**Effects of Mitochondria-Targeted Antioxidants on Real-time Blood Nitric Oxide and Hydrogen Peroxide Release in Hind Limb Ischemia and Reperfusion**

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**Introduction**

In the body, reperfusion of ischemic tissue with blood causes the release of reactive oxygen species (ROS), in part, from damaged mitochondria leading to endothelial and/or organ dysfunction. Endothelial dysfunction occurs within 5 min of reperfusion, is common to all vascular beds, and is characterized by increased hydrogen peroxide (H$_2$O$_2$) and decreased nitric oxide (NO) levels in the blood that further exacerbate reperfusion injury. Previous studies have shown that promoting endothelial NO synthase coupling during reperfusion increases blood NO and decreases blood H$_2$O$_2$ levels in hind limb I/R and attenuates myocardial I/R injury (1). This study specifically examines the effects mitochondria-targeted antioxidants, mitoquinone (mitoQ; Fig. 1), a cell permeable coenzyme Q analogue or SS-31 ((D-Arg)-Dmt-Lys-Phe-Arg; Genemed Synthesis, San Antonio, TX) (Fig.1), a cell permeable peptide, on inhibiting H$_2$O$_2$ release and increasing NO bioavailability in hind limb I/R. MitoQ (2) and SS-31 (3,4) are able to concentrate into the inner mitochondrial membrane via an electrical potential gradient or selective diffusion respectively, where they attenuate superoxide and subsequent H$_2$O$_2$ production thus allowing a concurrent increase in NO bioavailability (Fig.2).

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**Methods**

Male Sprague-Dawley (SD) rats (275-325 grams, Charles River, Springfield, MA) were anesthetized with an induction dose of 60mg/kg and maintenance dose 50mg/kg of sodium pentobarbital i.prenchestaldehyde (i.p.). The rats also received sodium heparin (1000 USP units/mL) i.v. to act as an anticoagulant. We measured blood H$_2$O$_2$ or NO release from femoral veins in real-time; one vein was subjected to 1R while the other was used as a non-ischemic sham control. The H$_2$O$_2$ or NO microsensors (100 μm, WPI Inc., Sarasota, FL) were connected to a free radical analyzer (Apollo 4000, WPI Inc.) and were inserted into a catheter placed in each femoral vein. Ischemia was induced by clamping the femoral artery/vein of one limb for 30 min followed by 45 min of reperfusion. MitoQ (2 mg/kg), SS-31 (2.5 mg/kg), or saline (for drug control group) was administered as a bolus injection via the jugular vein at the beginning of reperfusion. We continuously recorded the H$_2$O$_2$ or NO release and collected the data at 5 min intervals during a 15 min baseline period, 30 min ischemia and 45 min of reperfusion. The changes in H$_2$O$_2$ or NO release during reperfusion (in picomamps) are expressed as relative change to baseline after correction to the calibration curve of H$_2$O$_2$ (mM) or NO (nM) microsensors. Experimental groups were compared with Student’s t-test or ANOVA using post hoc analysis with the Student-Newman-Keuls test.

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**Results**

**Hypothesis**

We hypothesized that the femoral I/R vein will exhibit increased levels of H$_2$O$_2$ in the blood when compared to the sham vein in the same anesthetized rat. Moreover, we predict that there will be a concurrent decrease in levels of NO released in the femoral I/R vein compared to the sham vein. When mitoQ or SS-31 is given at reperfusion, we expect that the I/R limbs will show decreased H$_2$O$_2$ blood levels and increased NO blood levels compared to the non-drug treated saline controls. As a result, there will be a decrease in ROS and I/R injury.

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**Conclusions**

When mitoQ or SS-31 is given at the beginning of reperfusion, there is a significant reduction of blood H$_2$O$_2$ and a significant increase in endothelial-derived NO bioavailability compared to saline controls. The results of this study support our hypothesis that mitochondria-targeted antioxidant agents can significantly attenuate reperfusion induced ROS release and lead to an increase in NO bioavailability. Collectively, the data suggests that mitoQ or SS-31 can be effective tools in the clinical setting for attenuating post-ischemic insult and endothelial dysfunction. The results also suggest that mitochondrial derived ROS significantly contributes to increased blood H$_2$O$_2$ levels and decreased NO blood levels during reperfusion. Moreover, the mitochondria-targeted antioxidant agents mitoQ or SS-31 were able to attenuate the changes in blood H$_2$O$_2$ and NO levels suggesting that mitochondrial derived ROS are major contributors to oxidative stress in I/R injury.

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**References**