inhaled twice daily from day 0 to day 6, 14 times in total.

Animals in the intraperitoneal administration group were treated once with 5, 10 or 20 mg/kg of aminophylline ($n=6$, for each dose group).

Airway reactivity to OVA or ACh

Airway reactivity was determined by monitoring enhanced pause (Penh) units obtained from a whole body plethysmograph (BioSystem XA; Buxco Electronics, Suita, Japan) that measures respiratory function in unrestrained animals (Muraki et al., 2011). Guinea pigs were individually placed into the body plethysmograph for data collection.

In the antigen challenge test, animals (six per group) were forced to inhale OVA (1 mg/mL) for 1 min by using a nebulizer. To confirm the onset of a bronchial response, Penh was monitored before and at 1, 3, 5 and 30 min and 1 h after the inhalation. The antigen challenge was performed 5 min after aminophylline inhalation. In preliminary experiments, the blood levels of theophylline were at their peak at 5–20 min after inhalation and began a gradual decline at 30 min after inhalation (data not shown). Therefore, it was thought that inhaled aminophylline would show good efficacy at 5–30 min after inhalation. In a second test, the antigen challenge was performed 15 min after intraperitoneal administration of aminophylline because the peak blood concentration of theophylline occurred 10–20 min after administration (data not shown).

In airway reactivity tests to ACh, saline and ACh solutions (at concentrations of 39.0625 µg/mL $\times 2^n$ up to 625 µg/mL) were also sequentially inhaled for 1 min at intervals of 4 min to measure Penh. To investigate the suppressive effects on airway hyperreactivity via anti-inflammatory actions and not merely the bronchodilation effects, these tests were performed 24 h after final aminophylline inhalation (in both the single and long-term inhalation groups).

Measurement of blood concentrations of theophylline

After aminophylline inhalation for 30 min, guinea pigs ($n=3$) were deeply anesthetized by intraperitoneal injection of pentobarbital sodium (70 mg/kg) as described earlier. Blood samples were collected 5 min after aminophylline inhalation. Serum level of theophylline was measured using Dimension[®] clinical chemistry system, Flex cartridge theophylline THEO (Siemens Healthcare Diagnostics inc., Tokyo, Japan). Guinea pigs ($n=3$ each) were anesthetized 10 min after intraperitoneal injection with aminophylline and blood samples were collected 15 min after the administration.

Bronchoalveolar lavage and BAL cells

Bronchoalveolar lavage was performed on animals anesthetized by intraperitoneal injection of pentobarbital sodium ($n=6$). Briefly, after opening the trachea, a small cannula was inserted and BAL was performed four times using saline (5 mL/kg). Samson's staining solution (Muto Chemicals, Osaka, Japan) was added to aliquots of BAL fluid and the cells were counted. Other aliquots were stained with Diff-Quik stain (International Reagents corp., Kobe, Japan) to identify the various cell types (Muraki et al., 2011).

Histopathological evaluation of eosinophils

About 24 h or 7 days after exposure to OVA, the lungs were removed, distended with 10% buffered formalin via the trachea (10 cmH₂O) and immersed in a fixative. After dehydration and embedding in paraffin, histological sections (5 μ m 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) to count cells and calculate airway wall areas. A total of 10 fields per lung were randomly selected and analyzed in each group ($n=5$).

ROS production by BAL cells

Bronchoalveolar lavage was performed in sensitized guinea pigs ($n=4$) as described earlier and then airway-infiltrated cells were resuspended in Hank's balanced salt solution (HBSS, Whittaker Bioproducts, Walkersville, MD) at a concentration of 2×10^6 cells/mL and were used as BAL cells. Aminophylline solution was prepared at a final concentration of 5, 10, 20 or 40 μ g/mL. BAL cells (80 μ L) were incubated with 20 μ L of aminophylline or control solution (HBSS) at 37 °C for 10 min, followed by the addition of luminol solution (50 μ L, 2×10^{-4} M) (Futaba Medical Inc., Tokyo, Japan). BAL cells were stimulated with calcium ionophore A23187 (25 μ g/mL) (Sigma, St. Louis, MO). The ROS production by BAL cells stimulated with A23187 was measured by the luminol-dependent chemiluminescence (LDCL) method using a lumiphotometer (TD-4000: Lab Science Co. Ltd. Tokyo, Japan). The peak and integral values of LDCL were calculated by measuring LDCL at 37 °C for 10 min (Muraki et al., 1994).

