Phosphodiesterase 4 Inhibitor Roflumilast Improves the Bronchodilative Effect of Sevoflurane in Sensitized Airways

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ABSTRACT

Background: Although phosphodiesterase 4 inhibitors and the volatile anesthetic sevoflurane are known to have independent bronchodilator properties, the combined administration of these two agents may have the potential to exert an additive or synergistic bronchodilator effect. The authors tested this hypothesis and investigated the common site of this combined relaxation effect in a model of airway hyperresponsiveness with ovalbumin-sensitized guinea pigs.

Methods: Ovalbumin-sensitized animals (n = 138) were randomized into six groups: sensitized, sevoflurane, rolipram1.0, roflumilast1.0, sevoflurane/rolipram1.0, and sevoflurane/roflumilast1.0. Total lung resistance *in vivo*, airway smooth muscle tension *in vitro*, and intracellular cyclic adenosine monophosphate levels were measured to evaluate the relaxation effect.

Results: Among the six sensitized groups, total lung resistance was higher in the order of sensitized > sevoflurane > rolipram 1.0 > roflumilast1.0 > sevoflurane/rolipram1.0 > sevoflurane/roflumilast1.0, with an increase in acetylcholine concentration. Compared with the other five groups, the muscle tensions in the sevoflurane/roflumilast1.0 group were significantly lower at carbacholine doses of 10^{-7}, 10^{-6}, and 10^{-5} M; the cyclic adenosine monophosphate concentrations (means ± SD) in the sevoflurane/rolipram1.0 (1.61 ± 0.34) and sevoflurane/roflumilast1.0 (1.50 ± 0.20) groups were higher than that in the sensitized (0.52 ± 0.15) and sevoflurane (1.12 ± 0.32) groups.

Conclusions: The combined use of phosphodiesterase 4 inhibitors with the volatile anesthetic sevoflurane had an additive bronchodilator effect in ovalbumin-sensitized guinea pigs. The concurrent increase in cyclic adenosine monophosphate levels in sensitized airway smooth muscle might be a mechanism of this combined relaxation effect. **(ANESTHESIOLOGY 2014; 120:1152-9)**

THERE has been a worldwide increase in the recognition of patients with airway hyperreactivity (such as chronic obstructive pulmonary disease [COPD] and asthma) who, with increased perioperative respiratory morbidity, pose challenges to anesthetists.[1,2] Among the anesthetic options for the management of these patients, volatile anesthetics are usually regarded as first-line drugs for maintenance.[3] Sevoflurane, one of the volatile anesthetics, which are potent bronchodilators that can effectively reverse severe perioperative bronchospasm in patients with hyperreactive airway diseases, has been demonstrated to have bronchodilator properties.[4–6] Doi and Ikeda[7] reported that sevoflurane irritates the airways least among the four volatile anesthetic agents such as halothane, enflurane, isoflurane, and sevoflurane. Many authors have suggested the use of sevoflurane in patients with reactive airway diseases, and even in those with status asthmaticus.[8,9]

Phosphodiesterase 4 (PDE4), a member of the phosphodiesterase enzyme superfamily, specifically inactivates cyclic adenosine monophosphate (cAMP) and is considered to be a molecular target for a new class of drugs for pulmonary diseases.[10] PDE4 inhibitors maintain baseline levels of cAMP by interfering with the breakdown of cAMP to adenosine monophosphate, leading to its intracellular accumulation. The increased intracellular cAMP activates protein kinase A, which enhances phosphorylation of proteins, with subsequent inhibition of proinflammatory cells and mediators, inhibition of fibrosis, and relaxation of smooth muscle.[11] Recently, the novel PDE4 inhibitor roflumilast has been approved in the United States and Europe, as part of the Global Initiative for Chronic Obstructive Lung Disease,* for patients with stage 3 and 4 COPD. In Europe, roflumilast is indicated as a maintenance treatment in severe COPD associated with chronic

What We Already Know about This Topic

- Both phosphodiesterase 4 inhibitors and the volatile anesthetic sevoflurane are known to have independent bronchodilator properties

What This Article Tells Us That Is New

- In animals with sensitized airway, combination of phosphodiesterase 4 inhibitors, particularly a new generation roflumilast, and sevoflurane exerted additive airway relaxation *via* an increase in airway smooth muscle cyclic adenosine monophosphate levels

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* Available at: http://www.goldcopd.it/gruppi_lavoro/2011/GOLDPOCKETGUIDE2010.pdf. Accessed December 20, 2013.

