Diaphragm Force and Mitochondrial Function Ex Vivo Following GSNOR Inhibition In Vivo Preceding Mechanical Ventilation

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ABSTRACT

During mechanical ventilation (MV), force developed by the diaphragm is decreased over time much faster than locomotor muscles. This is known as ventilator-induced diaphragm dysfunction (VIDD), and VIDD may be accelerated by intramyofiber oxidative stress. An important free radical used for the treatment of acute respiratory distress syndrome (ARDS) is nitric oxide (NO) which can diffuse to diaphragm myofibers during treatment. However, little is known whether NO or NO by-products such as S-nitrosothiols (RSNO), can accelerate or prevent VIDD. PURPOSE: To investigate whether inhibiting S-nitrosoglutathione reductase (GSNORi) during MV could affect ex vivo diaphragm force and mitochondrial respiration. METHODS: Male (C57BL6J) mice (n=27) were anesthetized and subjected to MV for 2, 4, or 6h, and non-MV mice (0 h) were used as controls. Alternatively, mice were treated with PBS/10% DMSO (n=6) or 25 µg SPL-334 (GSNORi, n=6) or 25 µg SPL-334 + 1.7 mg isosorbide dinitrate (ISDN; n=6), and then subjected to MV for 2 h. After MV, mice were euthanized and diaphragm strips were used for force or for mitochondrial oxidative phosphorylation and reactive oxygen species generation measurements. RESULTS: Peak tetanic force was decreased by MV starting at 4 h (30 ± 2 N/cm² vs 26 ± 1 N/cm² vs 23 ± 2 N/cm² vs 18 ± 4 N/cm², for 0 vs 2 vs 4 vs 6h MV, P=0.0097 one-way ANOVA). Peak force was not different between DMSO vs GSNORi (P=0.3834). Leak respiration (Mann-Whitney p=0.26; CI95 7, 45 vs 10, 68 pmol/s/mg), coupled-phosphorylating mitochondrial respiration (Mann-Whitney p=0.91; CI95 121, 180 vs 107, 215 pmol/s/mg), and H2O2 flux in any of the respiratory states (e.g. coupled-phosphorylating Mann-Whitney p=0.26; CI95 30, 245 vs 4, 543 fmol/s/mg), were not different between DMSO vs GSNORi. CONCLUSION: VIDD was developed at 4 hours MV, but GSNORi treatment for 2 h did not produce any changes to VIDD and to mitochondrial function. These data suggest that if exogenous NO is not provided, inhibiting GSNOR in vivo alone does not affect diaphragm function ex vivo. Support: SDSU 2023 SEED Grant (to L.N.)