Statistical analysis

All values are the mean $\pm$ SEM. Statistical analysis was performed using Student's *t*-test with the statistical software JMP ver. 10.0.2 (SAS Institute Japan Inc., Tokyo, Japan). Differences of 0.05 or less were considered statistically significant.

Results

Action of aminophylline against ACh-induced contraction in isolated trachea

Aminophylline relaxed the tracheal smooth muscle contraction induced by 5 μ M ACh in a concentration-dependent manner ($p<0.05$) (Figure 1). The relaxation was marked at higher concentrations (≥ 20 μ g/mL).

Action of inhaled or intraperitoneally administered aminophylline against airway constriction induced by OVA challenge

Penh (an indicator of airway narrowing) immediately and rapidly rose after OVA inhalation and reached the maximum level at 3–5 min in the control group. Thereafter, Penh gradually decreased. The pretreatment of inhaled aminophylline significantly suppressed the increase in Penh (Figure 2). In contrast, intraperitoneal administration of 5 mg/kg aminophylline did not suppress the Penh increase. Intraperitoneal administration of 10 mg aminophylline significantly suppressed the increase in Penh at 5 min after OVA inhalation ($p<0.05$); 20 mg/kg aminophylline markedly suppressed the Penh increase (Figure 2). The suppressive effect of inhaled aminophylline was equivalent to that observed with intraperitoneal administration of 10–20 mg/kg.

Blood concentrations of theophylline

Serum levels of theophylline after inhalation and after intraperitoneal injection of 5, 10 and 20 mg/kg of theophylline are shown in Table 1.

Effects of inhaled aminophylline on airway hyperreactivity to ACh after OVA challenge

The ACh-induced airway constriction observed 24 h after OVA challenge (Figure 3, control [single]) was accelerated compared to that in guinea pigs not challenged with OVA ($p<0.05$). Single inhalation of aminophylline did not suppress the airway hyperreactivity to ACh observed after 24 h. Airway reactivity to ACh was significantly increased in these animals (Figure 3, control [repeated]; saline inhalation, twice daily for 7 days), even 7 days after challenge with OVA, compared to that in animals without OVA challenge ($p<0.05$). However, repeated inhalation of aminophylline twice daily for seven consecutive days significantly suppressed airway hyperreactivity compared to controls at ACh concentrations of 156 and 313 μ g/mL, even 24 h after the final inhalation ($p<0.05$).

Effects of inhaled aminophylline on inflammatory cells in BAL fluid

The intra-airway infiltration of inflammatory cells, particularly eosinophils, was markedly increased 24 h after OVA

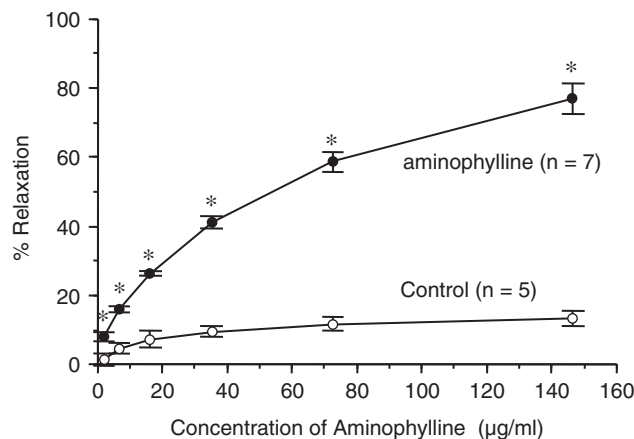


Figure 1. Effect of aminophylline on ACh-induced contraction in isolated trachea. Aminophylline relaxed the tracheal contraction induced by ACh in a concentration-dependent manner (* $p < 0.05$ vs. control).

