

Project Title: A trial of suppression of SARS-CoV-2-associated acute respiratory distress syndrome (ARDS) with the hHv1 blocker C6
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SARS-CoV-2 infection produces a wide range of symptoms. Acute respiratory distress syndrome (ARDS) is the leading cause of mortality in severe COVID-19 cases. ARDS is a rapidly progressing inflammatory disease characterized by the disruption of the lung endothelial and epithelial barriers, leading to accumulation of fluid in the airspaces and hence impaired lung function. There is no effective pharmacological strategy for reducing the mortality of ARDS. Human polymorphonuclear leukocytes (PMNs, predominantly neutrophils) have been identified as the primary mediators that initiate and propagate ARDS inflammation. SARS-CoV-2 infection induce alveolar endothelial cells and resident macrophages to secrete chemokines, such as IL-6, which recruit circulating PMNs to migrate into the lungs. In the lungs, PMNs release inflammatory mediators, including reactive oxygen species (ROS), proteases and neutrophil extracellular traps (NETs), contributing to the destruction of the alveolar-capillary barrier leading to accumulation of protein-rich fluid in the alveoli. The human voltage-gated proton channel (hHv1) is characterized by strong voltage and intracellular pH dependent activation and is present in PMN plasma membrane. Mouse Hv1 (mHv1) is suggested to maintain the membrane potential and intracellular pH of PMNs and to be essential to entry of calcium ions in response to chemokines to allow migration. In human PMNs, we have shown that hHv1 mediates the proton efflux required for sustained ROS production using C6, a designed venom toxin-derived peptide blocker of the channel. C6 has 41 residues including six cysteines that form three intramolecular disulfide bonds. C6 inhibits hHv1 currents with an affinity of 31 nM. In preliminary studies, we found C6 inhibited the production of ROS by human PMNs stimulated by the formylated bacterial peptide, fMLF. In addition, intravenous administration of C6 suppressed damage in the lipopolysaccharide (LPS)-induced acute lung-injury mouse model, vouching for its effectiveness in vivo. We hypothesize that C6 will also suppresses pulmonary inflammation and tissue damage in a COVID-19 mouse model. We will first assess the efficacy of C6 in suppressing inflammatory responses of human PMNs stimulated by the ARDS-associated proinflammatory cytokine IL-6. We will measure C6 inhibition on IL-6 induced PMN migration, ROS production, degranulation-protease release and NETs formation. In addition, we will collaborate with Dr. Lane to test the efficacy of C6 in suppressing pulmonary inflammation and alveolar damage in a genetically-engineered mice expressing the SARS-CoV-2 receptor, ACE2 (K18-hACE2 mice) after SARS-CoV-2 infection. These studies will lay a foundation seeking funding for studies to test C6 in humans. ARDS has been reported in ~20% of COVID-19 cases and respiratory failure from ARDS has a mortality rate of >40%. Beyond the immediate challenge of COVID-19, ARDS affects >100,000 patients in the U.S. annually. Regarding the translational potential of peptide therapies, >40 peptides (6 of them are venom peptide-derived) have been approved as drugs for human therapies thus far. The success of this project will offer a new strategy for treating COVID-19 associated ARDS and ARDS from other causes.