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bronchitis and a history of exacerbations as an add-on to bronchodilator treatment.[12]

Because PDE4 inhibitors and sevoflurane are commonly used in clinical practice, it is important to know the combined effect of these two agents for patients under PDE4 inhibitor treatment who need to be anesthetized with a sevoflurane-based general anesthetic. In the current study, we hypothesized that the combined use of these two kinds of agents has an additive bronchodilator effect, and we tested this hypothesis by measuring total lung resistance (R_L) and airway smooth muscle tension in a model of airway hyperresponsiveness using ovalbumin-sensitized guinea pigs. Furthermore, the intracellular cAMP concentrations of sensitized airway smooth muscle were investigated at the molecular level.

Materials and Methods

Animals

All experimental procedures were approved by the Animal Care and Use Committee of Sapporo Medical University (Sapporo, Hokkaido, Japan). Young (5-week-old), male, pathogen-free Hartley guinea pigs (Japan SLC, Hamamatsu, Japan), weighing approximately 300 g at the time of purchase, were housed in an air-conditioned room at a temperature of 23° ± 1°C and 60 ± 10% humidity, and the room was illuminated from 08:00 to 20:00. The guinea pigs were fed a standard laboratory diet and given water *ad libitum*. All the experiments were performed on the animals within 2 weeks of their purchase in a standard laboratory in which the volatile anesthetic could be used. Each animal was used in only one experiment. The weight was determined just before the experiments and there were no differences among groups (data not shown).

Sensitization Procedure

An experimental model of ovalbumin sensitization was established by using egg white ovalbumin as an antigen.[13] In brief, 2 ml of ovalbumin (0.5 mg/ml) was administered intraperitoneally in guinea pigs followed by an exposure to 10 ml of aerosolized ovalbumin (1 mg/ml) for 10 min three times at day 10. In the control group, animals received normal saline at the same volume.

Measurements of Total Lung Resistance (R_L) In Vivo

The guinea pigs were anesthetized intraperitoneally with urethane (1.5 g/kg). The tracheae of each animal were intubated with a cannula (outer diameter: 2.5 mm), and each animal was ventilated with a respirator (Harvard model 683; South Natick, MA) at a constant rate of 32 breaths/min with 100% oxygen and a tidal volume of approximate 6 to 8 ml/kg (volume controlled). The tidal volume was adjusted in each animal to maintain an end-tidal carbon dioxide partial pressure at approximately 40 mmHg by measuring end-tidal carbon dioxide continuously during the study period (5250 RGM; Datex-Ohmeda Japan, Tokyo, Japan). The right jugular vein was cannulated for intravenous injection. To abolish spontaneous breathing, rocuronium was administered at a continuous rate of 2 mg kg^{-1} h^{-1}.[14] Intrapleural pressure was measured through a water-filled cannula (PE-240), which was placed in the lower third of the esophagus and connected to one port of the differential pressure transducer. Transpulmonary pressure (P_{TP}) was determined by monitoring the difference between the pressure in the external end of the tracheal cannula and the esophageal cannula using a Statham differential transducer (DP-45; Validyne Engineering, Northridge, CA). A Fleisch pneumotachograph and a differential transducer were used to monitor respiratory flow rates (V') (PULMOS-II; Medical Interface Project Station Co., Osaka, Japan). Volume changes (V) were obtained by electronic integration of V' signals. Total lung resistance (R_L) and dynamic lung compliance (C_L) were obtained using the equation of motion of the respiratory system as follows:

$$p_{TP}(t) = C_L \cdot V + R_L \cdot V'(t), \text{ where t is time.}$$

All signals were recorded and R_L was analyzed by an on-line computer on a breath-by-breath basis continuously. In all guinea pigs, the resistance of the tracheal tube was not removed from R_L. Following this, a period of 15 min without any sensitization was allowed to establish steady-state conditions. A heating pad was placed under each animal and the rectal temperature was kept at approximately 37°C during the study period.

Then, the sensitized model in this study was validated in two groups of animals: a sensitized group (n = 8) of ovalbumin-sensitized guinea pigs and a control group (n = 8) of normal guinea pigs. After establishing the steady-state conditions, boluses of acetylcholine were injected intravenously at subsequent increasing doses from 1 to 6 μg/kg with 5-min intervals. The R_L was recorded continuously, which allowed the determination of the peak response to acetylcholine and the establishment of a dose–response curve.

