Diaphragmatic Fatigue is treated with Inhaled Aminophylline Therapy in an Experimental Canine procedure

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ABSTRACT

Background: Diaphragmatic fatigue may contribute to the development of respiratory failure. Although aminophylline administered IV has been widely used to treat diaphragmatic fatigue, to date it has not been used in aerosol formulation for this purpose. Objective: The aim of this study was to assess the efficacy of inhaled aminophylline on contractility of fatigued diaphragm in an experimental canine model.

Methods: This open-label, dose-ranging, pharmacologic study was conducted at the Department of Anesthesiology, University of Tsukuba Institute of Clinical Medicine, Tsukuba (Ibaraki, Japan). Diaphragmatic fatigue was induced in healthy, male, mongrel dogs by intermittent supramaximal bilateral electrophrenic stimulation at a low frequency (20 Hz) applied for 30 minutes. Immediately after the end of the fatigue-producing period, group 1 received inhaled vehicle only, group 2 received inhaled aminophylline 12.5 mg/mL, group 3 received inhaled aminophylline 25 mg/mL, and group 4 was infused with verapamil 0.1 mg/kg·min during inhalation of aminophylline 25 mg/mL. Diaphragmatic contractility was assessed using transdiaphragmatic pressure (Pdi).

Results: Twenty-eight dogs were used in the study (7 dogs were assigned to each treatment group). When fatigue was established, Pdi at low-frequency stimulation decreased significantly from baseline in all groups (all P 0.05), and no significant change in Pdi was found at high-frequency stimulation. In groups 2 and 3, during aminophylline inhalation, Pdi at 20-Hz stimulation increased significantly from fatigued values (both P 0.05). Pdi increased significantly more in group 3 than in group 2 (P 0.05). In group 4, infusion of verapamil offset the increase in Pdi seen with aerosolized aminophylline in fatigued diaphragm. The integrated electrical activity of the diaphragm did not change significantly in any group.

Conclusions: Inhaled aminophylline significantly improved contractility of fatigued diaphragm in a dose-related manner in this experimental canine model

INTRODUCTION

The diaphragm is the most important inspiratory muscle in the respiratory pump. Fatigue of respiratory muscles, especially that of the diaphragm, has been associated with respiratory failure in a variety of pulmonary diseases.1,2 Pharmacologic agents have been examined for the effects on diaphragmatic contractility. Methylxanthine, b2-agonists,digoxin, dopamine hydrochloride, dobutamine hydrochloride, and phosphodiesterase III inhibitors are effective in improving contractility in fatigued diaphragm.3–7 Among these drugs, aminophylline IV, a methylxanthine methyltransferase compound, has been widely used to increase contractility of fatigued diaphragm in healthy subjects8 and in patients with chronic obstructive pulmonary disease (COPD).9 Early studies10–12 suggested the efficacy of an inhaled form of methylxanthine, but, according to a MEDLINE search (key terms: aminophylline, methylxanthine, inhalation, and diaphragmatic fatigue; years: 1974–2002), this modality has not been used to treat diaphragmatic fatigue.

In the present study, we hypothesized that aminophylline could enhance contractility of fatigued diaphragm in dogs when administered as an aerosol. If so, inhaled aminophylline could be an effective therapy for diaphragmatic fatigue in humans. Also, inhalation therapy with aminophylline may play a role in patients with COPD if IV access cannot be attained.

MATERIALS AND METHODS

This open-label, dose-ranging, pharmacologic study was conducted at the Department of Anesthesiology, University of Tsukuba, Tsukuba (Ibaraki, Japan). The protocol was approved by the university's animal research committee, and the care of the animals was in agreement with guidelines for ethical animal research at the University of Tsukuba.6,7 The author performed all measurements and analyses in this study.

