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Short communication

High frequency mechanical ventilation affects respiratory system mechanics differently in C57BL/6J and BALB/c adult mice

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ABSTRACT

We tested the hypothesis that high frequency ventilation affects respiratory system mechanical functions in C57BL/6J and BALB/c mice. We measured respiratory mechanics by the forced oscillation technique over 1 h in anesthetized, intubated, ventilated BALB/c and C57BL/6J male mice.

We did not detect any change in airway resistance, Rn, tissue damping, G, tissue elastance, H and hysteresivity, eta in BALB/c mice during 1 h of ventilation at 150 or at 450 breaths/min; nor did we find a difference between BALB/c mice ventilated at 150 breaths/min compared with 450 breaths/min. Among C57BL/6J mice, except for H, all parameters remained unchanged over 1 h of ventilation in mice ventilated at 150 breaths/min. However, after 10 and 30 min of ventilation at 450 breaths/min, Rn, and respiratory system compliance were lower, and eta was higher, than their starting value. We conclude that high frequency mechanical ventilation affects respiratory system mechanics differently in C57BL/6J and BALB/c adult mice.

1. Introduction

The forced oscillation technique (FOT) allows measurement of respiratory system impedance and has been used to characterize respiratory mechanics in mice in many experimental protocols during physiologic and pathologic conditions (Kelada et al., 2011; Sly et al., 2003). In mice, lung impedance is measured during a 3–8 s apnea produced by a level of anesthesia deep enough to prevent spontaneous breathing, but not cause death. Therefore, to measure impedance in adult mice, some use high frequencies of ventilation ranging from 150 to 450 breaths/min to induce apnea (Cannizzaro et al., 2011; Ito et al., 2004; Zosky et al., 2008).

Ventilation at 450 breaths/min, is about three fold greater than the resting breathing rate of BALB/c and C57BL/6J mice (Tankersley et al., 1994), the two most common strains on which respiratory system impedance is measured. Based on two reports (Kemi et al., 2002; Lee-Young et al., 2011), it is unlikely mice can maintain a breathing frequency of 450 breaths/min because their VO_2 at maximal exercise increased by only 1.1 and 1.8 fold respectively, compared with their resting values.

Moreover, mechanical ventilation alters lung surfactant responsible for a decrease in lung compliance and atelectasis (Albert, 2012). As tidal volume remains the same, a 450 breaths/min ventilation frequency results in alveolar ventilation three times larger than a 150 breaths/min ventilation. Hyperventilation is responsible for an acute hypocapnic alkalosis which exaggerates acute lung injury via several mechanisms including increased lung permeability, decreased compliance secondary to surfactant inactivation, increase airway resistance, reduced hypoxic vasoconstriction and increased intrapulmonary shunt (Curley et al., 2010). Moreover, in vitro studies have demonstrated a deleterious effect of a high respiratory rate responsible for alveolar epithelial cell necrosis and lung injury (Hammerschmidt et al., 2004).

The FOT is widely used to explore different aspects of lung mechanics in many experimental protocols. Therefore, it is important to establish some experimental conditions as close as possible to the physiologic state of the lung. Because the high respiratory rate of ventilation used in some protocols has numerous potential deleterious effects on the lung structure and mechanics, we tested the hypothesis ventilation at high frequency ventilation (450 breaths/min) would affect respiratory mechanics in BALB/c and C57BL/6J male mice.

2. Materials and methods

2.1. Animals

We used C57BL/6J (C57) and BALB/c (BALB) male mice (Jackson Laboratory Bar Harbor, ME). C57 mice were 4–5 months old and weighed 32.7 ± 2.1 g. BALB mice were 3–4 months old and weighed 28.7 ± 1.6 g. All mice were housed four or five per cage in the Division of Comparative Medicine (Georgetown University

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School of Medicine), maintained on a 12 h dark–12 h light cycle, and allowed Rodent Chow 5001, and tap water ad libitum. All procedures were approved by the Georgetown University Animal Care and Use Committee, and comply with the National Institutes of Health guidelines.

Mice were anesthetized by the intra-peritoneal injection of xylazine (~15 mg/kg) and ketamine (~50 mg/kg). After achieving a surgical level of anesthesia (failure to withdraw from a toe pinch), we performed a tracheotomy, inserted an 18 gauge metal cannula into the trachea. The mice were then placed in a supine position on a heating mat, and connected via the tracheal cannula to a computer-controlled small animal ventilator (FlexiVent™, Scireq Montréal, Quebec, Canada). During the time on the ventilator the level of anesthesia was checked (loss of pedal withdrawal reflex) and additional anesthetic (xylazine 10 mg/kg, ketamine 30 mg/kg) was given if needed. Mice were ventilated at 150 breaths/min with room air at a tidal volume (Vt) of 10 ml/kg body mass at a positive end expiratory pressure (PEEP) of ~2.5 cm H_2O.