A total of 48 sensitized guinea pigs were investigated to determine whether the combined use of rolipram, a first-generation PDE4 inhibitor, with sevoflurane had an additive effect on R_L and whether these effects changed with an increasing dose of rolipram. The sensitized guinea pigs were randomized (envelope technique) into six groups of eight animals each: group S (sevoflurane 2% [1 minimum alveolar concentration = 2.0% for sevoflurane in guinea pigs]),[5] group R1.0 (rolipram 1.0 g/kg, intraperitoneally),[15] group S+R1.0 (sevoflurane 2% + rolipram 1.0 g/kg, intraperitoneally), group S+R2.0 (sevoflurane 2% + rolipram 2.0 g/kg, intraperitoneally), group S+R3.0 (sevoflurane 2% + rolipram 3.0 g/kg, intraperitoneally), and group S+R6.0 (sevoflurane 2% + rolipram 6.0 g/kg, intraperitoneally). Rolipram was dissolved in saline and administered intraperitoneally 30 min before the experiment.[15] Sevoflurane was given at 20 min after the steady-state conditions were established.

A total of 16 sensitized guinea pigs were used to test the combined effects of roflumilast, a second-generation phosphodiesterase inhibitor, with sevoflurane in two groups: group F1.0 (roflumilast 1.0 mg/kg, oral)[10] and group S+F1.0 (sevoflurane 2% + roflumilast 1.0 mg/kg, oral). Roflumilast was dissolved in purified water and administered orally 1 h before the experiment.[10]

Then, the same study protocol as for the validation of the model for acetylcholine administration was performed in the rolipram and roflumilast groups while 100% oxygen with or without sevoflurane was continuously administered. The peak responses to R_L after the administration of successive increasing boluses of acetylcholine were established to evaluate the potential bronchodilator protective properties of the studied agents.

Measurements of Airway Smooth Muscle Tension In Vitro

In this experiment, 48 sensitized guinea pigs were randomized (envelope technique) into six groups of eight animals each: the sensitized group, group S (bubbling with sevoflurane 2%), group R1.0 (rolipram 1.0 g/kg, intraperitoneally), group S+R1.0 (sevoflurane 2% bubbling + rolipram 1.0 g/kg, intraperitoneally), group F1.0 (roflumilast 1.0 mg/kg, oral), and group S+F1.0 (sevoflurane 2% bubbling + roflumilast 1.0 mg/kg, oral). Each animal was anesthetized with urethane and killed by exsanguination. The cartilage rings (5 mm), in a section from the midportion of the trachea, were placed immediately in physiological salt solution (136.9 mM NaCl, 5.4 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 23.9 mM $NaHCO_3$, and 5.5 mM glucose, at 37°C) that was aerated continuously with 5% carbon dioxide in oxygen, and dissected free from the connective tissue. The trachea rings were connected to a strain gauge transducer (ULA-10GR; Unipulse, Tokyo, Japan) using surgical wire, and the resting tension was adjusted to 1.5 g. After a 60-min equilibration period with three washes, the tissue bath was bubbled with or without sevoflurane for 20 min. Then, contractions were induced by carbacholine (from 10^{-8} to 10^{-5} M) in 5-min intervals while the muscle tissue was continuously bubbled with or without sevoflurane. The peak data of muscle tension after the administration of increasing carbacholine were recorded to evaluate the effect of each group *in vitro*.

Measurements of Intracellular cAMP Levels

To determine the cAMP levels in airway smooth muscle cells, six additional sensitized groups (n = 7 each) were investigated: a control sensitized group, group S, group R1.0, group F1.0, group S+R1.0, and group S+F1.0. After each animal received the studied agents (details are provided in Measurements of Total Lung Resistance (R_L) *In Vivo*, beginning with the second sentence of the fourth paragraph to the fifth paragraph), tracheal smooth muscle tissues were removed and dissected free from connective tissue using a dissecting microscope in physiological salt solution at 37°C, and then they were immediately plunged into liquid nitrogen and stored at −80°C until the assay was performed. In the cAMP assay, we first weighed the tissues and then added a corresponding volume of 0.1 N HCl, after which we homogenized the tissue and centrifuged the resulting solution at 25,000*g* for 5 min at 4°C. The supernatant of the homogenized solution was assayed directly with the kit reagents to measure the cAMP concentrations according to the protocol of the cAMP Activity Assay Kit (Biovision, Milpitas, CA).

Drugs

Roflumilast was purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Rolipram, urethane, ovalbumin, acetylcholine, and carbacholine were purchased from Sigma Chemicals (St. Louis, MO). Sevoflurane was purchased from Maruishi Pharmaceutical Company Limited (Osaka, Japan). Rocuronium was purchased from MSD Incorporated (Tokyo, Japan).

Statistical Analysis

All data were expressed as means ± SD and analyzed using Prism 5 (GraphPad Software, La Jolla, CA). The data for R_L and muscle tension were tested by two-way ANOVA for repeated measures with a Bonferroni correction. The cAMP concentrations were analyzed by one-way ANOVA with *post hoc* Bonferroni testing. The testing was two tailed, and *P* values less than 0.05 were considered significant.