Healthy, adult male mongrel dogs weighing 10 to 15 kg were anesthetized with pentobarbital at a 2-mg/kg·h IV maintenance dose, supplemented as necessary to prevent spontaneous movement. Muscle relaxants were not used. Animals were placed in the supine position. Tracheas were intubated with a cuffed tracheal tube, and the lungs were mechanically ventilated with a mixture of oxygen and room air (fraction of inspired oxygen, 0.4) to maintain arterial pressure of oxygen 100 mm Hg, arterial partial pressure of carbon dioxide 35 to40mm Hg,andarterial pH7.35 to7.45.

Therightfemoral arterywas cannulated to monitor arterial blood pressure and to obtain blood gas samples for the measurements. Arterial gas tensions were measured every 30 minutes throughout the study (~4 hours). The right femoral vein was cannulated to administer maintenance fluids (lactated Ringer's solution, 10 mL/kg·h) and pentobarbital 2 mg/kg·h. The left femoral vein was cannulated for the administration of verapamil 0.1 mg/kg·h. Rectal temperature was continuously monitored and maintained at 36.5C to 37.5C using a heating pad.

Both phrenic nerves were exposed at the neck, and the stimulating electrodes were placed around them. Diaphragmatic contractility was assessed using transdiaphragmatic pressure (Pdi) via 2 thin-walled latex balloons, 1 positioned in the stomach and the other positioned in the middle third of the esophagus. The balloons were connected to a differential pressure transducer (TP-604 T, Nihon Koden, Tokyo, Japan) and an amplifier (Model 1257, Nihondenki San-ei, Tokyo, Japan). Supramaximal electrical stimuli (10–15 V) of 0.1-ms duration were applied for 2 seconds at low frequency (20 Hz) and high frequency (100 Hz) with an electrical stimulator (SEN-3301, Nihon Koden). Isometric contractility of the diaphragm was assessed by measuring the maximal Pdi after airway occlusion at functional residual capacity. Transpulmonary pressure—the difference between airway and esophageal pressures—was maintained by measuring same-lung volume before each phrenic stimulation. End-expiratory diaphragmatic geometry and muscle fiber length during contraction were kept constant by placing a close-fitting plaster cast around the abdomen and lower third of the ribcage. Integrated diaphragmatic electrical activity of the crural (Edi-cru) and costal (Edi-cost) parts of the diaphragm was recorded by 2 pairs of fishhook electrodes placed through a midline laparotomy; electrodes were positioned into the anterior portion of the costal part (away from the zone of apposition) in the left hemidiaphragm. Each pair was placed in the parallel fibers 5 to 6 mm apart. The abdomen was then sutured in layers. The signal was rectified and integrated using an integrator with a time constant of 0.1 second and was regarded as the integrated diaphragmatic electrical activity (Edi-cru, Edi-cost).

The dogs were randomly allocated using the sealed-envelope method to 1 of 4 treatment groups of equal size. Baseline measurements of heart rate (HR), mean arterial pressure (MAP), Pdi, Edi-cru, and Edi-cost were recorded in each group. Diaphragmatic fatigue was then induced by intermittent supramaximal bilateral electrophrenic stimulation applied for 30 minutes at a frequency of 20 Hz, an entire cycle of 4 seconds, and a duty cycle of 0.5 (ie, low-frequency fatigue).13 When fatigue was established, vehicle (group 1), aminophylline 12.5 mg/mL (group 2), or aminophylline 25 mg/mL (groups 3 and 4) was inhaled from a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, Pennsylvania) operated by compressed air at 5 L/min. The nebulizer output was 0.14 mL/min. Two concentrations of aminophylline were prepared by dilution with saline 10 mL to 12.5 mg/mL (125 mg) and 25 mg/mL (250 mg), respectively. The dose of aminophylline chosen in this experiment was used in the study by Prigal et al.12 In group 4, verapamil 0.1 mg/kg·min was administered IV during aminophylline inhalation to inhibit calcium influx into the diaphragm muscle.14 Thirty minutes after the start of inhalation in each group, HR, MAP, Pdi, Edi-cru, and Edi-cost were measured. Also, the changes of Edi-cru and Edi-cost (%Edi-cru, %Edi-cost) from baseline were measured.

