

Effect of Prophylactic Bronchodilator Treatment with Intravenous Colforsin Daropate, a Water-soluble Forskolin Derivative, on Airway Resistance after Tracheal Intubation

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Background: **After induction of anesthesia, lung resistance increases. The authors hypothesized that prophylactic bronchodilator treatment with intravenous colforsin daropate, a water-soluble forskolin derivative, before tracheal intubation would result in decreased lung resistance and increased lung compliance after tracheal intubation when compared with placebo medication.**

Methods: **Forty-six adult patients were randomized to placebo or colforsin daropate treatment. Patients in the control group received normal saline; patients in the colforsin group received 0.75 $\mu g \cdot kg^{-1} \cdot min^{-1}$ colforsin daropate intravenously until the study ended. Thirty minutes after the study began, the authors administered 5 mg/kg thiamylal and 5 μg/kg fentanyl for induction of general anesthesia and 0.3 mg/kg vecuronium for muscle relaxation. A 15-$mg \cdot kg^{-1} \cdot h^{-1}$ continuous infusion of thiamylal followed anesthetic induction. Four, 8, 12, and 16 min after tracheal intubation, mean airway resistance (R_{awm}), expiratory airway resistance (R_{awe}), and dynamic lung compliance (C_{dyn}) were measured.**

Results: **Patients in the colforsin group had significantly lower R_{awm} and R_{awe} and higher C_{dyn} after intubation than those in the control group. Differences in R_{awm}, R_{awe}, and C_{dyn} between the two groups persisted through the final measurement at 16 min. At 4 min after intubation, smokers had a higher R_{awm} and a lower C_{dyn} than nonsmokers in the control group. After treatment by intravenous colforsin daropate, R_{awm}, R_{awe}, and C_{dyn} values were similar for smokers and nonsmokers after tracheal intubation.**

Conclusions: **Prophylactic treatment with colforsin daropate produced lower R_{awm} and R_{awe} and higher C_{dyn} after tracheal intubation when compared with placebo medication. Pretreatment before intubation may be beneficial and advantageous for middle-aged smokers.**

AFTER induction of anesthesia, tracheal intubation often causes lung resistance increases (bronchoconstriction).[1–5] This constrictive response presumably is initiated by activation of abundant laryngeal and tracheal receptors with reflex constriction of the peripheral airways.[2]

Forskolin, a direct activator of adenylate cyclase,[6] is known to cause relaxation of the airway smooth muscles similar to other agents that increase intracellular cAMP.[7–12] The results form experimental study by Hiramatsu *et al.*[13] using guinea pig tracheal smooth muscle and porcine tracheal myocytes suggested that this relaxation is mediated at least in part by opening the large-conductance calcium Ca^{2+}-activated potassium channels; however, the potential usefulness of forskolin in treating bronchospasm[14] is limited by its poor water solubility.

We recently reported that intravenous colforsin daropate, a novel and potent water-soluble forskolin derivative, prevents bronchoconstriction induced by intravenous administration of thiamylal and fentanyl in combination under tracheal intubation and found that colforsin daropate is a potent bronchodilator.[15] We hypothesized that prophylactic bronchodilator treatment with intravenous colforsin daropate, with a bronchodilating effect in animals[16–18] and humans,[15] before tracheal intubation would result in decreased airway resistance and increased lung compliance after placement of the endotracheal tube when compared with placebo medication. Thus, we also measured hemodynamics and catecholamines because these variables can be affected by intravenous colforsin daropate, which has positive inotropic and vasodilatory actions.[15]

Patients and Methods

Patients

After obtaining approval from our institutional review board (Chiba Hokusoh Hospital, Nippon Medical School, Chiba, Japan) and written informed consent from the study patients, 46 adult patients with American Society of Anesthesiologists physical status classification of I or II who were scheduled to undergo minor elective surgery were enrolled in the study. Patients who had a clinical or radiologic abnormality of the ventilatory system, had a suspected (history of atopy) or overt (history of wheezing) bronchial hypersensitivity, or were receiving treatment with a β-blocker were excluded from the study. A random number computer-generated program was used to assign study patients randomly to one of two groups: (1) a placebo (control) group (n = 23) or (2) a colforsin daropate group (n = 23).