Results

Measurements of Total Lung Resistance (R_L) In Vivo

Figure 1A depicts the peak responses obtained for R_L in sensitized guinea pigs after the administration of incremental concentrations of acetylcholine (1.0 to 6.0 μg/kg). The baseline values for R_L which were determined before administration of each dose of acetylcholine shifted slightly but did not reach statistical significance (data not shown). The sensitized model was tested with the dose–response curves to acetylcholine obtained for R_L (fig. 1B). In control and sensitized groups, the peak responses of R_L to each dose of acetylcholine were significantly different compared with their baseline (data not shown). When compared with the control group, the dose–response curve of R_L was increased significantly, which is consistent with the results of our previous report.[16]

Figure 2A shows the effects of rolipram (at a dose of 1.0 g/kg) and/or sevoflurane. Compared with the sensitized group, R_L in the other three groups was significantly different with increasing concentrations of acetylcholine; compared with the S group, the data in the S+R1.0 group were lower, by 13.3 and 16.4%, at acetylcholine 5.0 and 6.0 μg/kg, respectively. Subsequently, the effects of roflumilast with or without sevoflurane were tested (fig. 2B). Compared with the sensitized group, the results were similar to those shown in figure 2A; compared with the S group, the data in the S+F1.0 group were much lower. Then, the effects of rolipram (at a dose of 1.0 g/kg) and roflumilast with sevoflurane were compared (fig. 2C). There were no differences between the S+R1.0 and S+F1.0 groups; however, compared with the R1.0 group, the data in the S+F1.0 group were significantly different with

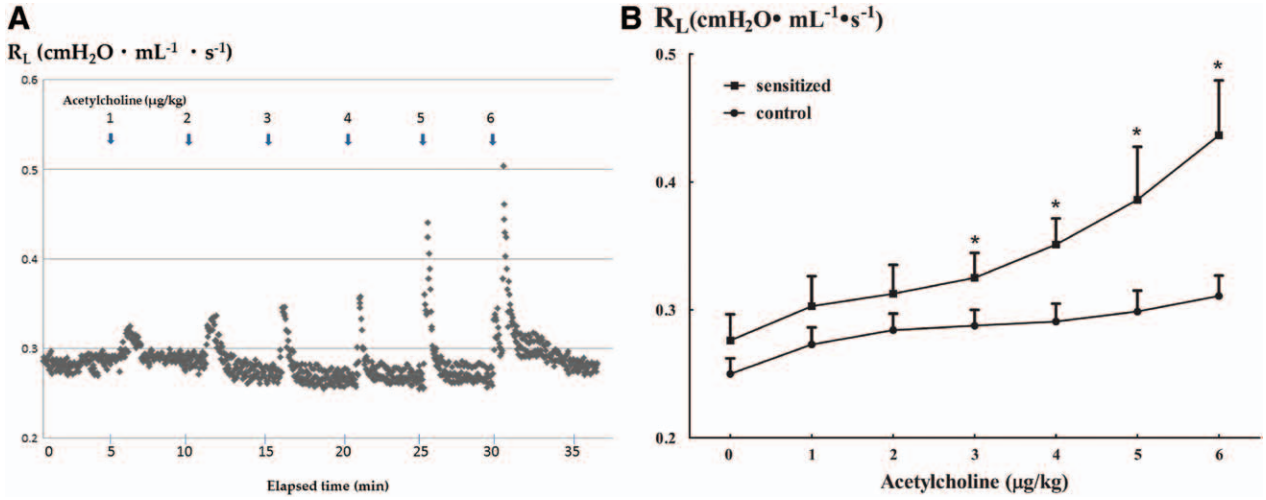

Fig. 1. (*A*) Representative raw data of sensitized guinea pigs to intravenous injections of acetylcholine (1.0–6.0 μg/kg) on the respiratory parameters of total lung resistance (R_L). (*B*) Dose–response curves of total lung resistance (R_L) to acetylcholine in sensitized and normal (control) guinea pigs (n = 8 each). * $P < 0.05$ *versus* control group at the same concentrations of acetylcholine.

increasing concentrations of acetylcholine (from 1.0 to 6.0 μg/kg), whereas the S+R1.0 group only showed differences at acetylcholine 5.0 and 6.0 μg/kg. Furthermore, there were no differences among S+R1.0, S+R2.0, S+R3.0, S+R6.0, and S+F1.0 groups (data not shown).