STATISTICAL ANALYSIS

Statistical analysis was performed using analysis of variance for repeated measurements followed by Bonferroni-Dunn test for multiple comparisons and the Student t test as appropriate. P 0.05 was considered significant. Analyses were performed using the Statistical Package for Social Sciences version 8.0 (SPSS Inc., Chicago, Illinois).

RESULTS

Twenty-eight dogs were included in the study; 7 dogs were assigned to each treatment group. No significant differences in baseline values of HR, MAP, Pdi, %Edi-cru, and %Edi-cost were observed between the groups (Table). In groups 1, 2, and 3, no significant changes were found in HR, MAP, %Edi-cru, or %Edi-cost after study drug administration. However, in group 4, HR and MAP decreased significantly compared with baseline (P 0.05). When fatigue was established in each group, Pdi at low-frequency stimulation decreased significantly from baseline(P 0.05)butP-diathigh-frequency(100-Hz)stimulationdidnotchange significantly. No significant changes in hemodynamic properties (HR and MAP) were observed in any group during fatigue-producing stimulation. With an inhalation of aminophylline in groups 2 and 3, Pdi at 20-Hz stimulation increased from fatigued values (both P 0.05). The mean Pdi value was significantly higher on inhalation in group 3 than in group 2 (P 0.05). In group 4, infusion of verapamil offset the increase in Pdi seen with aerosolized aminophylline in fatigued diaphragm. No significant changes in %Edi-cru and %Edi-cost were observed throughout the study in any group.

DISCUSSION

Low-frequency fatigue is of particular clinical importance because the spontaneous natural rate of phrenic nerve discharge occurs mainly in the low-frequency ranges (ie, 5–30 Hz).15 Therefore, the effect of inhaled aminophylline on contractility in fatigued diaphragm induced by 20-Hz stimulation (ie, low-frequency fatigue) was examined.The results of group 1, in which Pdi-was obtained without an inhalation of aminophylline in fatigued diaphragm, showed that recovery from diaphragmatic fatigue was delayed when stimulated at 20 Hz. This finding was in agreement with previous studies by me and my colleagues.6,7 In the present study, dogs were anesthetized with pentobarbital 2 mg/kg·h because it

Table. Mean (SD) values of hemodynamics, transdiaphragmatic pressure (Pdi), and integrated electrical activity of the crural (%Edi-cru) and costal (%Edi-cost) parts of the diaphragm.

HR heart rate; MAP mean arterial pressure.

*Group 1 inhaled vehicle; group 2 inhaled aminophylline 12.5 mg/mL; group 3 inhaled aminophylline 25 mg/mL; group 4 IV verapamil during 25-mg/mL aminophylline inhalation.

