SPECIFIC AIMS

Despite substantial progress in treatment over the past few decades,[1] myocardial infarctions (MIs) kill 120,000 Americans every year.[2] The best available treatment today is timely reperfusion of the heart using percutaneous intervention (PCI).[1,3] Upon reperfusion, however, the return of oxygenated blood stuns the myocardium, induces arrhythmias, and destroys additional myocytes. [4] This injury, known as ischemia-reperfusion (I/R) injury, explains roughly half of infarct size.[5,6] Molecularly, the pathology stems from a sudden burst of reactive oxygen species (ROS), including superoxide ($O_2^{\cdot-}$) and its rapidly formed dismutation product, hydrogen peroxide (H_2O_2).[7,8] Acutely, ROS damage biomolecules and induce cell death via multiple signaling pathways.[9] Chronically, they induce remodeling that leads to heart failure.[10–12] Antioxidants could ameliorate the damage, but trials of antioxidants have proven disappointing,[13] as they fail to target the cause of the injury effectively[14,15] and may perturb cell signaling in uninjured tissues.[9,16]

My long-term goal is to address the lack of effective therapies for MI and to develop an approach that mitigates damaging ROS production in the reperfused myocardium without perturbing redox balance elsewhere. The overall objective of this proposal is to develop a novel anti-ROS approach in which pathologic levels of H_2O_2 activate small molecule inhibitors of ROS generation exclusively in infarcted areas. Much of the H_2O_2 in I/R injury (and in subsequent heart failure) comes from NADPH oxidase 2 (Nox2) activity.[10,11,17,18] When activated, the proposed agents will inhibit Nox2. To achieve selectivity in drug activation, I will leverage my group's expertise in conditional control of cellular processes using "caging groups." These are nontoxic protecting groups that block a pharmacophore's function until removed via a chemical trigger. My proposed molecules use boronate ester caging group chemistry for selective activation in cells with elevated H_2O_2 levels. The activation process reduces H_2O_2 to water, and generates only non-toxic by-products. **Completion of the following specific aims will test my hypothesis that a novel boronate-based triggering mechanism will allow development of tissue-specific Nox2 inhibitors that selectively target cells accruing pathologic H_2O_2 levels.**

Specific Aim 1. Development and cell-based testing of H_2O_2-responsive Nox2 inhibitors. To ameliorate I/R injury in the heart without perturbing redox balance elsewhere, I will cage known Nox2 inhibitors to become active only in infarcted areas containing high levels of H_2O_2. **Sub-Aim 1A**: Synthesize a series of boronate-caged benzoisothiazolone Nox2 inhibitors. **Sub-Aim 1B**: Determine the kinetics of decaging and activation of the caged inhibitors. **Sub-Aim 1C:** Quantify inhibitor activation, ROS production, and cell survival/cardiac enzyme release upon treatment with caged inhibitors. Successful completion of Aim 1 will afford rapidly-activated Nox2 inhibitors that selectively inhibit ROS production in cells containing pathologic levels of H_2O_2.

Specific Aim 2. Evaluate caged Nox2 inhibitors in preventing acute and chronic injury via targeting injured tissue in a rat model of myocardial I/R injury. To validate this targeted therapy for I/R injury, I will determine the extent to which boron-caged Nox inhibitors protect the heart from excessive ROS production and resulting injury. **Sub-Aim 2A**: Measure Nox2 activity, infarct size, and apoptosis in the ischemic area in a rat model of myocardial I/R with and without caged Nox inhibitor treatment. **Sub-Aim 2B**: Quantify the extent of reductive stress in the uninjured myocardium in animals treated with the caged prodrug or with the active compound in order to assess specificity of the developed approach. **Sub-Aim 2C**: Assess cardiac function 6 weeks post-I/R injury in treated rats. Successful completion of Aim 2 will gauge the potential for caged Nox2 inhibitors to selectively reduce acute myocardial injury and to prevent subsequent heart failure.

Overall Impact: Although treatments are available to minimize damage from ischemia in MI, no treatments *selectively* target damage from I/R injury. As a first step in developing such treatments, I am using a novel strategy that targets injured cells by applying a nontoxic H_2O_2-triggered protecting group. If successful in the proposed studies, these compounds could reduce acute injury and chronic sequelae of MI without perturbing the redox balance in uninjured tissues. This work could provide a generally applicable starting point for treating other conditions involving I/R injury, including stroke, peripheral artery disease, and transplant surgery.

Contribution to training: The proposed research will build on my experience synthesizing caged biologically-active compounds and allow me to learn how to work with cell culture and animal models of I/R injury through my network of expert collaborators and consultants. Through this project, I will learn to develop new approaches to treating unmet clinical needs and to evaluate data from clinically-relevant cellular and animal models, which will prepare me for a career as a physician-scientist at the interface of chemistry and cardiology.