Measurements of Airway Smooth Muscle Tension In Vitro

Figure 3A shows the effects of rolipram and/or sevoflurane. Compared with the sensitized group, the muscle tensions in S, R1.0, and S+R1.0 groups were significantly different with increasing concentrations of carbacholine. The effects of roflumilast with or without sevoflurane were similar to those shown in figure 3A when compared with the sensitized group; one difference is that the data in the S+F1.0 group were significantly lower than in S and F1.0 groups (fig. 3B). Figure 3C shows a comparison of the effects of rolipram and roflumilast with sevoflurane. There was no difference between the S+R1.0 and S+F1.0 groups; however, the data in the S+F1.0 group were lower than in the R1.0 and F1.0 groups, whereas the S+R1.0 group showed no difference.

Measurements of Intracellular cAMP Levels

Figure 4 shows the cAMP concentrations with rolipram (fig. 4A), roflumilast (fig. 4B), and/or sevoflurane administration. Compared with the sensitized group, all the data in the other groups were higher; the S+R1.0 (1.61 ± 0.34 pmol/μl) and S+F1.0 (1.50 ± 0.20 pmol/μl) groups were significantly higher than the S group (1.12 ± 0.32 pmol/μl), whereas the R1.0 and F1.0 groups were not. There were no differences among the R1.0, F1.0, S+R1.0, and S+F1.0 groups (data not shown).

Discussion

In the current study, we first found that combined administration of PDE4 inhibitors with sevoflurane had an additive bronchodilator effect on total lung resistance *in vivo* and airway smooth muscle tension *in vitro*. Second, we tested the intracellular cAMP concentrations of the airway smooth muscle and concluded that the concurrent increase in cAMP levels might be a mechanism of the combined relaxation effect.

Additive Bronchodilator Effects on R_L by PDE4 Inhibitors with Sevoflurane

Bronchodilation induced by the volatile anesthetic sevoflurane[4,8] and PDE4 inhibitors[11,17,18] has been demonstrated clinically in humans. In animal studies, we previously reported that sevoflurane inhibits the contractility of hyperreactive airway smooth muscle in guinea pig models.[5] Nejman-Gryz *et al.*[15] concluded that the PDE4 inhibitor rolipram leads to bronchodilation effects on lung resistance in experimental asthma. In the current study, we obtained similar results and demonstrated that the combined use of the first-generation PDE4 inhibitor rolipram with sevoflurane had an additive relaxation effect on R_L (fig. 2A). Moreover, on the basis of the results that higher concentrations of rolipram with sevoflurane showed no greater effects, we concluded that the additive effects did not change with the increasing dose of rolipram. This result is consistent with a previous study in which Tang *et al.*[19] reported that the bronchodilator effect of rolipram is not dose dependent. We then expanded the study with roflumilast, a new-generation PDE4 inhibitor with the best pharmacological profile and good clinical efficacy at an effective dose of 1.0 mg/kg.[10] Roflumilast showed effects similar to rolipram when added to sevoflurane (fig. 2B). These additive bronchodilator effects indicated that patients under PDE4 inhibitors could be anesthetized safely with a sevoflurane-based general anesthetic and get better protection against R_L. Between the rolipram with sevoflurane and roflumilast with sevoflurane groups, there was no difference, even with a higher dosage of rolipram. However, compared