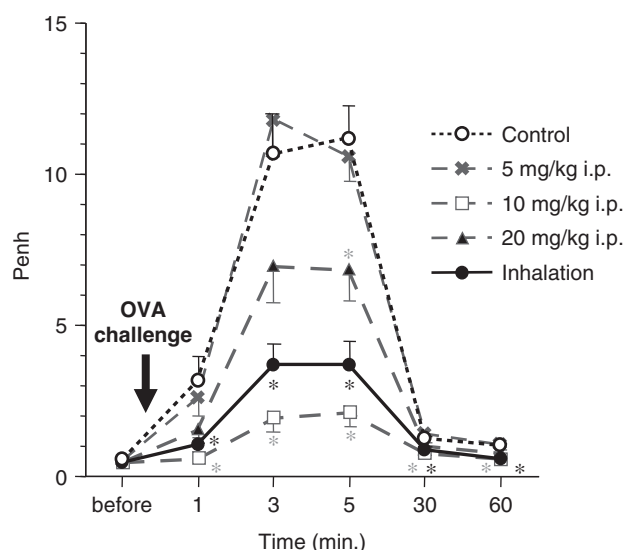


Figure 2. Airway response to OVA inhalation. Inhaled and higher concentrations of intraperitoneally injected aminophylline suppressed airway constriction induced by OVA challenge ($n = 6$ per group; * $p < 0.05$ versus control).

Table 1. Serum levels of theophylline (three per group).

Aminophylline	Blood concentration (µg/mL)
Inhaled	3.47 ± 0.29
5 mg/kg i.p.	8.50 ± 0.42
10 mg/kg i.p.	22.70 ± 2.04
20 mg/kg i.p.	45.17 ± 3.07

challenge; both the total number of cells and the proportion of eosinophils in BAL fluid were significantly increased in the control group (Figure 4, control [single]). Pretreatment using a single inhalation of aminophylline did not lower the total number of cells or decrease the proportion of eosinophils present in the infiltrate 24 h after OVA challenge.

In another control group (single OVA challenge and repeated saline inhalation *b.i.d.* for seven consecutive days), both the total number of cells and the proportion of eosinophils in BAL fluid were also markedly increased, even 7 days after OVA challenge, compared to the group

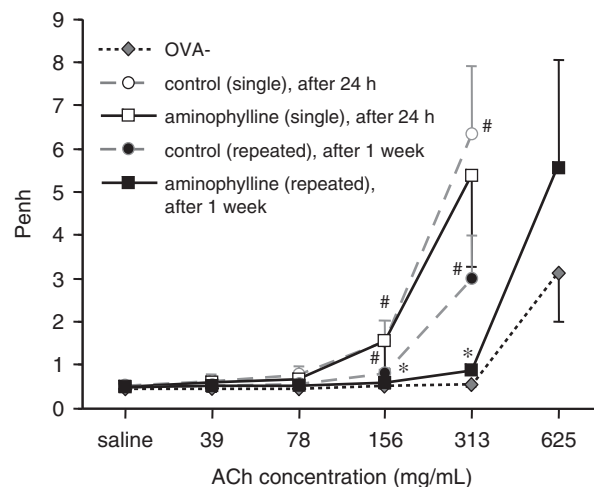


Figure 3. Effects of inhaled aminophylline on airway hyperreactivity to ACh 24 h or 1 week after OVA challenge. Pretreatment of single-dose inhaled aminophylline did not affect airway hyperreactivity to ACh at 24 h after OVA challenge. Repeated inhalation of aminophylline (twice daily for 7 days) suppressed airway hyperreactivity 1 week after OVA challenge (24 h after final inhalation). In the control group, there were no significant differences between 24 h and 1 week after OVA challenge. $n = 6$ per group; single, single-dose inhalation; repeated, repeated inhalation; after 24 h, 24 h after OVA challenge; after 1 week, 1 week after OVA challenge; * $p < 0.05$, versus control group in repeated inhalation with saline; # $p < 0.05$, control groups versus group without OVA challenge.

without OVA challenge (Figure 4, control [repeated]). Repeated inhalation of aminophylline decreased the total number of cells as well as the proportion of eosinophils 7 days after OVA challenge.

