STRONG CHILDREN'S RESEARCH CENTER

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ABSTRACT

Title: *The Effect of Mechanical Ventilation on Lung Thrombospondin-1 Expression and Platelet Activation*

Background: Approximately 93% of extremely low gestational age newborns (born <29weeks) experience respiratory distress, with most requiring mechanical ventilation support while in the NICU[1]. Mechanical ventilation is a life-sustaining procedure; however, it frequently results in lung injury and is correlated with increased incidences of BPD[2]. The mechanism through which ventilation leads to chronic lung disease is poorly understood; making it necessary to further investigate to optimize current treatments and to develop new therapies. Recently, the lung has been found to have a role in platelet production, with estimates that up to 50% of platelets originate from the lung[3]. Infants with BPD have been observed to have lower platelet counts and abnormal platelet volumes[4], and have been found to have high expression of the antiangiogenic protein TSP-1[5] which is highly expressed in platelets. We hypothesize that mechanical ventilation induces TSP-1 expression and increases platelet activation, leading to long-term vascular changes in the lung and BPD.

Objective: To investigate the impact of mechanical ventilation on TSP-1 expression and localization, and to determine if these molecular changes occur concurrently with changes in blood cell levels and platelet activation.

Methods: Adult transgenic *mT/mG;PF4-Cre* mice (n=3-6 per group) expressing fluorescent tdTomato in all tissues except platelets and megakaryocytes, which express GFP[6] were used. All mice were anesthetized with either ketamine-xylazine or isoflurane, and blood samples were collected by retro-orbital bleeding prior to ventilation. Three mice were assigned to each group, and tracheostomy was performed on experimental mice while a sham procedure was performed on controls. Mice were connected to the flexiVent (SCIREQ, Montréal, QC, CA) and exposed to 30 minutes of slow deep ventilation (tidal volume 30 mL/Kg, 50 breaths/min, PEEP 3 cm H_2O, FIO_2 21%). Post-ventilation, retro-orbital blood samples were collected, lungs were washed with PBS to collect BALF, and lung lobes were harvested. CBCs and flow cytometry were performed on blood samples, and lung tissue was used for TSP-1 qRT-PCR, western blotting, and immunohistochemistry. BALF was used to quantify total cell counts in the bronchoalveolar space. Data analyzed using one-way ANOVA and Student's t-test.

Results: *TSP-1* mRNA expression may be increased after mechanical ventilation compared to controls (p=0.1665), however, western blotting revealed no differences at the protein level. Immunohistochemistry suggests increased TSP-1 localization in vessels and the alveolar space. At the cellular level, mechanical ventilation did not alter platelet, white blood cell, monocyte, neutrophil, or lymphocyte levels. Platelet activation was not statistically significant from controls, and BALF cell counts were unchanged pre and post ventilation (p=0.2769).

Conclusion: Mechanical ventilation may induce lung injury and TSP-1 expression in platelets, however, cellular changes in blood are not observable within this timeframe. More data are needed to determine the effects of ventilation on platelet activation.

References:

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Abbreviations:

NICU - Neonatal Intensive Care Unit

BPD - Bronchopulmonary Dysplasia

TSP-1 - Thrombospondin-1

mT/mG – membrane-localized tdTomato/membrane-localized GFP

PF4 - Platelet Factor 4

GFP - Green Fluorescent Protein

PEEP - Positive End-Expiratory Pressure

FIO2 - Fraction of Inspired Oxygen

PBS - Phosphate Buffered Saline

BALF- Bronchoalveolar Lavage Fluid

CBC - Complete Blood Count

PE - Phycoerythrin

CD62P - P-selectin

qRT-PCR - Quantitative Real-Time PCR

DAPI - 4′,6-Diamidino-2-Phenylindole

ANOVA - Analysis of Variance