SPECIFIC AIMS: *The overarching goal of this proposal is to investigate the use of blood cells as a biomarker of early mitochondrial dysfunction and to investigate a novel therapeutic for the treatment of impaired cellular energy metabolism due to carbon monoxide (CO) poisoning.* CO is a colorless and odorless gas that is an important cause of poisoning annually with an estimated 50,000 emergency department visits occurring in the US. Sources include faulty heat generators, suicidal attempts, and fires. It is a leading cause of poisoning deaths globally. CO poisoning has high mortality and morbidity; and, although hyperbaric oxygen (HBO) is an established treatment of CO poisoning, it is technically challenging to provide and in those that survive over 50% develop long-term cardiac and neurological injuries. Carbon monoxide causes adverse effects by combining with hemoglobin to form carboxyhemoglobin (COHb); however, we have demonstrated additional lethal mechanisms of CO poisoning: mitochondrial dysfunction due to Complex IV (CIV) inhibition.

Significant knowledge gaps remain, including: (1) lack of biomarkers to gauge severity of disease; (2) limited mechanistic understanding of mitochondrial injury; (3) lack of complementary treatment strategies to HBO that target mitochondrial dysfunction in an attempt to mitigate cardiac and neurologic disability. This project will characterize the mitochondrial pathways involved in CO poisoning using peripheral blood mononuclear cells (PBMCs) and platelets (PLTs) against select tissues in a murine model of CO poisoning, furthering the mechanistic understanding of CO poisoning and developing PBMCs and PLTs as potential biomarkers to target organ mitochondrial function. We also propose to investigate an engineered succinate prodrug *in vivo* to improve mitochondrial respiration, sustain cellular function, and limit organ injury. Thus, we propose to address the following critical issues relevant to CO and mitochondrial injury:

- *Investigate and correlate tissue-specific changes in mitochondrial function in a murine model of CO poisoning and determine if blood cells can serve as a proxy for tissue-specific mitochondrial function.*
- *Determine if an engineered cell-permeable succinate prodrug can serve as an alternative source of mitochondrial substrates to attenuate cellular energy crisis and organ injury secondary to CO.*

The long-term goals of our proposed research are to define specific mitochondrial defects in CO poisoning and evaluate a novel mitochondria-directed therapeutic. We have assembled an accomplished team of complimentary researchers who specialize in medical toxicology, critical care, biomarkers in critical illness, drug development, and mitochondrial medicine.

Specific Aim 1: Assess tissue-specific and blood cell mitochondrial response in a murine model of carbon monoxide poisoning

We have demonstrated the potential of PBMCs as a clinical biomarker for CO poisoning in prior publications. In this aim, we will further elucidate the mechanisms of mitochondrial toxicity in CO poisoning, develop mitochondrial endpoints, and map the tissue specificity of the mitochondrial response. We hypothesize that blood cell mitochondrial metrics will exhibit a decrease in CIV respiration, increased reactive oxygen species (ROS), and increased mitochondrial fragmentation that will correlate with similar tissue mitochondrial metrics. We will utilize a dose range of CO exposure (mild, moderate, and severe) and duration that is clinically relevant.

Aim 1A. Characterize mitochondrial function in the two organs most severely affected in CO poisoning, the brain and the heart, by measuring mitochondrial respiration, mitochondrial ROS, and mitochondrial dynamics with the combination of cutting edge respirometry, western blotting, and confocal microscopy.

Aim 1B. Determine if alterations in peripheral blood cell mitochondrial function will correlate with target organ mitochondrial function following CO poisoning as a proof of concept for clinical biomarker development.

Specific Aim 2: Pharmacological treatment of CO-mediated mitochondrial dysfunction in a murine model of carbon monoxide poisoning

We have recently demonstrated a significant increase in bioenergetic efficiency in blood cell mitochondria from patients affected by CO through administering a novel cell-permeable succinate prodrug *ex vivo*. We hypothesize that this alternative substrate supply will increase mitochondrial respiration and decrease mitochondrial ROS, while restoring mitochondrial dynamics in select tissue and blood cells in CO-exposed rats. We will also assess the use of our engineered succinate prodrug for toxicity in a sham control group.

Aim 2. *A cell permeable succinate prodrug will be used post CO-exposure to evaluate mitochondrial functional metrics in both select tissue described in Aim 1 and blood cells.* We will also assess for adverse effects of our treatment in a group of control rodents.

Our proposal incorporates clinically relevant CO doses to examine for any dose-dependent changes in cellular function. Our engineered succinate prodrug represents a potential point of care treatment to address the gaps that currently exist. Our group's expertise in medical toxicology, cellular physiology and biomarkers as well as access to the *in vivo* test compounds will allow us to advance the treatment of CO poisoning.