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High-Level Pressure Support Ventilation Attenuates Ventilator-Induced Diaphragm Dysfunction in Rabbits

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Abstract: *Background:* The effects of different modes of mechanical ventilation in the same ventilatory support level on ventilator-induced diaphragm dysfunction onset were assessed in healthy rabbits. *Methods:* Twenty New Zealand rabbits were randomly assigned to 4 groups (n = 5 in each group). Group 1: no mechanical ventilation; group 2: controlled mechanical ventilation (CMV) for 24 hours; group 3: assist/control ventilation (A/C) mode for 24 hours; group 4: high-level pressure support ventilation (PSV) mode for 24 hours. Heart rate, mean arterial blood pressure, PH, partial pressure of arterial oxygen/fraction of inspired oxygen and partial pressure of arterial carbon dioxide were monitored and diaphragm electrical activity was analyzed in the 4 groups. Caspase-3 was evaluated by protein analysis and diaphragm ultra structure was assessed by electron microscopy. *Results:* The centroid frequency and the ratio of high frequency to low frequency were significantly reduced in the CMV, A/C and PSV groups ($P < 0.001$). The percent change in centroid frequency was significantly lower in the PSV group than in the CMV and A/C groups ($P = 0.001$ and $P = 0.028$, respectively). Electromyography of diaphragm integral amplitude decreased by 90% ± 1.48%, 67.8% ± 3.13% and 70.2% ± 4.72% in the CMV, A/C and PSV groups, respectively ($P < 0.001$). Caspase-3 protein activation was attenuated in the PSV group compared with the CMV and A/C groups ($P = 0.035$ and $P = 0.033$, respectively). Irregular swelling of mitochondria along with fractured and fuzzy cristae was observed in the CMV group, whereas mitochondrial cristae were dense and rich in the PSV group. The mitochondrial injury scores (Flameng scores) in the PSV group were the lowest among the 3 ventilatory groups (0.93 ± 0.09 in PSV versus 2.69 ± 0.05 in the CMV [$P < 0.01$] and PSV versus A/C groups [2.02 ± 0.08, $P < 0.01$]). *Conclusions:* The diaphragm myoelectric activity was reduced in the PSV group, although excessive oxidative stress and ultrastructural changes of diaphragm were found. However, partial diaphragm electrical activity was retained and diaphragm injury was minimized using the PSV mode.

Key Indexing Terms: Diaphram electric activity; Caspase-3; Injury; Mechanical ventilation; Pressure support ventilation; Modes; Diaphragm; Mitochondrial injury. **[Am J Med Sci 2015;350(6):471–478.]**

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Overwhelming evidence has shown that controlled mechanical ventilation (CMV) induces inactivity of the diaphragm, which activates pathophysiological cascades leading to a loss of contractile force and muscle mass. This phenomenon is referred to as ventilator-induced diaphragm dysfunction (VIDD). VIDD has been observed not only to occur in animals but also in humans.[1–3] Some studies have shown that pressure support ventilation (PSV) reduces the onset of VIDD due to retention of diaphragm motion.[4,5] However, diaphragm electrical activity (Edi) characteristics under different modes of ventilation, set at the same level of ventilator support, are still unknown. Although various modes of ventilation at the identical levels of ventilatory support may all cause VIDD during PSV, the patient triggers each breath and the ventilator delivers a flow up to a preset pressure limit. Thus, it is possible that Edi characteristics, and impact on the diaphragm, are different with PSV compared with CMV and assist/control ventilation (A/C). To date, there has not been sufficient evidence to confirm this phenomenon.

The objective of this study was to explore the effects of PSV on the diaphragm in an animal model and to compare PSV with other modes of mechanical ventilation with all modes providing the same level of support. The data presented herein may provide guidance when selecting a mode of mechanical ventilation.