Histopathological evaluation of lungs

The numbers of eosinophils in the airway walls are shown in Figure 5. Marked eosinophil infiltration in the airway walls was observed 24 h after OVA challenge and was also observed 7 days after OVA challenge. Single-dose inhalation of aminophylline did not affect the infiltration of eosinophils in the airway walls observed at 24 h after OVA challenge. Although eosinophilic infiltrate in the airway walls was also observed after repeated inhalation of aminophylline, the number of eosinophils was significantly less than that of the saline-treated control group.

Effects of aminophylline on ROS production by BAL cells

Aminophylline inhibited both LDCL peaks and areas in a concentration-dependent manner (Figure 6). There were significant differences at higher concentrations of aminophylline (≥ 20 µg/mL).

Discussion

The adverse effects of systemic theophylline, including gastrointestinal symptoms, loose stools, cardiac arrhythmias, seizures and even death, can be reduced by careful dose selection (Global Initiative for Asthma, 2012). However, the use of theophylline has decreased since the introduction of long-acting inhaled β_2 -agonists and leukotriene modifiers because of theophylline's narrow therapeutic range and weak

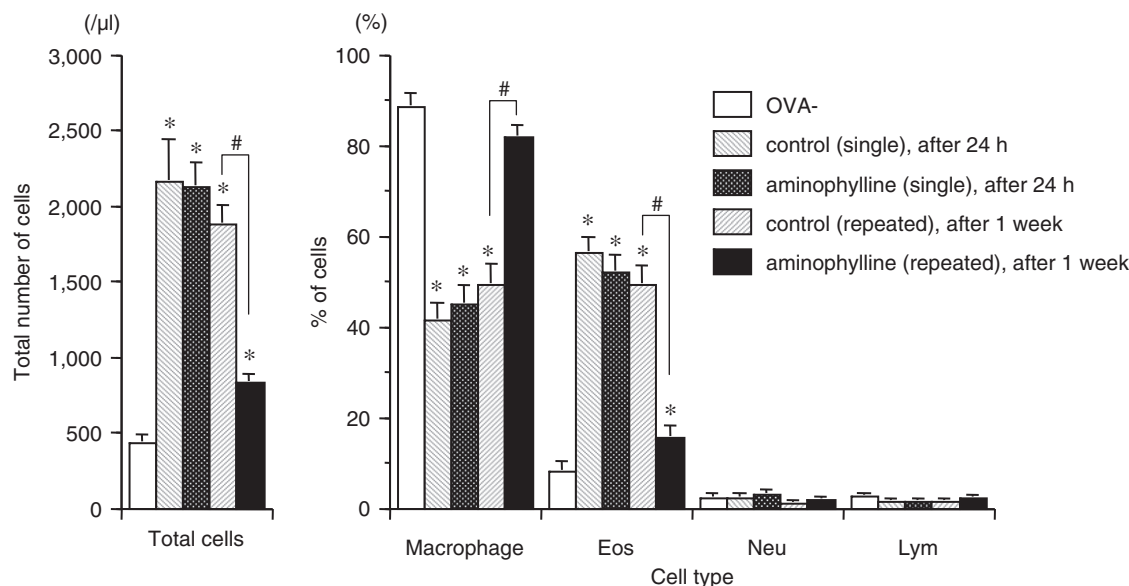


Figure 4. Effects of inhaled aminophylline on inflammatory cells in BAL fluid ($n=6$ per group; single, single inhalation; repeated, repeated inhalation; after 24 h, 24 h after OVA challenge; after 1 week, 1 week after OVA challenge; # $p<0.05$, repeated inhalation group with aminophylline versus control group in repeated inhalation with saline; * $p<0.05$, versus group without OVA challenge).

