

# Variable ventilation decreases airway responsiveness and improves ventilation efficiency in a rat model of asthma

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## ABSTRACT

The optimal ventilation strategy in patients receiving mechanical ventilation for severe asthma remains unclear. The effect of conventional ventilation (with constant tidal volume and respiratory rate) and variable ventilation (with the same average but variable tidal volume and respiratory rate) on peak airway pressure and airway exacerbation induced by increasing doses of methacholine was compared in a rat model of asthma. The respiratory rate and tidal volume data were obtained from a spontaneously breathing intact rat during immobility using a whole-body plethysmograph. Peak airway pressure and airway responsiveness to cumulative doses of methacholine were significantly affected by ventilation mode and they were lower in variable ventilation group than in the conventional ventilation group. Also, variable ventilation improved oxygen saturation compared to conventional ventilation. Our results indicate that variable ventilation decreases airway responsiveness and enhances ventilation efficiency in a rat model of asthma. We suggest further investigations on beneficial effects of variable ventilation strategy in mechanically ventilated patients with severe asthma.

## 1. Introduction

Acute exacerbations of asthma can lead to significant morbidity and mortality requiring ventilatory assistance. Approximately 30% of all intensive care unit admissions for acute exacerbation of asthma need mechanical ventilatory support with mortality of 8% (McFadden, 2003). Although mechanical ventilation is life-saving, patients receiving mechanical ventilation for severe asthma are at risk for lung injury, may be due to high peak inspiratory pressures (PIP) (Abd-Allah et al., 2003). Moreover, prior intubation and mechanical ventilation has been known as the strongest risk factor for death from subsequent asthma exacerbations (Pendergraft et al., 2004). However, the optimal approach to reduce morbidity and mortality in mechanically ventilated patients with severe asthma remains unclear.

Variable mechanical ventilation, introduced by Lefevre et al. (1996), seems to be a viable ventilatory strategy for improving respiratory function without increased lung injury compared to conventional ventilation with constant respiratory rate and tidal volume. Variable ventilation, characterized by cycle to cycle variation of respiratory rate and tidal volume, mimics the physiological fluctuation of the breathing pattern in the healthy resting state and can be considered as a promising approach in protective ventilation (Raoufy et al., 2016). Several lines of evidence suggest that variable ventilation is superior to conventional ventilation in terms of respiratory mechanics in

experimental models (Mutch et al., 2007; Huhle et al., 2016; Soluri-Martins et al., 2017). For instance, Mutch et al. have reported that variable ventilation improves lung compliance and reduces mean peak inspiratory pressures and total respiratory system resistance compared to conventional ventilation in an experimental model of severe bronchospasm (Mutch et al., 2007). However, the impact of variable and conventional mechanical ventilation on peak airway pressure and airway exacerbation, which contribute to morbidity and mortality in mechanically ventilated patients with severe asthma, remains unknown.

Based on these observations, we hypothesized that variable ventilation might reduce peak airway pressure and airway exacerbation in an animal model of asthma. To assess this, we evaluated the effect of conventional ventilation (with constant tidal volume and respiratory rate) and variable ventilation (with the same average but variable tidal volume and respiratory rate) on peak airway pressure and airway exacerbation induced by increasing doses of methacholine in a rat model of asthma.

## 2. Material and methods

All protocols were approved by the “Ethical Committee of Tarbiat Modares University”.

Specific pathogen-free male Sprague-Dawley rats (n = 14, weight

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270–300 g) received two intraperitoneal (i.p.) doses of ovalbumin-Al(OH)<sub>3</sub> [1 mg ovalbumin (Grade III; Sigma) and 200 mg Al(OH)<sub>3</sub> in 0.5 ml saline] on Day 0 and Day 7 and eight inhalations of ovalbumin (1 wt%/vol) using a compressor nebulizer (Pari, Starnberg, Germany) for 30 min, every 2 d from Day 14 to Day 28, as previously described (Eslami-Behroozi et al., 2018).

Twenty-four h after the last exposure, animals were anaesthetized with intraperitoneal urethane (1.5 g/kg), tracheally cannulated, and ventilated with a volume controlled ventilator (Harvard Apparatus, Holliston, MA). Pancuronium bromide was intraperitoneally administered to prevent efforts against mechanical ventilation (0.2 mg/kg initial dose, supplemented every 20 min by 0.05 mg/kg). Animals were placed on a heated pad to maintain the core body temperature at 37 °C throughout the experiment. Oxygen saturation (SpO<sub>2</sub>) and pulse rate were continuously monitored using pulse oximetry. Animals were randomly assigned to 60 min of conventional ventilation (CV, *n* = 7) with a constant tidal volume and respiratory rate or variable ventilation (VV, *n* = 7) with the same average but a variable tidal volume and respiratory rate to obtain a minute ventilation equal to that during CV. Positive end-expiratory pressure (PEEP) was always set at 5 cm H<sub>2</sub>O.