MATERIALS AND METHODS

Ethics Statement

The study was conducted with the approval of the Animal Care Committee of Zhejiang University (China). All animal procedures were performed in accordance with National Research Council's Guide for the Care and Use of Laboratory Animals.[6]

Animals

Twenty New Zealand White (NZW) rabbits, weighing 2.0 to 2.3 kg, were used in this study. All animals were randomized to 4 groups, according to a random numbers table. Rabbits were anesthetized with 4 mL/kg of 8% chloral hydrate (Shanghai Jinjinle Industry Co, Shanghai, China) administered through intraperitoneal injection. After reaching a surgical plane of anesthesia, each animal was weighed and intubated (inner diameter of 4 mm) through a tracheostomy. A flow sensor was connected to the endotracheal tube and ventilator circuit. Two 20-gauge catheters were placed in 2 marginal ear veins for fluid and drug administration. One catheter was for saline (0.9% saline) and the other was for the sodium midazolam ($1\ mg \cdot kg^{-1} \cdot hr^{-1}$) also used, which provided mild sedation that preserved the drinking reflex. This ensured consistency with the clinical situation and reduced the effect of the sedatives on respiration. Another catheter was inserted into the left common carotid artery to monitor blood pressure and heart rate and for sampling of arterial blood gases, including PH, partial pressure of arterial carbon dioxide ($PaCO_2$) and partial pressure of arterial oxygen. A laparotomy was performed

during the experiment and a pair of wire electrodes was inserted into the 2 flanks of the central tendon portion of the diaphragm for measurement of diaphragmatic electrical activity (RM6240 multi-channel physiological signal acquisition and processing systems, Chengdu Medical Instrument Company, Chengdu, China). All surgical procedures were performed using aseptic techniques. Blood samples were taken from all animals (Cobas B 221 Blood Gas System; Roche Ltd.) at baseline (0 hours) and after 12 and 24 hours of mechanical ventilation. The settings for the ventilation rate were adjusted according to the ABG results. Body temperature was monitored and maintained at 37 ± 1°C with a heat lamp. During the experimental period, animals were kept in a humidity controlled, adequately insulated, quiet environment. Prevention of lung atelectasis was also ensured.

Experimental Groups

Animals were randomly assigned to 4 groups (n = 5 per group) and the experiment time was 24 hours.[7] Group 1: non-ventilated animals (Control); group 2: CMV; group 3: A/C; and group 4: PSV. Animals were mechanically ventilated using a 840 Puritan Bennett Ventilator with a neonate circuit (Neonatal type: Tyco Healthcare Group LP Nellcor Puritan Bennett Division, Ireland, England). The inspired air was humidified.

Ventilator Settings

Control Group: animals were sedated for 24 hours without mechanical ventilation.

CMV Group (volume control ventilation–control mechanical ventilation, VCV–CMV): animals were mechanically ventilated for 24 hours using time-triggered and volume-target ventilation. The tidal volume was 10 mL/kg of body weight and the respiratory rate (RR) was 35 to 40 breaths per minute with a fraction of inspired oxygen (FiO_2) of 21% and 1 cmH_2O positive end-expiratory pressure (PEEP). RR was adjusted to maintain $PaCO_2$ within 35 to 45 mm Hg.

A/C Group (pressure control ventilation-assist/control, PCV-A/C): animals were mechanically ventilated using time triggered and volume target ventilation (10 mL/kg) and assessed plateau pressure (Pplat). The ventilator was then changed to A/C mode and was time/flow triggered. The pressure target was set according to Pplat in the CMV mode (range from 10 to 12 cmH_2O). The trigger was 0.1 L/min; the RR was 35 to 40 breaths per minute, with a FiO_2 of 21% and 1 cmH_2O PEEP. Inspiration time (Ti) 0.5 seconds and the RR was adjusted to maintain $PaCO_2$ within 35 to 45 mm Hg.

