Research Proposal

Background

Many pathophysiological states can result in a person’s inability to adequately ventilate their lungs with spontaneous breathing, which makes mechanical ventilation a necessary clinical intervention. These include Acute Lung Injury (ALI), Acute Respiratory Distress Syndrome (ARDS), pneumonia, asthma, and Chronic Obstructive Pulmonary Disease (COPD). It is estimated that annually there are 800 thousand hospitalizations in the United States requiring mechanical ventilation with an estimated inhospital mortality rate of 34.5%. This represents 2.7 episodes of mechanical ventilation per 1000 population and .9 deaths per 1000 population. This means that pathological conditions requiring mechanical ventilation lead to more deaths annually than breast cancer and prostate cancer combined. The national costs of mechanical ventilation are estimated at $27 billion dollars per year and account for 12% of all hospital costs in the United States. The largest population of patients requiring mechanical ventilation (53%) is the elderly. And incidence of mechanical ventilation and inhospital mortality both sharply rise with patient age.

Despite its necessity, mechanical ventilation can result in ventilator induced lung injury (VILI). The four proposed mechanisms of lung injury in VILI are over distention of the alveoli (volutrauma) exposure of the alveoli to excessive pressure (barotrauma), cyclic recruitment and derecruitment of the alveoli (atelectatrauma), and secondary injury caused by stretch induced release of inflammatory cytokines from the alveoli (biotrauma). The aging lung undergoes changes in both mechanical properties and response to injuries and inflammation. Over time the chest wall becomes less compliant, the lung loses elasticity, the average alveolar diameter increases, and overall lung capacity diminishes. Over time the lungs capacity to recover from injury diminishes. These physiological changes in the aging lung correlate to each of the proposed mechanisms of VILI. Accordingly age is a predictive factor in the severity of VILI however; the exact relationship between age and the severity of VILI is currently unknown. In our current phase of the VILI project we are conducting experiments to investigate the effects of age on the severity of VILI in an aging mouse model. To accomplish this we are ventilating young and old mice with either protective or injurious ventilator settings and measuring the severity of VILI in each mouse. Our preliminary data supports our hypothesis that increasing age increases the severity of VILI in our mouse model.

We know that in both humans and in mice that VILI often leads to poor alveolar fluid clearance which can develop into pulmonary edema and we know that VILI causes an immune and inflammatory response that can mimic or even lead to Sepsis. These two processes are both known to be important in the disease process of VILI. Our own preliminary data have shown a significant age related decrease in the survival rates of mechanically ventilated mice, which correlate with increases in the severity of pulmonary edema, and can be attenuated by reduced fluid support strategies. It has also recently been shown that both alveolar fluid clearance/edema formation and the development of Sepsis are greatly attenuated in mice treated with high dose Vitamin-C. We hypothesize that high dose Vitamin-C treatments will reduce the severity of VILI and increase the survival of older ventilated mice.

Methods

In our proposed project we will be protectively and injuriously mechanically ventilating young and elderly mice for 4 hour time periods. 30 minutes prior to the ventilation each mouse will either be given an IP injection of 100 microliters of saline or 100 microliters of Vitamin-C at a concentration of 200 mg/kg. We then anesthetize each mouse with an IP injection of 80 mg/kg of pentobarbital. We then place the mouse on a warming blanket and intubate the mouse’s trachea with cannula attached to the port a flexivent small animal ventilator. Just prior to intubation we will begin running either a protective (10mg/kg) or injurious (25mg/kg) ventilation pattern on the ventilator. Immediately after the intubation we paralyze the mouse with an IP injection of 30 mg/kg pancurium bromide. We then attach the thigh clip sensor of the Starr Life MouseOx device to the mouse’s thigh. We wait for 5 minutes for the paralytic to take effect then use the MouseOx device to measure the baseline anesthetized heart rate for the mouse and begin the timer for our four hour ventilation.
At the 0, 2 hour and 4 hour time points we use the ventilation and included software to perform the following forced inspiration maneuvers: Deep Inflation v7.0, Snapshot-150 v7.0, Quick Prime-3 v7.0, Prime-8 v7.0, PVs-V v7.0, and PVs-P v7.0. From which gather the following measurements: tissue damping, tissue elastance, airway resistance, airway compliance, airway elastance, quasi-static lung compliance, Salazar-Knowles parameters for the PV loops performed, inspiratory capacity, PV loop area, and Newtonian resistance of the airways. These measurements will be used to determine the interaction of age and protective/injurious ventilation patterns on the lung mechanics.