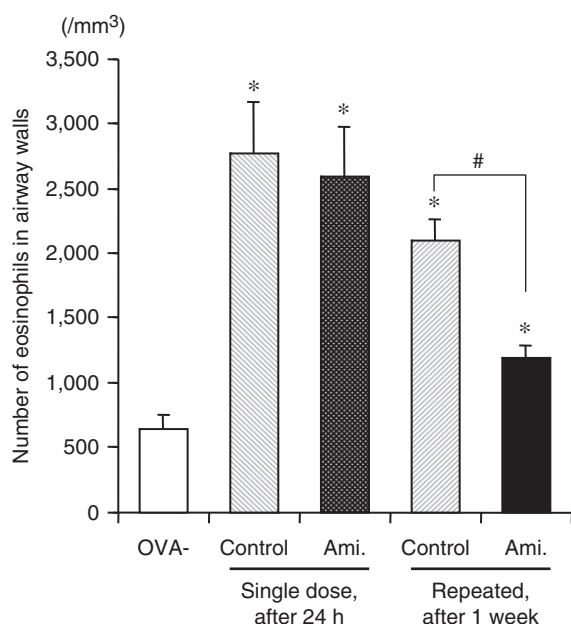


Figure 5. Numbers of eosinophils in the airway walls ($n=5$ per group; Ami, aminophylline; single, single inhalation; repeated, repeated inhalation; after 24 h, 24 h after OVA challenge; after 1 week, 1 week after OVA challenge; # $p<0.05$, repeated inhalation group with aminophylline versus control group in repeated inhalation with saline; * $p<0.05$, versus group without OVA challenge).

bronchodilating effects within the therapeutic range (Wieshammer et al., 2010). Bronchodilating doses of theophylline (10–20 $\mu\text{g/mL}$) are also hazardous in the elderly (Reed, 2010). On the other hand, theophylline has a variety of favorable anti-inflammatory effects on eosinophils, including the suppression of superoxide production (Piacentini et al., 2005), migration (Kikuchi et al., 2007) and eosinophilopoiesis (Wang et al., 2003), and the induction of apoptosis (Chung et al., 2000). Theophylline has further been reported to increase the activity of histone deacetylase 2 (HDAC2)

(Tamimi et al., 2012) and is expected to be effective in the treatment of smoking-induced asthma (Spears et al., 2009) and COPD (Cosio et al., 2009).

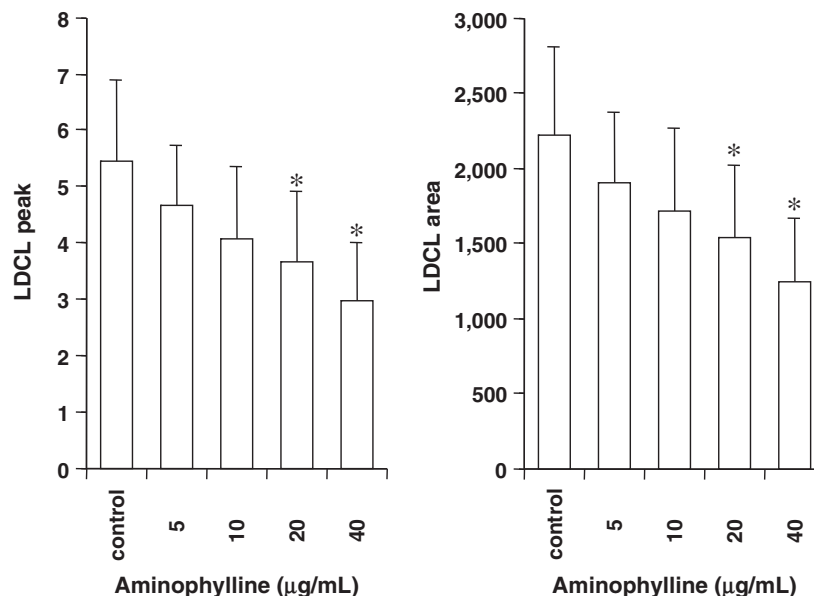
In this study, the possibility of using inhaled aminophylline was investigated. The efficacy of inhaled aminophylline is controversial, with conflicting reports in animal models (Dahlbäck et al., 1990; Ali et al., 1992) and in patients with asthma (Stewart & Block, 1976; Cushley & Holgate, 1985). These reports, regardless of their positive or negative association, only studied single-dose administration of inhaled theophylline. The present study thus examines the bronchodilating and anti-inflammatory effects of both single- and multiple-dose inhalation of aminophylline.

