Effects of Low Tidal Volume Ventilation in a Murine Model of Ventilator-induced Diaphragmatic Dysfunction

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Introduction

✓ Mechanical ventilation (MV) is a life-saving procedure in many critically ill patients.

✓ Short period of MV has been associated with ventilator-induced lung injury even in healthy lungs.

Wolthuis EK, et al., ANESTHESIOLOGY 2008
Controlled MV

- Maintains the diaphragm at rest
- Has been implicated in the development of diaphragmatic dysfunction
- Not only in animal models but also in humans.

Ventilator-Induced Diaphragmatic Dysfunction (VIDD)

• Play an important role in the difficulties in weaning critically ill patients from the ventilator.
  Schultz MJ. ANESTHESIOLOGY 2010

• Multiple mechanisms appear to be involved in VIDD
  – increase of reactive oxygen species
  – mitochondrial dysfunction
  – inhibition of the insulin-like growth factor pathway
  – activation of different proteolytic systems, such as the calpains and caspase 3
    • Calpains and caspase 3 are ubiquitous nonlysosomal proteases, and their expression may be up-regulated early in the time course of VIDD.
  Goll DE, et al. Physiol Rev 2003
✓ All of these mechanisms culminate in
  ➢ muscle atrophy
  ➢ injury
  ➢ loss of diaphragmatic force-generating capacity

VIDD according to Tidal Volume

✓ Low tidal volume ventilation (LTVV)

- Important concept of lung protective strategy from ventilator associated lung injury

- However, there was no evidence that LTVV has protective effect on diaphragm, especially on ventilator induced diaphragmatic dysfunction.
Study Objectives

✓ To evaluate the effects of LTVV in murine VIDD model by exploring both histologic and the main protease pathway after MV
MATERIALS AND METHODS
Materials and methods

Healthy male C57/BL6 mice (10-12 weeks, 25-30g)

1) Higher tidal volume MV for 6 h (HTV group, n=6)
   • TV: 10 ul/mg BW in HTV

2) Lower tidal volume MV for 6 h (LTV group, n=6)
   • TV: 6 ul/mg BW in LTV

3) Controls (Control group, n=6).
✓ Anesthesia:
  - IP inj of pentobarbital sodium (50mg/kg body weight)
✓ Tracheostomy:
  - 22-gauge angiocatheter was inserted
✓ Hemodynamic stability
  - 0.05ml RL solution IP every hour
✓ Other general care:
  - bladder expression
  - ocular lubrication
  - passive limb movement
Experimental protocol for MV

✓ Small animal ventilator
  : Flexivent®, SCIREQ Inc, Canada

✓ Ventilator setting:
  CMV mode
  FiO2: 0.21
  RR: 150 rpm
  PEEP: 3-4 cm H2O
  Tidal volume
  – 10ul/mg BW in HTV
  – 6ul/mg BW in LTV
1. Lung Function Measurement
   • In 3 groups
   • **Forced oscillation technique in FlexiVent system**
     – Airway resistance \([R_n]\)
     – Tissue damping (resistance)\([G]\)
     – Tissue elasticity \([H]\)

2. Blood collection & Tissue Collection (Diaphragm)
   • By thoracotomy and laparotomy
3. Measurement of Diaphragm contractile Properties

- The force-frequency relationship
  : Enhanced contractility at higher rates of stimulation
    - at 10, 20, 30, 50, 60, 80, 100, 120 Hz
    - for 600 ms
    - with 1 min between the stimulation trains.

- The fatigability assessment
  - measured by loss of force to repeated stimuli
    - over 10 min
    - with 30 Hz
    - 300 ms duration
4. Measurement of inflammation and atrophy

- Stained by Hematoxylin and Eosin
- The complete area was evaluated by a blinded investigator for invasion of neutrophils and lymphocyte

5. Measurement of the sarcolemmal injury

- Exposed to a fluorescent tracer dye
  - Procion orange; Sigma, St. Louis, MO
6. Biochemical Evaluation

✓ Total levels of **calpains 1 and 2**

✓ **Immonoblot analysis** was performed.