Parameter/Group*		Baseline	Fatigued	Inhalation
HR, bpm	1	141 (9)	140 (8)	142 (9)
	2	140 (10)	142 (9)	141 (10)
	3	139 (8)	140 (8)	142 (8)
	4	143 (9)	143 (8)	130 (9)†‡§||¶
MAP, mm Hg				
	1	124 (7)	126 (8)	125 (8)
	2	122 (8)	123 (9)	123 (7)
	3	125 (10)	125 (9)	124 (10)
	4	123 (6)	122 (7)	112 (8)†‡§||¶
Pdi, cm H2O				
	20-Hz stimulation 1		15.6 (1.6)	11.4 (1.1)†
11.6 (1.3)†				
	2	15.8 (1.6)	11.9 (0.7)†	13.6 (0.9)†‡§
	3	15.7 (1.7)	11.8 (1.1)†	14.3 (0.9)‡§||
	4	15.8 (1.6)	11.9 (1.3)†	12.0 (1.5)†||¶
	100-Hz stimulation 1		22.4 (1.9)	22.0 (1.8)
22.3 (1.9)				
	2	22.4 (2.2)	22.0 (1.8)	22.6 (2.1)
	3	22.1 (1.9)	21.9 (2.3)	22.5 (2.0)
	4	22.3 (1.5)	22.1 (1.7)	22.0 (2.3)
%Edi-cru				
	20-Hz stimulation 1		100.0 (0.0)	99.6 (6.1)
99.6 (6.1)				
	2	100.0 (0.0)	99.7 (6.4)	99.7 (6.4)
	3	100.0 (0.0)	98.8 (3.0)	100.7 (6.2)
	4	100.0 (0.0)	98.6 (6.9)	98.6 (6.9)
	100-Hz stimulation 1		100.0 (0.0)	99.4 (1.5)
99.0 (2.0)				
	2	100.0 (0.0)	98.4 (2.8)	100.1 (2.6)
	3	100.0 (0.0)	99.0 (2.6)	100.4 (1.1)
	4	100.0 (0.0)	98.6 (2.7)	98.6 (2.7)
%Edi-cost				
	20-Hz stimulation 1		100.0 (0.0)	98.3 (4.5)
98.3 (4.5)				
	2	100.0 (0.0)	98.6 (3.8)	100.0 (5.8)
	3	100.0 (0.0)	99.3 (1.9)	100.7 (4.5)
	4	100.0 (0.0)	98.6 (6.9)	98.6 (6.9)

does not affect diaphragmatic contractility.16 Also, the results of group 1, in which Pdi was obtained during vehicle inhalation, showed that Pdi to each stimulus did not change significantly from baseline throughout the study.

In a previous animal study,14 aminophylline administered IV at therapeutic doses (10 mg/kg) increased diaphragmatic contractility by 25% at low-frequency (20-Hz) stimulation. The results of this study suggest that inhaled aminophylline enhances contractility in fatigued diaphragm in a dose-dependent manner. However, in the present study, augmentation (20%) of Pdi during aminophylline 25 mg/mL inhalation was less than that obtained by administering aminophylline IV. The reason for this difference is not known.

Aminophylline administered IV enhances contractility of the diaphragm.3,8,9,14 The mechanism for this is unknown, but extracellular calcium may be necessary for aminophylline to have a potentiating effect on diaphragmatic contractility.14 In the present study, we showed that inhaled aminophylline significantly increased contractility in fatigued diaphragm (groups 2 and 3). However, the mechanism is not clear. To examine the role of transmembrane calcium movement in the potentiation of diaphragmatic contractility by aminophylline, verapamil, acalciumchannel antagonist,was administered IVduring aminophylline inhalation.

As shown in group 4, the augmentation of Pdi by aerosolized aminophylline was offset by verapamil. Thus, inhaled aminophylline augments the generation of force of fatigued diaphragm, perhaps by affecting transmembrane calcium movement. Aminophylline can induce tachycardia and hypotension through positive inotropic and vasodilatory actions.17 In this experiment, however, no significant changes in HR and MAP were found during aminophylline inhalation in groups 2, 3, and 4. When inhaled aminophylline was administered at a concentration 25 mg/mL,

cardiovascular effects were not seen.

According to the MEDLINE search, this is the first report to examine the effect of inhaled aminophylline on diaphragm muscle function. However, the followinglimitations of the study should be considered. First, the plasma concentrations of aminophylline were not measured throughout the experiment. The toxicity of aminophylline severely limits its use because of cardiac dysrhythmias.17 In this experiment, no cardiac dysrhythmias were found when aminophylline was administered as an aerosol, suggesting that inhaled aminophylline at doses 25 mg/mL may not causecardiac dysrhythmias. Regardless of the lack of this measurement, our findings have important therapeutic implications for the developmentofrespiratorymusclefatigue. Second, wedidnotdeterminethe optimal dose of aminophylline by inhalation matched with IV administration. Further studies should consider these limitations.

CONCLUSION

Inhaled aminophylline significantly improved contractility of fatigued diaphragm in a dose-related manner in this experimental canine model (P 0.05). Its potent effect may be caused by transmembrane calcium movement.

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