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Title: Diaphragm force and mitochondrial function following enhanced nitric oxide availability during mechanical ventilation

Introduction: During mechanical ventilation (MV), the force developed by the diaphragm decreases over time much faster than locomotor muscles, a condition known as ventilator-induced diaphragm dysfunction (VIDD), and it is accompanied by an increase in intramyofiber protein S-nitrosylation, a modification of cysteines by nitric oxide (NO). During acute respiratory distress syndrome (ARDS) treatment, inhaled NO is commonly used to improve gas exchange, but little is known about the effects of increased NO availability to the diaphragm during MV and the intracellular mechanisms to protect against excessive S-nitrosylation.

Hypothesis: We hypothesize that the enzyme S-nitrosoglutathione reductase (GSNOR) protects against excessive protein S-nitrosylation in diaphragm myofibers where NO availability is enhanced during MV, thereby preserving contractile function.

Strategy and Methods: Male (C57BL/6J) 3-4 month-old mice (n=27) were anesthetized and subjected to MV for 0 (non-MV), 2, 4, or 6h (150 breaths/min, 10 cmH_2O PIP, 3.5 cmH_2O PEEP. 8 mL/kg V_T). A group of mice were treated with PBS/10% DMSO (DMSO, n=6) or 25 µg SPL-334 in PBS/DMSO ($GSNOR_i$, n=6) or 25 µg SPL-334 + 1.7 mg ISDN in PBS/DMSO ($GSNOR_i$-ISDN; n=6), and then subjected to MV for 2 or 4 hours. After MV, mice were euthanized and diaphragm strips were used for ex-vivo contractility measurements, and permeabilized fiber bundles for mitochondrial oxidative phosphorylation and H_2O_2 generation measurements.

Results: Peak tetanic force was decreased by MV ~23% at 4h and ~40% at 6h (P=0.0183 and P=0.0062, respectively, one-way ANOVA, Tukey post-test) vs 0 h. However, peak force was not different between DMSO, GSNORi and $GSNOR_i$-ISDN at 2h MV. Coupled-phosphorylating mitochondrial respiration (Kruskal-Wallis H=2.2, p=0.35) was not different but H_2O_2 flux was highest in GSNORi-ISDN vs DMSO and GSNORi (Kruskal-Wallis H=6.6, p=0.03).

Conclusions: Ex-vivo force of diaphragm strips was statistically reduced at 4 hours of mechanical ventilation. Treating mice with GSNORi or ISDN + GSNORi did not affect ex-vivo force of diaphragm strips during mechanical ventilation but increased leak and complex I respiration in permeabilized myofiber mitochondria. However, after 4h of MV, the increase was only present in uncoupled respiration. GSNORi + ISDN increased H_2O_2 production in myofibers after 2 hours of mechanical ventilation but not after 4h of MV. The data suggest that increases in NO-dependent GSNO availability enhanced mitochondrial respiration and ROS production but did not accelerate VIDD during the short period of mechanical ventilation.

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