  - **Calpain 1**: ab 49652 1:1,000 dilution in 3% BSA/TBST, 80KDa; Abcam, Cambridge, MA
  - **Calpain 2**: ab39165 1:1000 dilution with TBST containing 5% skim-milk, 76 KDa; Abcam, Cambridge, MA
Statistical Analysis

- Data are presented as mean ± standard deviation.
- Kruskal-Wallis test
  - additional analysis between 2 groups was performed using Mann-Whitney test.
- Repeated measure of ANOVA.
  - Differences in muscle contractility force-frequency response and muscle contractility for fatigability between groups
- Significance was established at P<0.05.
- Statistical analysis was performed using SPSS 21.0.
RESULTS
# Animal characteristics

**Table 1.** Animal characteristics of all 3 groups and mean peak inspiratory pressure (PIP), positive end expiratory pressure (PEEP), and tidal volume (TV) in ventilated groups (HTV and LTV).

<table>
<thead>
<tr>
<th></th>
<th>HTV (n=6)</th>
<th>LTV (n=6)</th>
<th>Control (n=6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>22.0 ± 0.9</td>
<td>21.8 ± 1.1</td>
<td>23.6 ± 2.1</td>
<td>0.273</td>
</tr>
<tr>
<td>Ventilator</td>
<td>3.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIP</td>
<td>11.0 ± 0.9</td>
<td>8.9 ± 0.6</td>
<td>-</td>
<td>0.006</td>
</tr>
<tr>
<td>PEEP</td>
<td>2.5 ± 1.1</td>
<td>2.9 ± 0.03</td>
<td>-</td>
<td>0.873</td>
</tr>
<tr>
<td>TV</td>
<td>0.2 ± 0.007</td>
<td>0.12 ± 0.003</td>
<td>-</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SE.

HTV = high tidal volume; LTV = low tidal volume; PIP = peak inspiratory pressure; PEEP – positive end expiratory pressure; TV = tidal volume.
## Arterial blood gas analyses

<table>
<thead>
<tr>
<th></th>
<th>PH</th>
<th>PaO₂ (mmHg)</th>
<th>PaCO₂ (mmHg)</th>
<th>HCO₃⁻ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTV (n=6)</td>
<td>7.4 ± 0.1</td>
<td>65.2 ± 19.7</td>
<td>21.6 ± 4.4</td>
<td>20.0 ± 5.0</td>
</tr>
<tr>
<td>LTV (n=6)</td>
<td>7.4 ± 0.1</td>
<td>84.5 ± 11.6</td>
<td>21.0 ± 2.3</td>
<td>22.6 ± 0.9</td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>7.4 ± 0.2</td>
<td>92.4 ± 25.3</td>
<td>19.7 ± 5.9</td>
<td>26.1 ± 3.2</td>
</tr>
</tbody>
</table>

Data are represented as mean ± SE.

HTV = high tidal volume; LTV = low tidal volume

No significant differences were observed between groups.
Invasive Lung Function Measurement by Forced oscillation technique (FlexiVent)

<table>
<thead>
<tr>
<th></th>
<th>Airway resistance (RN), cmH2O.s/ml</th>
<th>Tissue Damping (G), cm H2O/ml</th>
<th>Tissue Elasticity (H), cm H2O/ml</th>
<th>P  value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTV (n=6)</td>
<td>0.37±0.09</td>
<td>4.48 ± 1.15</td>
<td>33.1 ± 5.79</td>
<td>0.343</td>
</tr>
<tr>
<td>LTV (n=6)</td>
<td>0.38 ± 0.05</td>
<td>5.10± 0.71</td>
<td>39.2 ± 4.95</td>
<td>0.158</td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>0.32 ± 0.07</td>
<td>4.25 ± 0.77</td>
<td>31.5 ± 7.16</td>
<td>0.087</td>
</tr>
</tbody>
</table>

NS
Force-Frequency Relationship

Enhanced contractility at higher rates of stimulation

* P<0.05, HTV vs control
§ P<0.05, HTV vs LTV
Fatigability Assessment:
Loss of absolute force production of the diaphragm during the fatigue test

*P<0.05, HTV vs LTV and control groups.
Histologic findings for inflammation in diaphragm

No differences between groups
Quantification of the sarcolemmal injury in diaphragm

No differences between groups
Expression of calpain isoforms in diaphragm

No differences between groups
Conclusion

✓ Low tidal volume ventilation
  ✓ partially attenuates the development of VIDD in murine model

✓ Reducing ventilatory support to only that level necessary for respiratory system recovery while avoiding harm is just as true for VIDD as it is for VILI.