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Procedure

The control group patients received a 7.5-ml/h continuous infusion of normal saline, and the colforsin group patients received a 0.75-$\mu g \cdot kg^{-1} \cdot min^{-1}$ (7.5 ml/h) continuous infusion of colforsin daropate (Adehl®Inj.; Nippon Kayaku Co., Ltd., Tokyo, Japan) until the study ended. Thirty minutes after the study began, we administered 5 mg/kg thiamylal and 5 μg/kg fentanyl for induction of general anesthesia and 0.3 mg/kg vecuronium for muscle relaxation to facilitate oral tracheal intubation with a disposable endotracheal tube (ID, 8 mm). A 15-mg $\cdot kg^{-1} \cdot h^{-1}$ continuous infusion of thiamylal followed anesthetic induction. Controlled ventilation was maintained, delivering 50% oxygen with a semiclosed circle system (Ohmeda Modulus® CD Anesthesia System; Ohmeda, Madison, WI) at a fresh gas flow rate of 6 l/min. The ventilatory parameters were set at a tidal volume of 8 ml/kg, an inspiratory-to-expiratory ratio of 1:2, and a respiratory rate of 10 breaths/min. Four, 8, 12, and 16 min after tracheal intubation, mean airway resistance (R_{awm}), expiratory airway resistance (R_{awe}), and dynamic lung compliance (C_{dyn}) were measured. These respiratory variables (R_{awm}, R_{awe}, and C_{dyn}) were measured and analyzed with a CP-100 pulmonary function monitor (Bicore, Irvine, CA) attached to a flow transducer (VarFlex®; Bear Medical Systems, Inc., Palm Springs, CA) and an esophageal balloon catheter (SmartCath®; Bear Medical Systems, Inc.)[15,19–26]

We drew arterial blood to measure plasma epinephrine and norepinephrine at baseline (just before the study began) and 30 min after the study began (just before anesthesia induction). The study was completed before the elective surgery was initiated. We analyzed differences between smokers and nonsmokers after completion of this study.

Statistical Analysis

For statistical analyses, we used chi-square analysis to compare differences in sex between the control group and the colforsin group. Unpaired *t* tests were applied to compare differences in age, weight, and height between the control group and the colforsin group. Unpaired *t* tests were also applied to compare the differences in cigarettes per day between the control group and the colforsin group. Intragroup comparisons of systolic and diastolic arterial pressure, heart rate, R_{awm}, R_{awe}, and C_{dyn} were performed by two-way analysis of variance with repeated measures and paired *t* tests with the Bonferroni correction. Between-group comparisons were made at each time point by unpaired *t* test. Paired and unpaired *t* tests were used to compare differences in plasma epinephrine and norepinephrine. The mean ± SD is given for each value. A *P* value less than 0.05 was considered statistically significant.

Table 1. Demographic Data of Control and Colforsin Groups

	Control Group	Colforsin Group
Sex (M/F)	8/15	10/13
Age (yr)	42 ± 10	40 ± 13
Weight (kg)	59 ± 13	58 ± 8
Height (cm)	164 ± 9	163 ± 7
Smokers (n)	10	10
Smoking (cigarettes/day)	21 ± 10	15 ± 7

Data are presented as mean ± SD unless otherwise indicated.

Results

Preoperative pulmonary function test results did not differ between the control group and the colforsin group, and they did not differ between smokers and nonsmokers (data not shown).

Table 1 shows the demographic data for the two groups. There were no statistical differences between the two groups. Demographic data for smokers and nonsmokers are shown in table 2.

Arterial blood pressure did not change in either group after treatment (fig. 1, *top*). Although heart rate increased after colforsin daropate infusion, it did not change after normal saline infusion (fig. 1, *bottom*). After anesthesia induction, heart rate decreased compared to baseline values in the control group; however, it remained increased after treatment in the colforsin group (fig. 1, *bottom*).

Patients receiving colforsin daropate had significantly lower R_{awm} and R_{awe} at 4, 8, 12, and 16 min after intubation (fig. 2, *top* and *center*), and they had significantly higher C_{dyn} at 4, 8, 12, and 16 min after intubation (fig. 2, *bottom*). In the control group, R_{awm} decreased at 8, 12, and 16 min after intubation when compared with

Table 2. Demographic Data for Smokers *vs.* Nonsmokers in the Study Groups

	Control Group		Colforsin Group	
	Smokers	Nonsmokers	Smokers	Nonsmokers
Sex (M/F)	6/4	2/11	7/3	3/10
Age (yr)	38 ± 13	42 ± 7	38 ± 18	45 ± 9
Weight (kg)	69 ± 17	55 ± 8	60 ± 10	57 ± 6
Height (cm)	170 ± 8	161 ± 8	164 ± 8	162 ± 7
Smoking (cigarettes/day)	21 ± 10		15 ± 7	

Data are presented as mean ± SD unless otherwise indicated.