Aminophylline concentration-dependently suppressed ACh-induced contraction in the present study, similar to that observed in previous reports (Karlsson & Persson, 1981; Gallos et al., 2008). However, this study shows that overdose-levels rather than clinical doses ($\geq 20 \mu\text{g/mL}$ in serum) are required to achieve a strong relaxing effect.

The suppressive effect of single-inhalation aminophylline on antigen-induced airway constriction was evaluated. The blood concentration of theophylline after inhalation was low in this study. However, compared to the blood concentrations obtained through inhalation, those obtained after intraperitoneal administration were in the range of clinical overdose (Wieshammer et al., 2010), achieving a similar degree of bronchodilation. Serum levels were at 22.7 and 45.17 $\mu\text{g/mL}$ after intraperitoneal injection of 10 and 20 mg/kg of aminophylline, respectively. Therefore, inhaled aminophylline produces reliable bronchodilation and may potentially have fewer systemic side effects than does systemic administration.

Pretreatment with single-dose inhaled aminophylline did not affect airway infiltration by inflammatory cells (in both BAL fluids and airway tissues) or airway hyperreactivity to ACh at 24 h or 1 week after OVA challenge (data not shown). Thus, single-dose inhalation of aminophylline is transient, having no long-lasting bronchodilation or anti-inflammatory

Figure 6. ROS production by BAL cells. Aminophylline inhibited both LDCL peaks and areas in a concentration-dependent manner. $n = 4$; $*p < 0.05$ versus control.



effects. However, repeated inhalation of aminophylline (twice daily for 7 days) suppressed eosinophilic inflammation and airway hyperreactivity even 24 h after the final inhalation. Many reports have shown the effectiveness of repeated systemic administration of theophylline on eosinophilic airway infiltration and airway hyperreactivity (Aizawa et al., 2000; Wang et al., 2005).

Regretfully, the retention time of inhaled aminophylline in airway tissue could not be measured in the present study. The retention time seemed to be short, as evidenced by poor anti-inflammatory effects and lack of airway hyperreactivity suppression only 24 h after aminophylline treatment. However, repeated inhalation of aminophylline suppressed airway inflammation and had a transient bronchodilating effect. We believe that the anti-inflammatory effects resulted in the suppression of airway hyperreactivity, indicating that repeated inhalation of aminophylline could be an effective anti-inflammatory medication.

Higher concentrations of aminophylline ($\geq 20 \mu\text{g/mL}$) inhibited ROS production in BAL cells stimulated with A23187 in a concentration-dependent manner. In the present study, clinical doses of inhaled aminophylline had a bronchodilatory effect equal to that of overdose levels of systemic administration. The anti-inflammatory effects of aminophylline on ROS production may be due to the achievement of high local concentrations in airways.

A limitation of the present study is that the immediate effects of inhaled aminophylline on ongoing airway constriction could not be evaluated owing to the long time required for aminophylline inhalation. The effective duration of bronchodilation by inhaled aminophylline may also not be long acting according to a previous report (Cushley & Holgate, 1985).

In summary, the inhalation of aminophylline prevented allergen-induced airway constriction, and repeated inhalation suppressed eosinophilic airway inflammation and airway hyperreactivity. Furthermore, aminophylline inhibited ROS production by BAL cells. Aminophylline as an inhaled medication not only has the potential to reduce systemic

side effects but may also deliver higher concentrations of the drug into airway tissues. We hope to conduct further studies to evaluate the effective duration of inhaled aminophylline using models of smoking.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