During the ventilation if the mouse’s heat rate increases by more than 10% a redo of 30mg/ml of pentobarbital is administered IP. At the 2 hour time point a redo of 30 mg/kg pancurium bromide is administered IP. At 1, 2, 3 hours 100 microliters of saline are administered subcutaneously. At the 4 hour time point the mouse is removed from the ventilator. The mouse’s vena cava is exposed and the mouse is exsanguinated. The blood is analyzed using a siemens blood gas analyzer and the PH, PO2, and PCO2 are measured. These measurements will be used to determine the difference in the effectiveness of the protective/injurious mechanical ventilation in the young/elderly mice.

We then lavage the mouse’s airway by re-intubating with approximately .3 meters of tubing connected to an open (stopper removed) 10ml syringe. We add 5 ml of saline to the open syringe and hold it directly above the mouse and allow saline to fill the lung until the saline stops flowing. We then remove the syringe from the tube and invert the mouse and end of the tube. The saline flows naturally out of the tracheal tube into a collection tube. We hold in this position until the saline stops flowing naturally and repeat the lavage procedure an additional 2 times. We then expose the mouse’s lungs and heart. We perfuse the lungs saline by injecting 10mls of saline into the mouse’s left ventricle repeating up to two additional times as needed. We then clamp of one half of the lung and fill the remaining half of the lung with 4% PFA (10% formalin) using the same gravity feed method previously used to fill the lung with saline. Once one half of the lung is filled with PFA that half of the lung is removed and placed in a 15ml conical tube with 10mls of PFA.

This half of the lung will be analyzed using standard histology technique and damage to the lung resulting from the mechanical ventilation will be measured at the level of the alveoli. The other half of the lung is snap frozen in liquid nitrogen stored at -80 degrees for future protein analysis. The remaining blood is spun at 14k RPMs for 10 minutes and the plasma is collected and stored at -80 for future pro-inflammatory cytokine analysis. The lung lavage fluid is spun at 1k RPM’s for 8 minutes. The supernatant is removed and the cell pellet is re-suspended in 600 mls of saline. The cells are then counted using hemocytometry. If the cell concentration is too high to count with a hemocytometer the cells are further diluted. Every 150mls of the resulting volume of cells are cytopspun onto glass slides, stained, and mounted. These slides are then analyzed using microscopy to determine the ratios of lymphocytes, leukocytes, and macrophages respectively. An N of 6 will be collected for each experimental group to ensure statistical significance.

**Limitations**

The main limitation of this of this study is that mice naturally produce their own Vitamin-C. This means that all control groups will have serum Vitamin-C levels equal to the upper limit of serum Vitamin-C levels that humans can obtain from taking Vitamin-C orally. This means that this model will not be able to make any direct comparisons between our control mouse groups and Vitamin-C deficient human patients. We will however be able to determine if the high dose Vitamin-C treatment conveys protection against VILI for the old mice and potentially for the elderly human patients as well.

**Future Directions of Research**

There are also transgenic mice that are incapable of producing their own Vitamin-C. We are already working collaboratively with a lab that has a colony of Vitamin-C knockout mice and we are planning to ventilate Vitamin C deficient mice as soon as we are able. We are also planning on collaborating with a pulmonary lab at Duke University who has developed a procedure to install a glass window into the chest wall of a mouse. This will permit real time in vivo microscopy of the alveoli of a mouse. The will allow a direct measurement of any physical/physiological changes that occur at the alveolar level of the mouse lung as a result of age, VILI, or Vitamin-C levels.