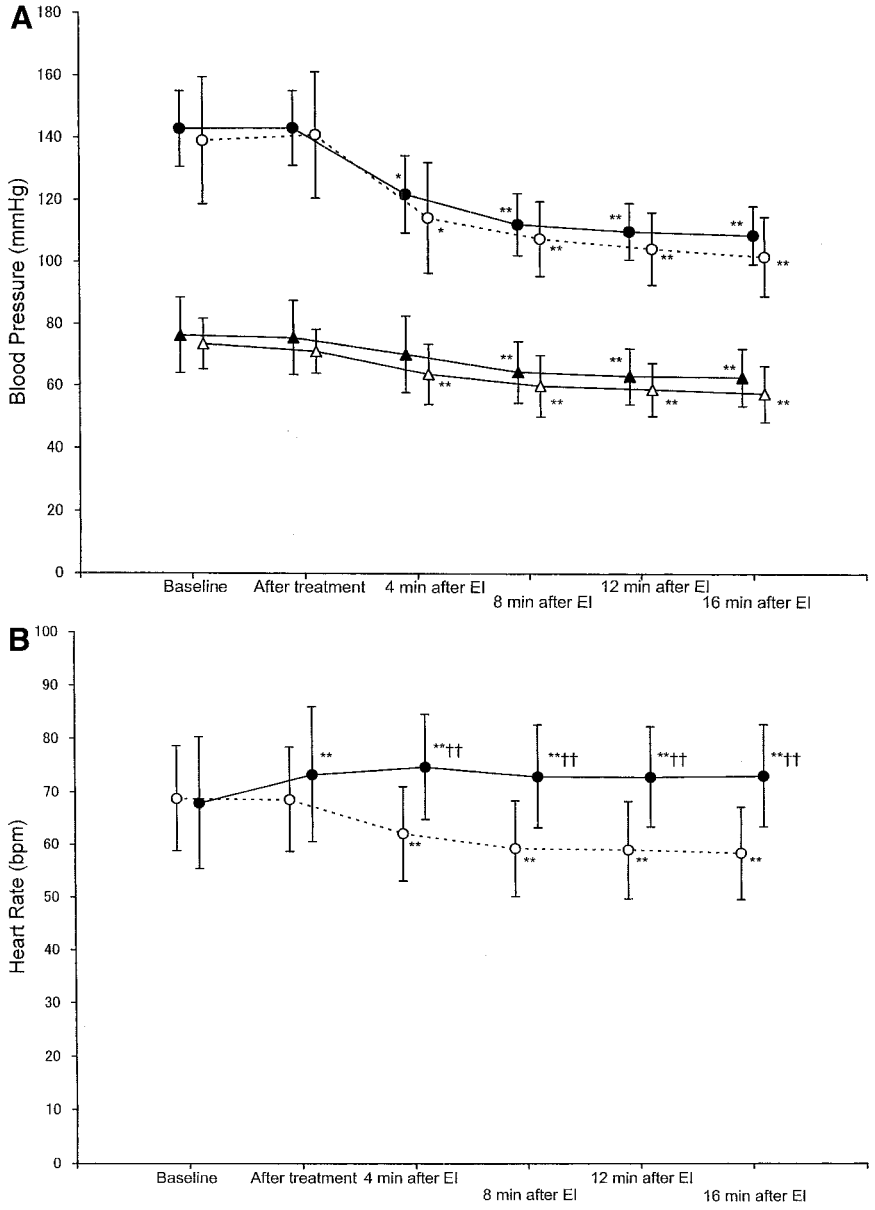

Fig. 1. (*A*) Arterial blood pressure measurements over time in control and colforsin groups. bpm = beats/min; DAP = diastolic arterial pressure; EI = tracheal intubation; SAP = systolic arterial pressure. ● = SAP in control group; ○ = SAP in colforsin group; ▲ = DAP in control group; △ = DAP in colforsin group. *P < 0.01, **P < 0.01 *vs.* baseline. (*B*) Heart rate measurements over time in control (○) and colforsin (●) groups. **P < 0.01 *vs.* baseline. ††P < 0.01 *vs.* control group.

R_{awm} 4 min after intubation (fig. 2, *top*), and R_{awe} decreased at 8 and 12 min after intubation when compared with R_{awe} at 4 min after intubation (fig. 2, *center*). However, R_{awm} and R_{awe} remained unchanged in the colforsin group (fig. 2, *top* and *center*). In the colforsin group, C_{dyn} decreased at 16 min after intubation when compared to C_{dyn} at 4 min after intubation (fig. 2, *bottom*).

Smokers in the control group had a R_{awm} value higher than that of nonsmokers at 4 min after intubation (fig. 3, *top*). After 4 min, R_{awm} was similar in these two group (fig. 3, *top*). However, smokers and nonsmokers in the colforsin group both had a similar R_{awm} at all time points (fig. 3, *bottom*).

Smokers and nonsmokers in both the control and colforsin groups had a similar R_{awe} after intubation (fig. 4).

Smokers in the control group had a lower C_{dyn} than that of nonsmokers 4 min after intubation (fig. 5, *top*). After 4 min, both had a similar C_{dyn} (fig. 5, *top*). In the colforsin group, however, C_{dyn} at all time points was similar for smokers and nonsmokers (fig. 5, *bottom*).

Both groups had comparable baseline concentrations of plasma epinephrine, and the plasma epinephrine concentration remained unchanged after injection in both groups (table 3). Both groups had comparable baseline concentrations of plasma norepinephrine, and even though plasma norepinephrine concentrations increased after medication in the colforsin group, the concentrations did not change in the control group even after injection (table 4).