- Aizawa H, Iwanaga T, Inoue H, et al. (2000). Once-daily theophylline reduces serum eosinophil cationic protein and eosinophil levels in induced sputum of asthmatics. *Int Arch Allergy Immunol* 121:123–8.
- Ali S, Mustafa SJ, Metzger WJ. (1992). Modification of allergen-induced airway obstruction and bronchial hyperresponsiveness in the allergic rabbit by theophylline aerosol. *Agents Actions* 37:168–70.
- Chung IY, Nam-Kung EK, Lee NM, et al. (2000). The downregulation of Bcl-2 expression is necessary for theophylline-induced apoptosis of eosinophil. *Cell Immunol* 203:95–102.
- Cosio BG, Iglesias A, Rios A, et al. (2009). Low-dose theophylline enhances the anti-inflammatory effects of steroids during exacerbations of COPD. *Thorax* 64:424–9.
- Cushley MJ, Holgate ST. (1985). Bronchodilator actions of xanthine derivatives administered by inhalation in asthma. *Thorax* 40:176–9.
- Dahlbäck M, Karlberg IB, Prytz M, Jarby R. (1990). Antigen-induced dyspnea in Sprague Dawley rats. Effects of local treatment with anti-asthmatic drugs. *Allergy* 45:487–96.
- Gallos G, Gleason NR, Zhang Y, et al. (2008). Activation of endogenous GABAA channels on airway smooth muscle potentiates isoproterenol-mediated relaxation. *Am J Physiol Lung Cell Mol Physiol* 295: L1040–7.
- Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention 2012 (update). Available at: www.ginasthma.org.
- Karlsson JA, Persson CG. (1981). Influence of tracheal contraction on relaxant effects in vitro of theophylline and isoprenaline. *Br J Pharmacol* 74:73–9.
- Kikuchi I, Kikuchi S, Kobayashi T, et al. (2007). Theophylline attenuates the neutrophil-dependent augmentation of eosinophil trans-basement membrane migration. *Int Arch Allergy Immunol* 143:44–9.
- Muraki M, Tohda Y, Sugihara R, et al. (1994). The effect of TYB-2285 on dual phase bronchoconstriction and airway hypersensitivity in guinea-pigs actively sensitized with ovalbumin. *J Pharm Pharmacol* 46:883–6.

- Muraki M, Imbe S, Santo H, et al. (2011). Effects of a cysteinyl leukotriene dual 1/2 receptor antagonist on antigen-induced airway hypersensitivity and airway inflammation in a guinea pig asthma model. *Int Arch Allergy Immunol* 155: 90–5.
- Persson CG. (1986). Overview of effects of theophylline. *J Allergy Clin Immunol* 78:780–7.
- Piacentini GL, Peroni DG, Bodini A, et al. (2005). Theophylline inhibits integrin-dependent eosinophil superoxide production. *Allergy Asthma Proc* 26:47–51.
- Reed CE. (2010). Asthma in the elderly: diagnosis and management. *J Allergy Clin Immunol* 126:681–7.
- Spears M, Donnelly I, Jolly L, et al. (2009). Effect of low-dose theophylline plus beclometasone on lung function in smokers with asthma: a pilot study. *Eur Respir J* 33:1010–17.
- Stewart BN, Block AJ. (1976). A trial of aerosolized theophylline in relieving bronchospasm. *Chest* 69:718–21.
- Tamimi A, Serdarevic D, Hanania NA. (2012). The effects of cigarette smoke on airway inflammation in asthma and COPD: therapeutic implications. *Respir Med* 106:319–28.
- Tsukiyama M, Akaishi T, Ueki T, et al. (2007). The extract from *Nandina domestica* THUNBERG inhibits histamine- and serotonin-induced contraction in isolated guinea pig trachea. *Biol Pharm Bull* 30:2063–8.
- Wang CH, Lin HC, Lin CH, et al. (2003). Effect of theophylline and specific phosphodiesterase IV inhibition on proliferation and apoptosis of progenitor cells in bronchial asthma. *Br J Pharmacol* 138: 1147–55.
- Wang Y, Wang CZ, Lin KX, et al. (2005). Comparison of inhaled corticosteroid combined with theophylline and double-dose inhaled corticosteroid in moderate to severe asthma. *Respirology* 10:189–95.
- Wieshammer S, Dreyhaupt J, Basler B. (2010). Theophylline and cardiac stress in patients with dyspnea: an observational study. *Pharmacology* 86:189–95.