PSV Group: animals were mechanically ventilated using time triggered and volume target ventilation (10 mL/kg) and Pplat was assessed. The ventilator was then changed to PSV mode. The pressure target was set according to Pplat in the CMV mode (range from 10 to 12 cmH_2O). The flow trigger was 0.1 L/min, with a FiO_2 of 21% and 1 cmH_2O PEEP. The expiratory trigger was fixed at 25% of expiration peak flow. Back-up settings were the same as in the A/C group.

Main Outcome Measures

All animals were euthenized after 24 hours. The diaphragm of each animal was then quickly removed and split into 3 portions. One section was frozen in liquid nitrogen and stored at −80°C for caspase-3 activity detection by Western blot assay and the 2nd section was immersed in 10% formalin for qualitative observation of caspase-3 protein expression elevation through immunohistochemistry since caspase-3 has been extensively characterized as a regulator of cellular DNA fragmentation in numerous models in skeletal muscle atrophy.[8] A third section was dipped in 2.5% glutaraldehyde solution at 4°C for observation of diaphragm cell ultra-microstructure through electron microscopy.

Electromyography of Diaphragm Analysis

Diaphragmatic EMG included measurements of low frequency (L) electrical activity (20 ~ 40 Hz), high frequency (H) electrical activity (150 ~ 350 Hz) and centroid frequency (Fc, Hz). The ratio of high frequency to low frequency power (H/L) of the diaphragmatic EMG was calculated. The electromyography of diaphragm integral amplitude was analyzed.

Caspase-3 Protein Expression Detection by Western Blot

Activation of caspase-3 protein expression was quantified using a Western blot. The procedure was as follows: extraction of total protein in the diaphragm, determination of protein content, sodium dodecyl sulfate polyacrylamide gel electrophoresis, transfer film, immune response, chemiluminescence and imaging and analysis. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the reference antibody. Quantity 1 software, using Western Blot protein bands, was used to calculate the gray value of a relative expression level of gray scale values. In other words, the target protein expression level = the target band gray value/reference material strip gray value. Anticaspase 3 activated polyclonal antibody was obtained from Abcam Company (USA). The blot contained evidence of AB binding.

Immunohistochemistry (IHC) Analysis

Caspase-3 expression was observed by IHC in the diaphragm cells. Active caspase-3 protein expression was assessed in the diaphragm muscle. Ten high-power fields were randomly observed and at least 1,000 cells were counted. Scoring was done according to high-power, field-positive cell percentage as follows: ≤10% positive cells = 0, 10% to 50% positive cells = 1, 51%–75% positive cells = 2 and ≥75% positive cells = 3 points. Cells were also scored according to color intensity: no coloring = 0 points, weakly stained, pale yellow = 1 point, medium yellow = 2 points; intensely stained, brownish yellow = 3 points. The product of the 2 scores (defined as Apoptotic index, AI) determined the level of protein expression: <4 points was considered low expression and ≥6 points was considered high expression.

Analysis of Diaphragm Ultra-Structure Injury Through Electron Microscopy (EM)

Diaphragm samples were fixed in a 2.5% glutaraldehyde solution for subsequent assay. The fixative was discarded and tissues were then dehydrated, embedded and sliced. Tissues were imaged using an electron microscope (JEM-1200EX; JEOL Company, Japan). Ultra-structural changes of the diaphragm tissue were observed and documented.

Mitochondrial injury scores (Flameng scores) were evaluated to assess the extent of mitochondrial damage. Five fields were obtained from every sample (20 mitochondria were selected in every field) for quantitative analysis. Scoring was done as follows: Grade 0 (0 points): mitochondrial structures are normal and their particles are intact. Grade I (1 point): mitochondrial structures are normal, but the particles are lost. There is also mild swelling, reduced density matrix and ridge separation; grade II (2 points): mitochondrial swelling (slight swelling), but the matrices are transparent and clear. Grade III (3 points): breaks in mitochondrial ridges and the matrix solidification. Grade IV (4 points):