Discussion

These observations suggest that prophylactic bronchodilator treatment with intravenous colforsin daropate

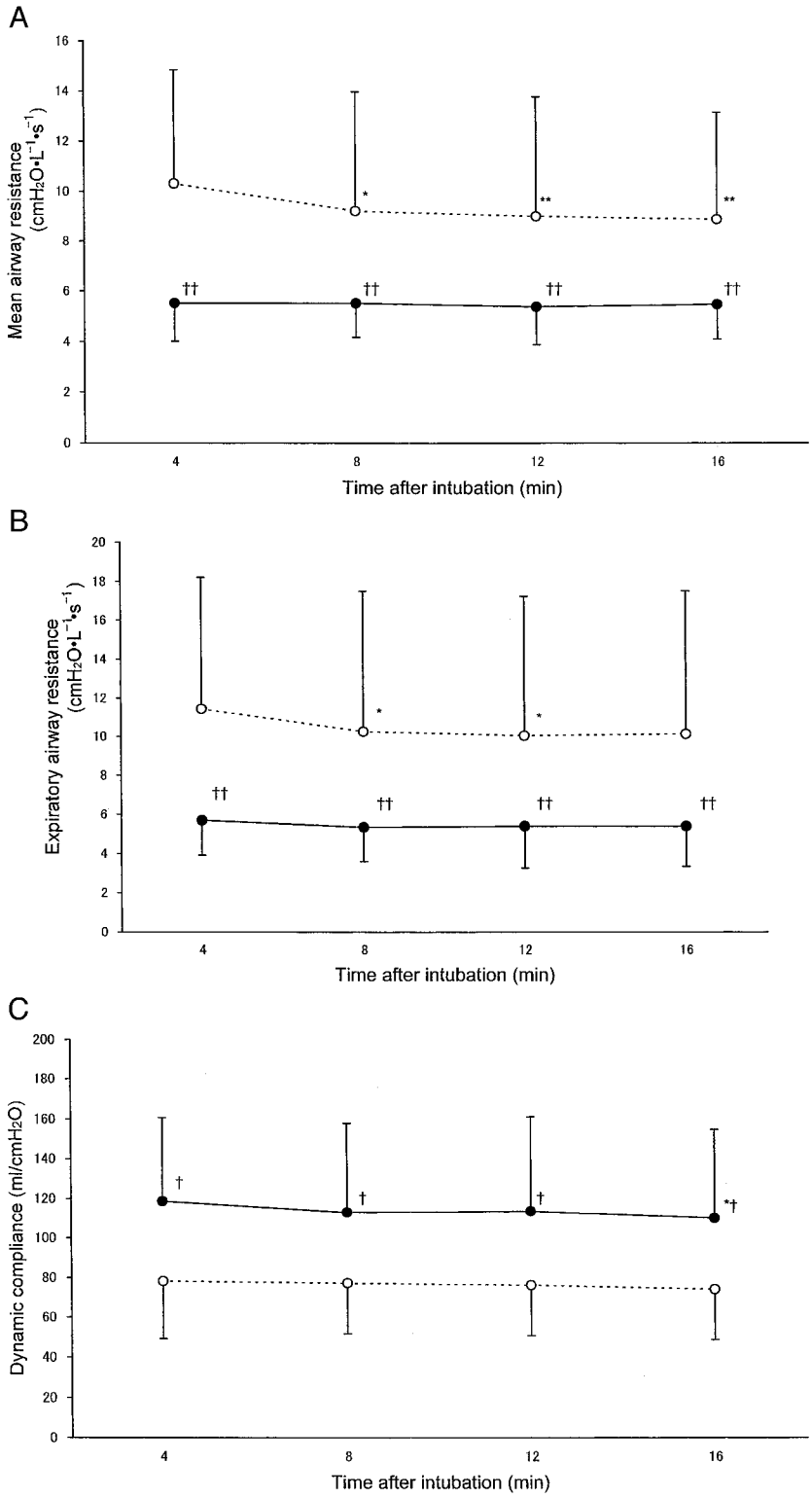

Fig. 2. (*A*) Mean airway resistance (R_{awm}) measurements over time in the control (○) and colforsin (●) groups. **P* < 0.05, ***P* < 0.01 *vs.* 4 min after intubation. ††*P* < 0.01 *vs.* control group. (*B*) Expiratory airway resistance (R_{awe}) measurements over time in the control (○) and colforsin (●) groups. **P* < 0.05 *vs.* 4 min after intubation. ††*P* < 0.01 *vs.* control group. (*C*) Dynamic compliance (C_{dyn}) measurements over time in the control (○) and colforsin (●) groups. **P* < 0.05 *vs.* 4 min after intubation. †*P* < 0.05 *vs.* control group.

before tracheal intubation resulted in lower airway resistance and greater dynamic lung compliance after placement of the endotracheal tube when compared with placebo medication. Moreover, R_{awm} was higher for smokers than for nonsmokers in the control group, and it was similar for smokers and nonsmokers in the colforsin group. C_{dyn} was lower for smokers than for nonsmokers in the control group, and it was similar for both smokers and nonsmokers in the colforsin group.