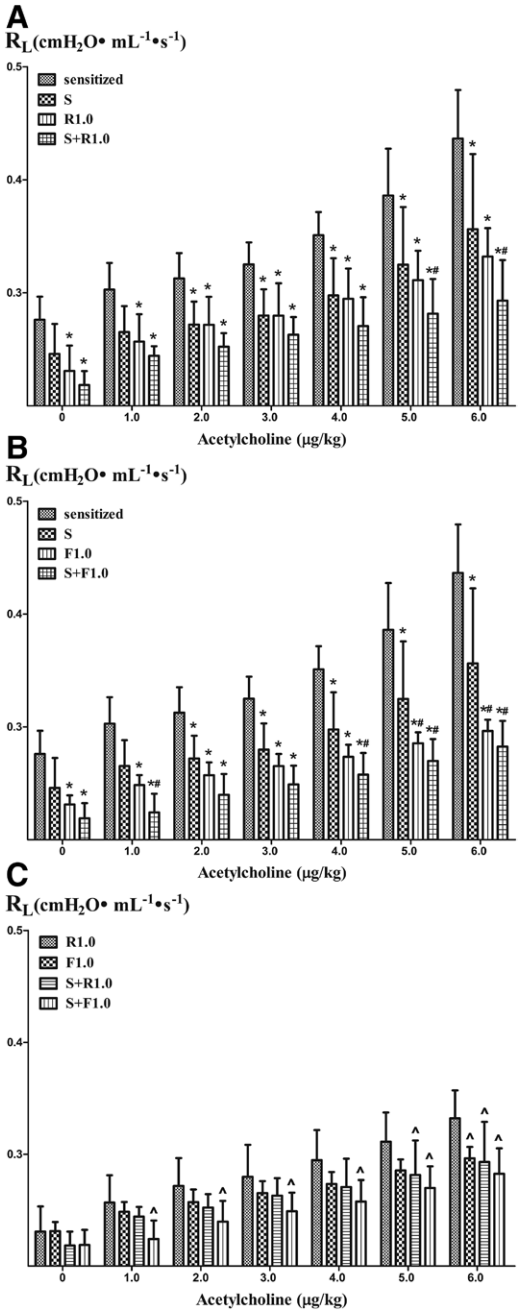

Fig. 2. (*A*) Peak responses of total lung resistance (R_L) *in vivo* to acetylcholine in the four groups of sensitized guinea pigs (n = 8 each). S = sevoflurane; R1.0 = rolipram1.0; S+R1.0 = sevoflurane/rolipram1.0. **P* < 0.05 *versus* the sensitized group; #*P* < 0.05 *versus* the S group, at the same concentrations of acetylcholine. (*B*) Peak responses of total lung resistance (R_L) *in vivo* to acetylcholine in the four groups of sensitized guinea pigs (n = 8 each). S = sevoflurane; F1.0 = roflumilast1.0; S+F1.0 = sevoflurane/roflumilast1.0. **P* < 0.05 *versus* the sensitized group; #*P* < 0.05 *versus* the S group, at the same concentrations of acetylcholine. (*C*) Peak responses of total lung resistance (R_L) *in vivo* to acetylcholine in the four groups of sensitized guinea pigs (n = 8 each). R1.0 = rolipram1.0; F1.0 = roflumilast1.0; S+R1.0 = sevoflurane/rolipram1.0; S+F1.0 = sevoflurane/roflumilast1.0. ^*P* < 0.05 *versus* the R1.0 group, at the same concentrations of acetylcholine.