**CV group:** animals were ventilated with a constant tidal volume = 1.7 ml and respiratory rate = 77 breaths/min. **VV group:** animals were ventilated with a variable tidal volume (mean = 1.7 ml, coefficient of variation [CV] = 7.4%, *n* = 1000) and respiratory rate (mean = 77 breaths/min, coefficient of variation [CV] = 7.1%, *n* = 1000). The respiratory rate and tidal volume data were obtained from a spontaneously breathing intact rat during immobility using a whole-body plethysmograph (BIODAC-R172, Trita Wavegram Co., Iran). Nonlinear analysis reveals that these time series had irregular and fractal dynamics (Eslami-Behroozi et al., 2018; Pazhoohan et al., 2017). These respiratory rate and tidal volume data were used to control the serial command output to the ventilator which was connected to a PC computer through an RS232C serial interface. We developed an Application Programming Interface (API) in MATLAB software which communicates with the ventilator and allows change ventilation attributes directly from the MATLAB command line. The 1000 cycles of respiratory rate and tidal volume continuously looped itself until the end of the experiments.

For measurement of peak airway pressure, the ventilator's airway pressure output was digitized and saved using LabChart software at a sampling rate of 1 KHz (Powerlab, ADInstruments, Australia). Airway exacerbation induced by increasing doses of methacholine (MCh) was evaluated immediately after 60 min of ventilation. For this purpose, increasing concentrations (0.25–0.5–1–2–4 mg/ml in saline) of MCh were inhaled for 60 s at 5-min intervals. Airway responsiveness to cumulative doses of MCh was expressed as the percentage change in peak airway pressure from basal pressure obtained by saline administration (Eslami-Behroozi et al., 2018; Pazhoohan et al., 2017).

The GraphPad Prism V6.01 (GraphPad Software, San Diego, CA) was used for statistical analysis of data. The significance of differences in SpO<sub>2</sub> and peak airway between groups was assessed by unpaired *t*-test. Airway responsiveness to cumulative doses of MCh for groups were compared using repeated measures two-way ANOVA, with the Bonferroni post hoc test. All results are presented as mean ± standard error of the mean (SEM). *P*-values less than 0.05 were considered statistically significant.

### 3. Results

An example of the respiratory rate and tidal volume sequences for the first 60 cycles of VV is shown in Fig. 1. A representative tracing of airway pressure during CV and VV is illustrated in Fig. 1c. Mean tidal volume, respiratory rate and minute ventilation did not differ between groups during the experiment.

Fig. 2a depicts the percentage change in peak airway pressure after 60 min of ventilation using CV and VV in a rat model of asthma. Each

point is calculated from the percentage change in the average of peak airway pressure in 55–60 min from the average of basal peak airway pressure (0–5 min). Peak airway pressure was significantly affected by ventilation mode and it was higher in CV group than in the VV group (10.3 ± 0.9 vs 4.7 ± 0.7, *p* < 0.001, respectively). Also, VV group had higher SpO<sub>2</sub> compared to CV group after 60 min of ventilation (92.3 ± 1.2 vs. 85.4 ± 1.7, *p* < 0.01, respectively).

Fig. 2b shows the effect of ventilation mode on airway exacerbation induced by increasing doses of MCh. Airway responsiveness to cumulative doses of MCh significantly depended on the mode of ventilation. VV led to lower airway responsiveness as compared with CV in asthmatic rats (*F*(1, 12) = 9.1, *p* < 0.01). Also, SpO<sub>2</sub> was decreased with MCh administration in both groups but remained significantly greater in the VV group than in the CV group (63.2 ± 1.5 vs. 51.4 ± 1.6, *p* < 0.001, respectively).

### 4. Discussion

This study aimed to investigate the effect of VV on peak airway pressure and airway exacerbation induced by increasing doses of MCh in a rat model of asthma. Compared to CV with constant tidal volume and respiratory rate, VV, with the same average but variable tidal volume and respiratory rate, decreased airway responsiveness and enhanced ventilation efficiency via improved SpO<sub>2</sub> and decreased peak airway pressure. To our knowledge, this is the first study addressing the physiological benefits of VV in an animal model of asthma. Our findings provide evidence that ventilation with variable tidal volume and respiratory rate could be considered as an appropriate ventilation strategy in patients receiving mechanical ventilation for severe asthma. However, experimental investigations are required to validate the results of this study, to further evaluate physiological responses to VV (such as gas exchange, respiratory mechanics and inflammatory cytokines), and to rule out the possible adverse effects of VV when applied over a longer time-period.