Thus, colforsin daropate was shown to be an effective bronchodilator in humans. Forskolin is a direct activator of adenylate cyclase[6] and is known to cause a relaxation

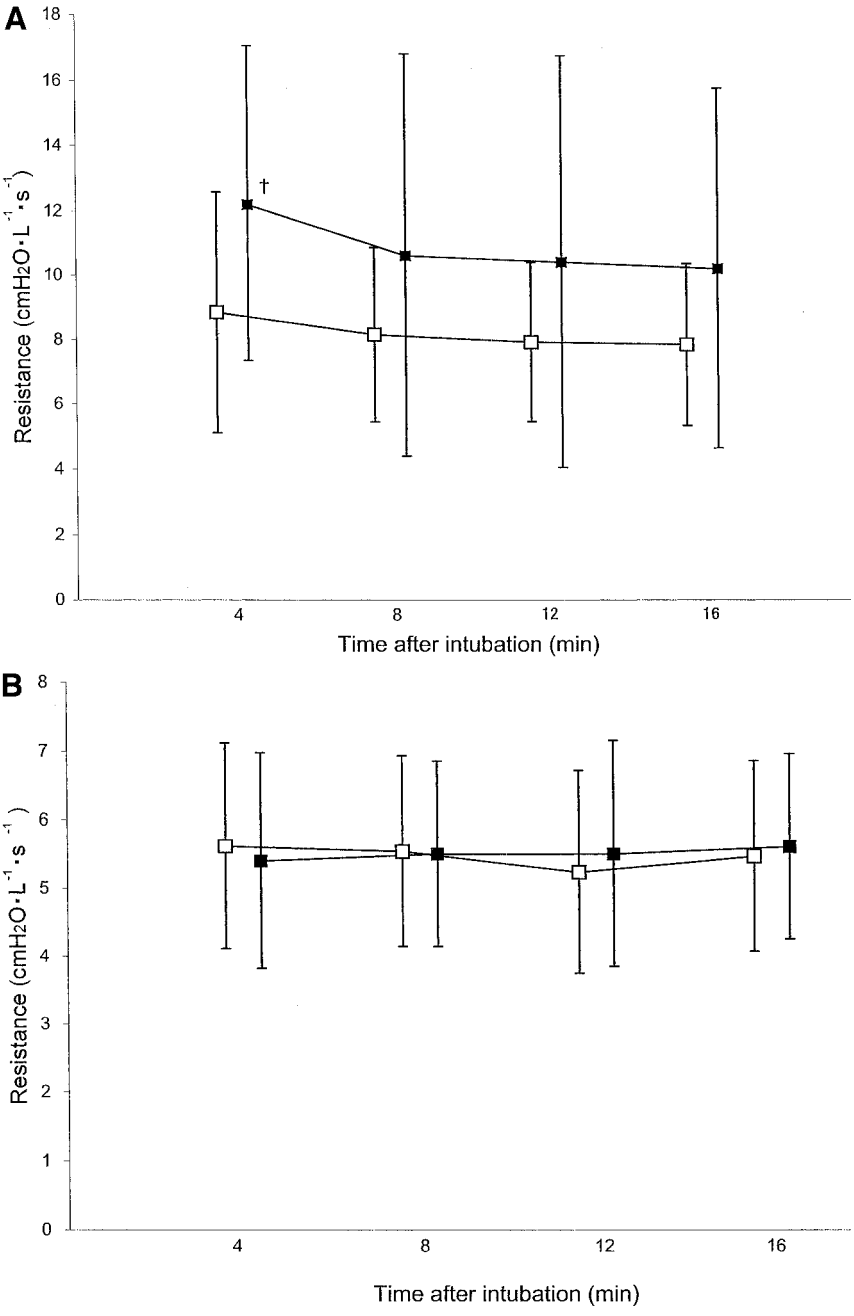

Fig. 3. (*A*) Comparison between smokers (■) and nonsmokers (□) in the control group for mean airway resistance. †$P < 0.05$ *vs.* nonsmokers. (*B*) Comparison between smokers (■) and nonsmokers (□) in the colforsin group for mean airway resistance.

of airway smooth muscle similar to other agents that increase intracellular cyclic adenosine monophosphate (cAMP).[7–12,27,28] Although it is well acknowledged that an increase in the level of cAMP is associated with the relaxation of tracheal smooth muscle, the precise molecular events underlying cAMP-mediated relaxation are not known.[17,29] The involvement of many different mechanisms has been suggested. For example, an increase in cAMP may reduce the affinity of myosin light chain kinase for the Ca^{2+}-calmodulin complex through the phosphorylation of myosin light chain kinase by cAMP-activated protein kinase. This would result in a decrease in the Ca^{2+} sensitivity of the contractile elements.[30] Alternatively, cAMP may reduce intracellular Ca^{2+} by enhancing the Ca^{2+} extrusion to the extracellular space *via* an activation of sarcolemmal Ca^{2+}–adenosine triphosphatase and/or an increase in sodium–Ca^{2+} exchange secondary to the activation of sodium–potassium pump.[29] Ca^{2+} sequestration into intracellular storage sites may also be facilitated by cAMP, leading to a decrease in intracellular Ca^{2+}.[29] Observations using patch clamp techniques have shown that large-conductance Ca^{2+}-activated potassium channels are distributed abundantly in the surface of airway smooth muscle cells[31,32] and that these channels are stimulated *via* cAMP-dependent phosphorylation as well as by a cAMP-independent, membrane-delimited signal transduction process.[32–34] Activation of Ca^{2+}-activated potassium channels should