2.2. Experimental protocol

After an initial inflation to 25 cm H_2O, all mice were ventilated at 150 breaths/min, Vt 10 ml/kg, PEEP ~2.5 cm H_2O for 2 min. Baseline measurements of impedance and static compliance were recorded, before mice were ventilated with the following ventilation protocol. C57 and BALB were ventilated at a tidal volume (Vt) of 10 ml/kg, PEEP ~2.5 cm H_2O with recruitment maneuvers (one inflation to 25 cm H_2O) every 5 min at a ventilation frequency of either 150 breaths/min (C57 VF150, BALB VF150) or 450 breaths/min (C57 VF450, BALB VF 450). We performed breathing measurements after 10, 30 and 60 min of ventilation in mice ventilated at 150 breaths/min (BALB VF150 $n = 9$, C57 VF 150 $n = 15$) and mice ventilated at 450 breaths/min (BALB VF450 $n = 7$, C57 VF450 $n = 15$). After 1 h of ventilation and measurements, the mice were removed from the ventilator, and we observed the mice to determine if they resumed spontaneous breathing, thereby indicating they were alive during the entire procedure. Data from mice that did not resume spontaneous breathing were not included in this report.

2.3. Measurements of mechanical properties of the respiratory system

Measurements of impedance were performed using the forced oscillation technique provided by the FlexiVent™ (Scireq Montréal, Quebec, Canada). After 2 min of ventilation, volume history was standardized by inflating the lung until a pressure at the airway opening of 25 cm H_2O was obtained. After 3 consecutive inflations to 25 cm H_2O, ventilation was resumed for 15 s then stopped. An 8 s volume perturbation, made up of 19 mutually primed sinusoids ranging from 0.25 to 19.5 Hz was then applied to the airway opening by the FlexiVent™, after which ventilation was immediately reinstituted. The constant phase model (Hantos et al., 1992) was fitted to the impedance data to obtain Newtonian airway resistance (Rn) indicating airway resistance, inertance (I), and the constant phase parameters of tissue damping (G) representing energy dissipation and tissue elastance (H) which represents the elastic energy storage within the tissue (Hantos et al., 1992). Tissue hysteresivity (eta) was calculated as G/H. The FlexiVent™ software corrects the values of Rn for the resistance of the tracheal cannula. Most of the inertance (I) resides in the cannula; it was negligible and not reported.

After measurement of impedance, ventilation was resumed for 15 s and static compliance was measured during a slow volume control stepwise inflation to 25 cm H_2O and deflation back to 2.5 cm H_2O.

2.4. Statistical analysis

All statistics were analyzed using GraphPad Prism version 5.04 for Windows (GraphPad Software, San Diego, CA, USA, www.graphpad.com). For each parameter measured, the values for individual animals were averaged per experimental group and the standard deviation of the group mean was calculated. One way analysis of variance (ANOVA) was used for comparisons between baseline and subsequent periods of ventilation within the same group; the Bonferroni post hoc test was used for multiple comparisons. The statistical significance of the difference between C57 mice ventilated at 150 breaths/min and 450 breaths/min, and BALB ventilated at 150 compared to 450 breaths/min at each duration of ventilation, was obtained by an unpaired two-tailed *t*-test.

3. Results

3.1. Airway resistance

Airway resistance Rn remained unchanged over the hour of ventilation in C57 mice ventilated at 150 breaths/min (Fig. 1 column A), and in BALB ventilated at 150 and 450 breaths/min (Fig. 1 column B). However, airway resistance of C57 mice ventilated at 450 breaths/min decreased to a statistically significant extent compared with its baseline value, by 10 min, and remained diminished at 30 and 60 min of ventilation.

3.2. Tissue damping

In C57 mice ventilated at 150 breaths/min, tissue damping G was lower than its baseline value after 60 min of ventilation (Fig. 1 column A). In C57 mice ventilated at 450 breaths/min, G remained unchanged compared with its baseline value. But after 10, 30 and 60 min of ventilation tissue damping was higher than the value observed after the same period of ventilation, in the group ventilated at 150 breaths/min. Tissue damping remained unchanged during the hour of ventilation in the two groups of BALB mice ventilated at 150 and 450 breaths/min (Fig. 1 column B).

3.3. Tissue elastance

In C57 ventilated at 150 and 450 breaths/min, tissue elastance, H, decreased compared with its baseline value; most of the fall occurring within the first 10 min of ventilation (Fig. 1 column A). By contrast, tissue elastance remained constant during the hour of ventilation among BALB mice ventilated at 150 and 450 breaths/min (Fig. 1 column B).

3.4. Tissue hysteresivity

In C57 mice, after 10 min of ventilation at 450 breaths/min, tissue hysteresivity, eta, increased from its baseline value and, in addition, was significantly higher than in C57 mice ventilated at 150 breaths/min (Fig. 1 Column A). This response was also present after 30 min of ventilation, but not after 60 min. During the hour of ventilation, eta remained unchanged in C57 mice ventilated at 150 breaths/min and BALB mice ventilated at 150 and 450 breaths/min (Fig. 1 Column A and B).

3.5. Static compliance

Static compliance (Cs) remained constant in C57 mice ventilated at 150 breaths/min, fell within the first 10 min in C57 mice ventilated at 450 breaths/min, and was significantly lower compared with C57 mice ventilated at 150 breaths/min after 10, 30