Our findings confirm previous studies suggesting beneficial effects of VV to improve respiratory function. In a first report on biologically variable mechanical ventilation, Lefevre and colleagues (Lefevre et al., 1996) observed an improvement in respiratory mechanics and oxygenation without increased lung injury through variation of tidal volume and respiratory rate, as compared with CV. In this context, Mutch et al. reported that VV is superior to CV in terms of gas exchange and respiratory mechanics in an experimental model of severe bronchospasm induced by MCh (Mutch et al., 2007). In the present study on a rat model of asthma, we found that VV improves SpO<sub>2</sub> as well as reduces peak airway pressure and airway exacerbation, which contribute to morbidity and mortality in mechanically ventilated patients with severe asthma (Abd-Allah et al., 2003; Pendergraft et al., 2004). However, the beneficial effect of VV strategy to reduce lung injury in mechanically ventilated patients with severe asthma needs further investigations.

Asthma leads to the decreased fractal dimension of bronchial tree and heterogeneity in the distribution of lung ventilation (Boser et al., 2005). The improvement of SpO<sub>2</sub> and peak airway pressure with VV suggests that enhanced airway opening and alveolar recruitment via stochastic resonance phenomenon played an important role in beneficial effects of VV (Suki et al., 1998). The recruitment of atelectatic lung leads to more homogenous distribution of ventilation and improved mechanical properties. However, some other mechanisms such as improved ventilation/perfusion matching (Gama de Abreu et al., 2008), increased release of surfactant (Arold et al., 2003), and enhanced respiratory sinus arrhythmia (Mutch et al., 2005) can also explain the improved physiological outcomes of VV as compared with CV.

In addition, asthma has episodic behavior and clinical symptoms which may be associated with greater distance from normal fluctuation and decreased irregularity of both respiratory rhythm and volume (Raoufy et al., 2016). There is evidence indicating that controllability, severity and prognosis of asthma are accompanied by alteration in