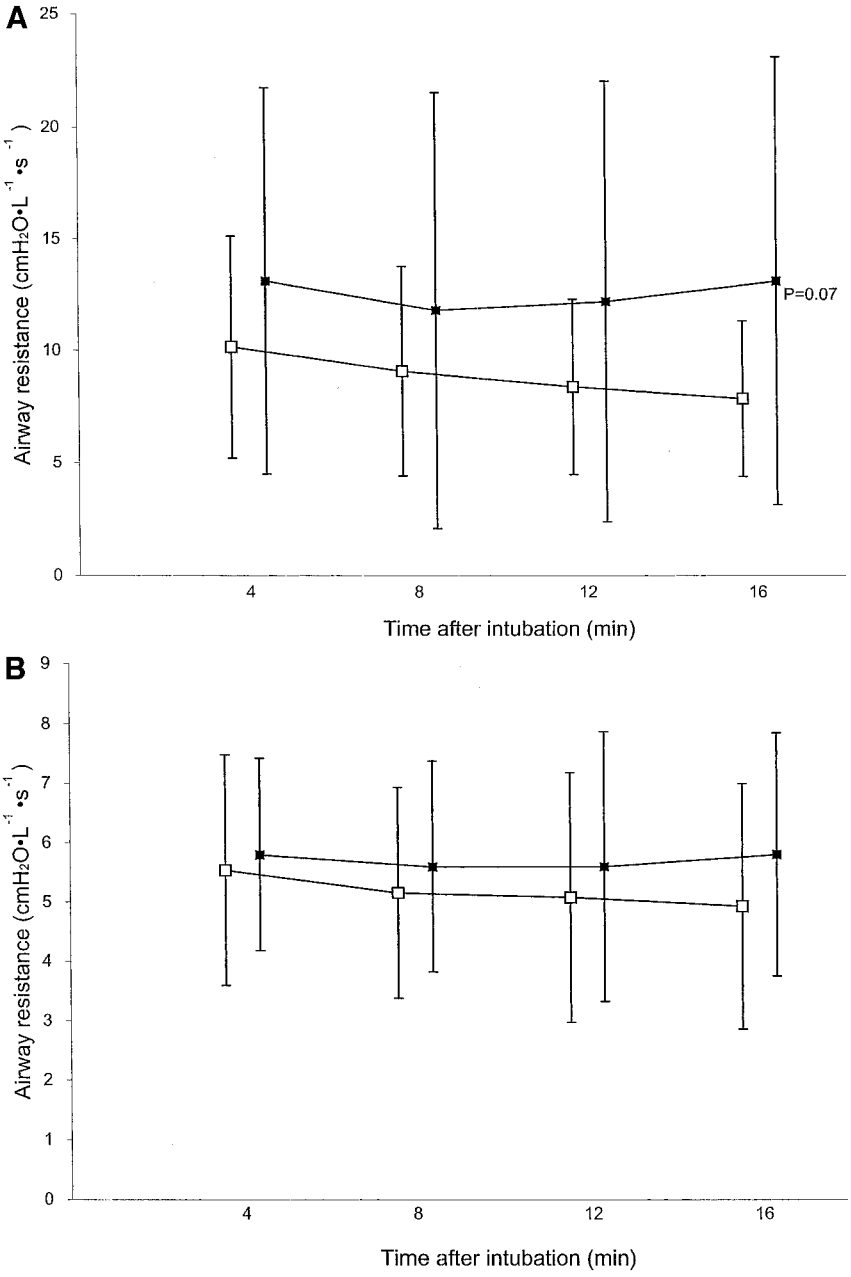

Fig. 4. (*A*) Comparison between smokers (■) and nonsmokers (□) in the control group for expiratory airway resistance. (*B*) Comparison between smokers (■) and nonsmokers (□) in the colforsin group for expiratory airway resistance.

cause the membrane hyperpolarization of smooth muscle cells; this hyperpolarization is expected to inhibit the Ca^{2+} influx through voltage-dependent Ca^{2+} channels.[35] Satake *et al.*[17] reported that with use of guinea-pig isolated tracheas, the bronchorelaxant action of colforsin daropate may result, at least in part, from activation of Ca^{2+}-activated potassium channels, which may cause a hyperpolarization of smooth muscle cell membranes and a secondary decrease in Ca^{2+} influx through voltage-dependent Ca^{2+} channels, leading to a decrease in intracellular Ca^{2+}.