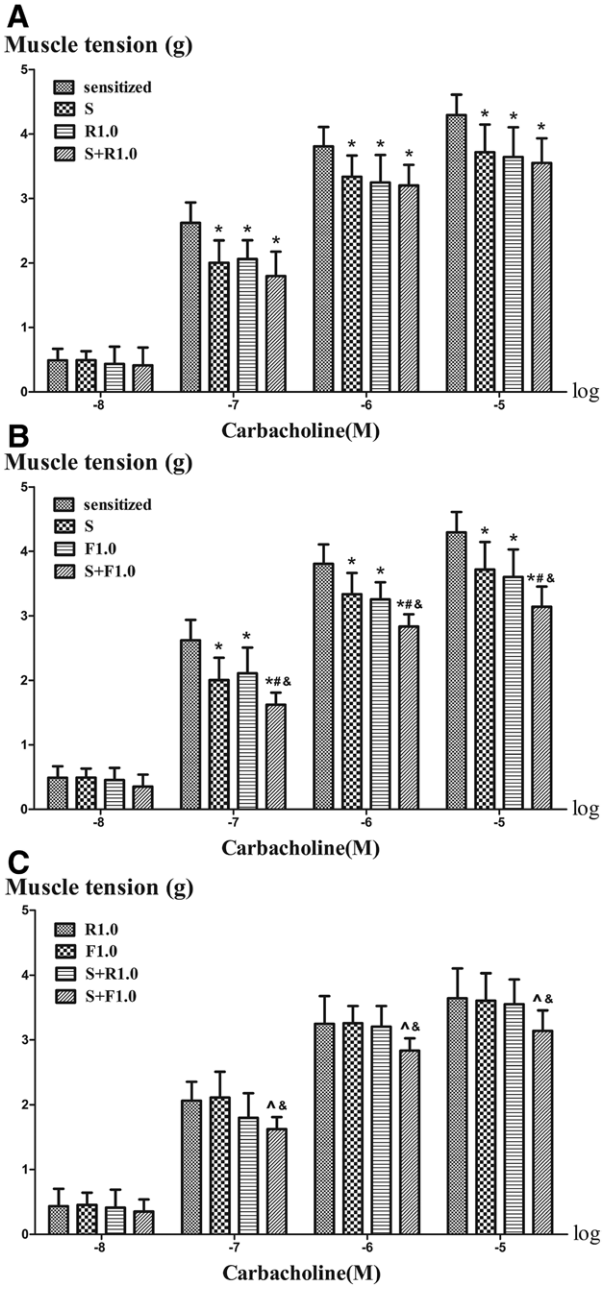

Fig. 3. (*A*) Peak responses of airway smooth muscle tension *in vitro* to carbacholine in the four groups of sensitized guinea pigs (n = 8 each). S = sevoflurane; R1.0 = rolipram1.0; S+R1.0 = sevoflurane/rolipram1.0. **P* < 0.05 *versus* the sensitized group, at the same concentrations of carbacholine. (*B*) Peak responses of airway smooth muscle tension *in vitro* to carbacholine in the four groups of sensitized guinea pigs (n = 8 each). S = sevoflurane; F1.0 = roflumilast1.0; S+F1.0 = sevoflurane/roflumilast1.0. **P* < 0.05 *versus* the sensitized group; #*P* < 0.05 *versus* the S group; &*P* < 0.05 *versus* the F1.0 group, at the same concentrations of carbacholine. (*C*) Peak responses of airway smooth muscle tension *in vitro* to carbacholine in the four groups of sensitized guinea pigs (n = 8 each). R1.0 = rolipram1.0; F1.0 = roflumilast1.0; S+R1.0 = sevoflurane/rolipram1.0; S+F1.0 = sevoflurane/roflumilast1.0. ^*P* < 0.05 *versus* the R1.0 group; &*P* < 0.05 *versus* the F1.0 group, at the same concentrations of carbacholine.

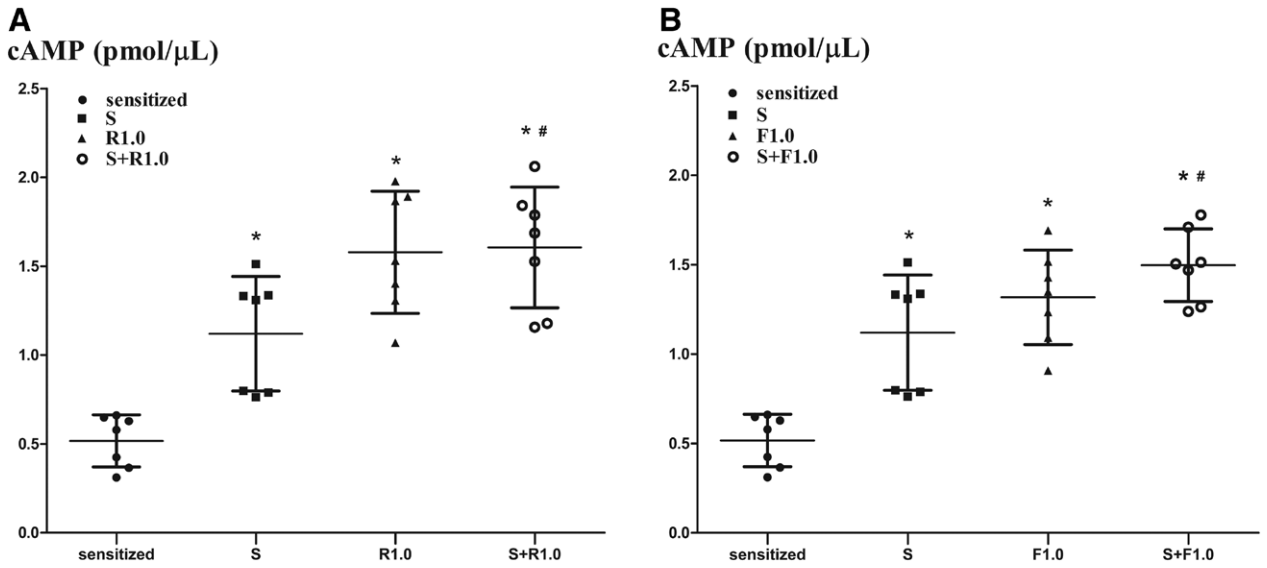

Fig. 4. (*A*) Intracellular cyclic adenosine monophosphate (cAMP) concentrations of airway smooth muscle in the four groups of sensitized guinea pigs (n = 7 each). S = sevoflurane; R1.0 = rolipram1.0; S+R1.0 = sevoflurane/rolipram1.0. *P < 0.05 *versus* the sensitized group; #P < 0.05 *versus* the S group. (*B*) Intracellular cAMP concentrations of airway smooth muscle in the four groups of sensitized guinea pigs (n = 7 each). S = sevoflurane; F1.0 = roflumilast1.0; S+F1.0 = sevoflurane/roflumilast1.0. *P < 0.05 *versus* the sensitized group; #P < 0.05 *versus* the S group.

with the rolipram with sevoflurane group, the difference of roflumilast with sevoflurane was achieved much earlier (fig. 2C). It is known that PDE4 has two conformations, with high ($PDE4_H$) and low ($PDE4_L$) affinities, respectively. $PDE4_H$ is generally expressed in the central nervous system, producing the adverse effects of nausea and vomiting, whereas $PDE4_L$ is present in airway smooth muscle and is associated with anti-inflammatory activity and relaxation.[20] Rolipram has high and low affinity to $PDE4_H$ and $PDE4_L$, respectively. Roflumilast, in contrast, shows much lower potency for $PDE4_H$ and much higher potency for $PDE4_L$, which results in its greater selectivity and higher therapeutic ratio in airway diseases.[21] Therefore, the high potency and selectivity of roflumilast[11,22,23] could be a reason for the different effects between rolipram and roflumilast with or without sevoflurane in this study.