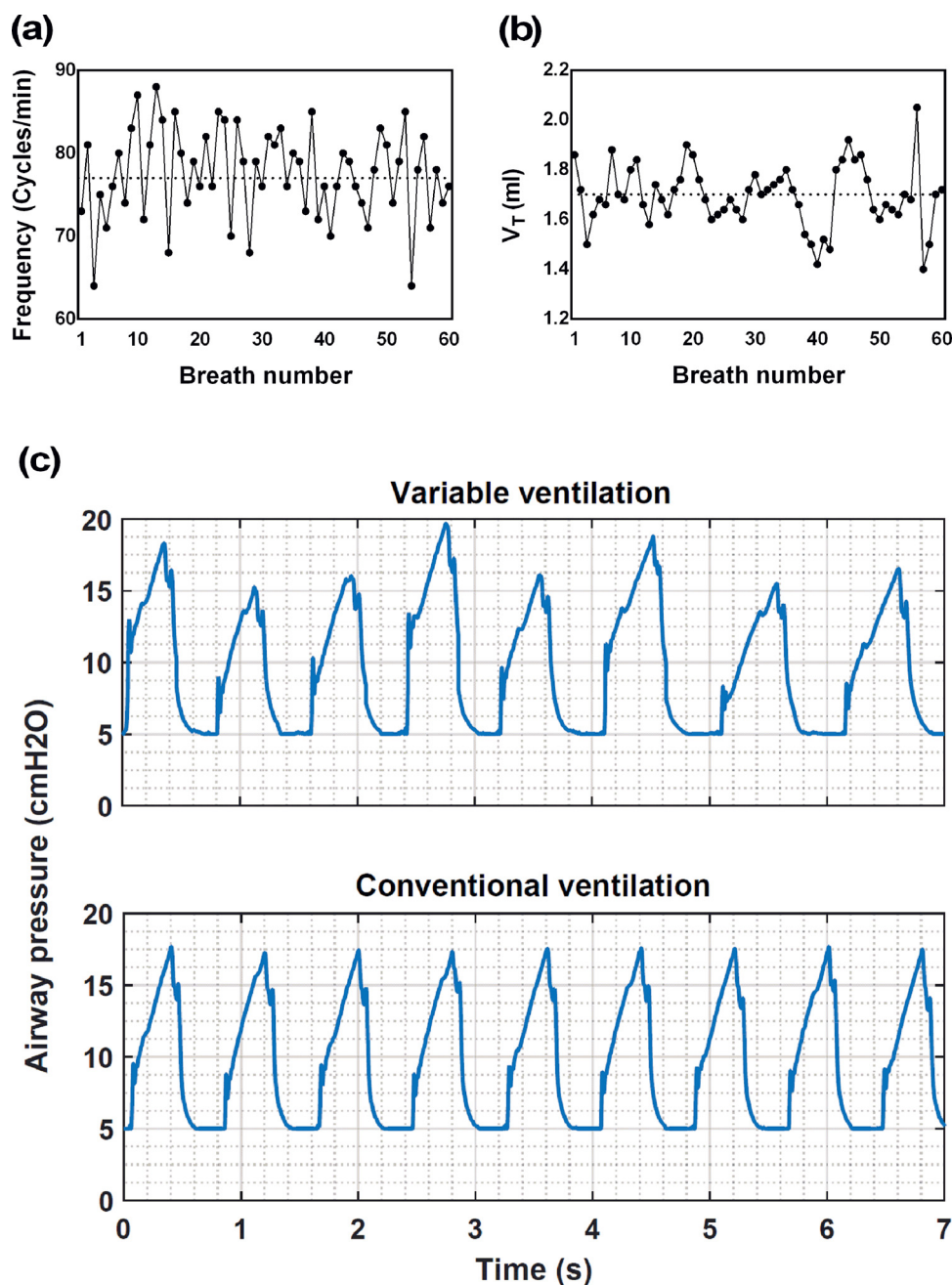


**Fig. 1.** Representative segment of variable ventilation parameters.

(a) and (b), The respiratory rate and tidal volume ( $V_T$ ) sequences during the first 60 cycles of variable ventilation. The mean is shown as the dot horizontal line. (c), A representative tracing of airway pressure during variable ventilation (top) and conventional ventilation (bottom).

variability of breathing pattern and lung function (Thamrin et al., 2016; Raoufy et al., 2017). Based on our findings, ventilation with variable tidal volume and respiratory rate improves airway responsiveness to MCh compared to monotonously regular ventilation in asthmatic rats. Taken together, it seems that asthma leads to increased regularity of respiratory dynamics (i.e. variability of respiratory rhythm and volume) and this alteration might be involved in impairment of respiratory function in asthma. Therefore, a breathing retraining method based on the normal fluctuation of breathing pattern and also ventilation with this variable pattern may decrease the airway responsiveness and even improve the lung function in patients with asthma.

In conclusion, ventilation with variable tidal volume and respiratory rate improved SpO<sub>2</sub> and peak airway pressure as well as airway responsiveness to MCh compared to CV in a rat model of asthma.

However, it remains to be determined whether these beneficial effects of VV can have important implications for future ventilation strategies in mechanically ventilated patients with severe asthma.

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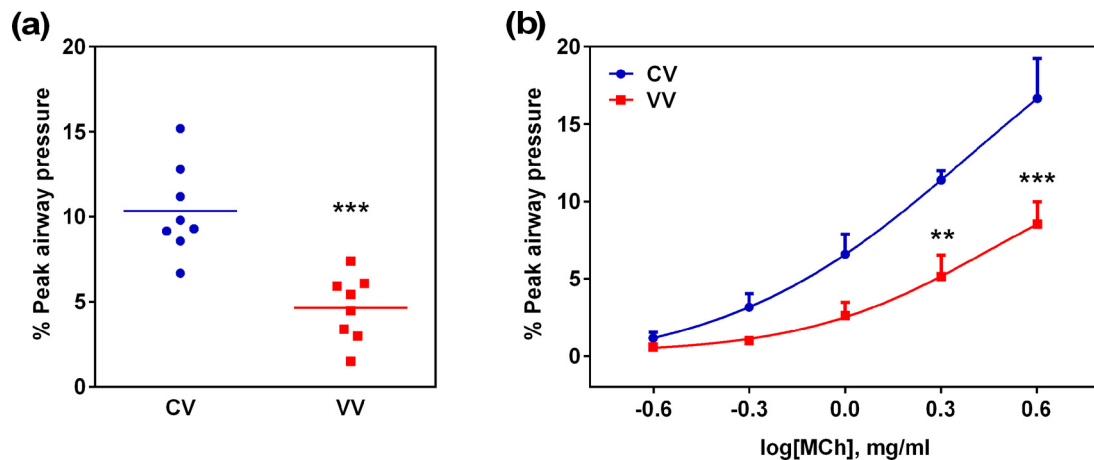


**Fig. 2.** Effect of ventilation mode on peak airway pressure and airway responsiveness in a rat model of asthma.

(a), Percentage change in peak airway pressure after 60 min of conventional ventilation (CV) and variable ventilation (VV). The horizontal bars represent mean values. The significance of differences between groups was assessed by unpaired *t*-test. (b), Effect of ventilation mode on airway exacerbation induced by increasing doses of methacholine (MCh). The values are mean  $\pm$  SEM ( $n = 7$ ); analyzed by repeated measures two-way ANOVA, with the Bonferroni post-test.  $n = 7$ ; \*  $p < 0.01$ , \*\*\*  $p < 0.001$

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