Kil *et al.*[1] showed that there was no difference in lung resistance between smokers and nonsmokers in their placebo group; however, in our study, 4 min after intubation, smokers had higher levels of R_{awm} (fig. 3, *top*) and lower levels of C_{dyn} (fig. 5, *top*) than did nonsmokers in the control group. Although we cannot explain why our results differed from those of Kil *et al.*,[1] we speculate that differences in the study groups may be responsible. The smoking patients in the study of Kil *et al.*[1] had lower ratio of 1-s forced expiratory volume to vital capacity (percent) and forced expiratory flow after 25–75% of expelled vital capacity (percent predicted) values than nonsmoking patients, and the patient group had a moderate degree of obstructive lung disease, especially among smokers. Moreover, their patients were approximately 20 yr older than ours.

Kil *et al.*[1] also showed that after treatment with ipratropium bromide, an anticholinergic bronchodilator, and albuterol, a β_2-adrenergic agonist, postintubation

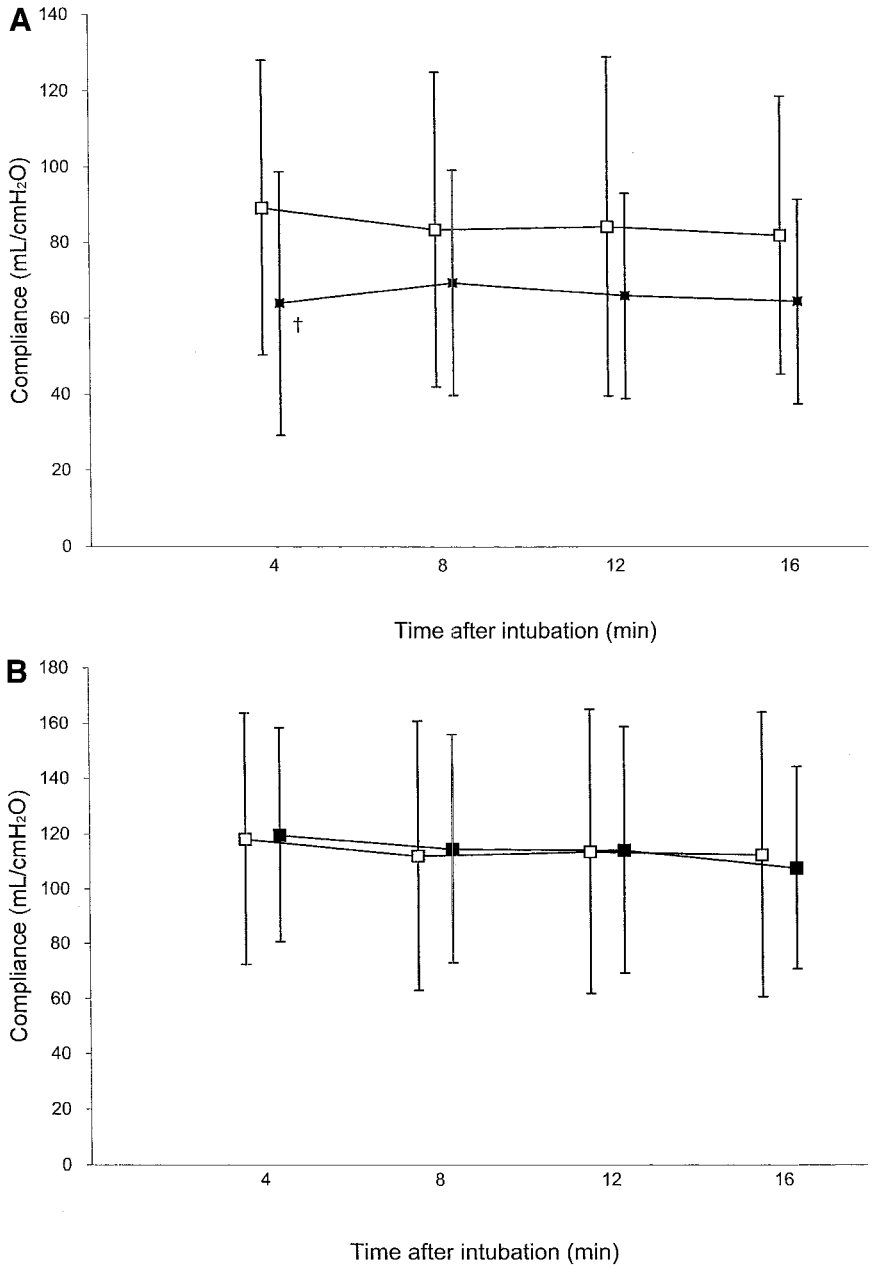

Fig. 5. (*A*) Comparison between smokers (■) and nonsmokers (□) in the control group for dynamic compliance. †$P < 0.05$ *vs.* nonsmokers. (*B*) Comparison between smokers (■) and nonsmokers (□) in the colforsin group for dynamic compliance.