Combined Relaxation of Airway Smooth Muscle Tension by PDE4 Inhibitors with Sevoflurane

It is well known that volatile anesthetics such as sevoflurane are potent bronchodilators, and that the bronchodilation occurs indirectly by the inhibition of reflex neural pathways[24] and directly by airway smooth muscle cells.[25] In the current study, a lack of influence by reflex nerves on isolated smooth muscle was necessary; therefore, we measured the airway smooth muscle tension *in vitro*. All of the results concerning sevoflurane, rolipram, roflumilast, or a combination of two agents showed relaxation effects compared with the sensitized group, which is consistent with previous studies showing that airway smooth muscle tension is relaxed with sevoflurane[5,26] or PDE4 inhibitors.[27,28] In particular, the different results between roflumilast and rolipram with sevoflurane not only confirmed that the combined use of roflumilast with sevoflurane has an additive relaxation effect but also showed that the additive effect is preferable to roflumilast and sevoflurane, suggesting that this may be the better choice for patients.

Concurrent Increase of cAMP Levels by PDE4 Inhibitors with Sevoflurane

Intracellular levels of cAMP regulate the function of cells, contributing to the pathogenesis of respiratory diseases such as asthma and COPD.[29] PDE4, an enzyme capable of mediating cAMP hydrolysis, is being explored as a molecular target for novel antiasthmatic agents and pulmonary inflammatory diseases. The validity of this approach has been borne out by the clinical development of PDE4 inhibitors such as roflumilast.[30–32] Lipworth[17] summarized the work done with PDE4 inhibitors for asthma and COPD and speculated that restraining the breakdown of cAMP with PDE4 inhibitors might potentiate the effect of long-acting β_2 agonists, which in turn might result in a synergistic outcome. Although sevoflurane does not belong to the β_2 agonists, the additivity of PDE4 inhibitors with sevoflurane is similar to that shown in studies of PDE4 inhibitors and β_2 agonists.[33,34] To investigate a possible common mechanism of PDE4 inhibitors and sevoflurane, we measured the cAMP levels in airway smooth muscle. Interestingly, the concentration of cAMP in the sevoflurane group was increased significantly. Previous studies have suggested that volatile anesthetics such as sevoflurane antagonize muscarinic receptors[35,36] and then reduce cAMP levels *via* an inhibitory G protein.[37] This discrepancy might be due to the numerous signaling pathways stimulated by sevoflurane,[25,35] and in airway smooth muscle, sevoflurane may increase cAMP

levels through other receptors or ion channels. For example, it has been reported that sevoflurane can decrease the hydrolysis of cAMP by inhibiting the activity of calmodulin.[38] Further investigations are needed to clarify these complex mechanisms. In the current study, considering that the concentrations of cAMP were higher than with sevoflurane alone, rolipram and roflumilast with sevoflurane demonstrated an additive effect at the molecular level, and this result suggested that cAMP-mediated airway smooth muscle relaxation might be one of the mechanisms of the combined relaxation effect of PDE4 inhibitors with sevoflurane.

Limitations

There were several limitations to this study. First, urethane could have some effects on the respiratory parameters because it increases airway resistance and potentiates the bronchoconstrictor effects of constrictor agonists, especially acetylcholine.[39] Second, R_L might be affected by secretions from small airway and pulmonary tissues, which could be decreased with sevoflurane.[40] Third, there is the possibility that deep anesthesia and hypotension might alter oxygen consumption, and the corresponding smaller tidal volumes might change R_L. To test these possibilities, further studies are needed.

Conclusions

In conclusion, the current investigation revealed that combined administration of a PDE4 inhibitor, roflumilast, and a volatile anesthetic, sevoflurane, exerted additive relaxation in an animal model of airway hyperresponsiveness. These findings suggest that those patients under PDE4 inhibitors treatment could be anesthetized safely with sevoflurane-based general anesthesia, and that a combination of these two agents might provide a better protection against airway disease.

Acknowledgments

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Competing Interests

The authors declare no competing interests.

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