lung resistance was lower for treated nonsmokers than for treated smokers. Our results showed that after treatment by intravenous colforsin daropate, R_{awm}, R_{awe}, and C_{dyn} were similar for smokers and nonsmokers after tracheal intubation (figs. 3 through 5, *bottom*). These results suggest that intravenous colforsin daropate treatment before intubation is beneficial and advantageous for smokers. Kil *et al.*[1] commented as follows: "The lesser response to bronchodilators in smokers are often said to have reactive airways. However, this result may reflect a higher fixed resistance in smokers that in nonsmokers. The airway response to tracheal intubation may be a normal reflex response that may even be blunted in smokers by the presence of chronic irritation

Table 3. Plasma Epinephrine Levels

Group	Baseline	After Injection	*P* Value
Control (ng/ml)	0.08 ± 0.04	0.08 ± 0.04	NS
Colforsin (ng/ml)	0.07 ± 0.03	0.08 ± 0.04	NS

Data are presented as mean ± SD.
NS = not significant.

Table 4. Plasma Norepinephrine Levels

Group	Baseline	After Injection	*P* Value
Control (ng/ml)	0.30 ± 0.13	0.31 ± 0.12	NS
Colforsin (ng/ml)	0.28 ± 0.11	0.30 ± 0.12	< 0.005

Data are presented as mean ± SD.
NS = not significant.

and inflammation. However, this remains speculation." The differences in study groups noted above may be a reason for this difference.

Although they administered inhaled ipratropium bromide and albuterol to their patients, we gave colforsin daropate intravenously. Intravenous administration for 30 min may not be the best method; inhalational may be an alternative or better method. We believe that a suitable inhalational method needs to be developed. In this study, we chose 0.75 $\mu g \cdot kg^{-1} \cdot min^{-1}$ as the dosage of colforsin daropate because the appropriate clinical dose range of intravenous colforsin daropate for acute heart failure is considered to be from 0.25 to 0.75 $\mu g \cdot kg^{-1} \cdot min^{-1}$.[36] However, we could not determine the optimal dose of colforsin daropate in the current study, and further investigation is needed. Colforsin daropate induced tachycardia (fig. 1, *bottom*), which was consistent with previous reports.[37,38] This drug has positive inotropic and vasodilatory actions, makes the cardiac index increase, the pulmonary capillary wedge pressure decrease, the stroke volume increase, and the systemic vascular resistance decrease.[37] Thus, the drug improves hemodynamics in patients with acute congestive heart failure.[37] The effects of this drug are mediated by an increase in intracellular cAMP concentration caused by the stimulatory action of this drug on adenylate cyclase and not through β-adrenoreceptors. Results from several animal experiments suggest that this agent may be effective in patients with severe heart failure who fail to respond to β-stimulants or phosphodiesterase inhibitors.[37]

We recently showed the thiamylal–fentanyl combination induces bronchoconstriction.[15] Cigarini *et al.*[39] reported that 5 mg/kg thiopental followed by a 15-mg $\cdot kg^{-1} \cdot h^{-1}$ continuous infusion with a 5-μg/kg fentanyl bolus injection induces bronchoconstriction. Cigarini *et al.*[39] showed that, under thiopental anesthesia, fentanyl induced a small but highly significant increase in maximum tracheal pressure and respiratory resistance associated with a decrease in respiratory compliance. We suggested that the release of histamine is probably not involved in the bronchoconstriction induced by thiamylal and fentanyl.[15]

Plasma epinephrine concentrations remained unchanged after intravenous colforsin daropate (table 3), and plasma norepinephrine concentrations increased significantly after treatment in the colforsin group (table 4), but we consider the change to be very small and not clinically relevant. These results show that catecholamines are not involved with the lower levels of R_{awm} and R_{awe} and higher levels of C_{dyn} in the colforsin group after intubation compared with those in the control group. Hirota *et al.*[18] noted recently in dogs that catecholamine release may not be responsible for colforsin daropate–induced relaxation.

The results of our study suggest that colforsin daropate is a bronchodilator in humans, and we speculate that colforsin daropate may be useful as a bronchodilator in the treatment of bronchial asthma, similar to other agents that increase intracellular cAMP. Adrenergic down-regulation can occur rapidly in many tissues. Therefore, β_2-agonists might have a rapidly decreasing effect in time, which is a potential problem for the treatment of bronchial asthma.[40] Colforsin daropate may even be effective in patients with bronchial asthma who fail to respond to β-stimulants because the action of this drug is not mediated through β-adrenoreceptors. However, further clinical investigation is required to confirm such speculation.

In conclusion, prophylactic treatment with colforsin daropate produced lower airway resistance and higher dynamic lung compliance after tracheal intubation when compared with placebo medication. Moreover, our results suggest that treatment with colforsin daropate before anesthesia induction and tracheal intubation is beneficial and advantageous for middle-aged and smokers without chronic obstructive pulmonary disease.

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