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

T. Galbreath, Q. Chen, R. Ondrasik, M. Bertolet, R. Barsotti, L. Young

Department of Pathology, Microbiology, Immunology & Forensic Medicine, Philadelphia College of Osteopathic Medicine

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 organ dysfunction. Endothelial dysfunction occurs within 5 min of reperfusion, is common to all vascular beds, and is characterized by increased hydrogen peroxide (H_2O_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_2O_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-Amide; Genemed Synthesis, San Antonio, TX) (Fig.1), a cell permeable peptide, on inhibiting H_2O_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_2O_2 production thus allowing a concurrent increase in NO bioavailability (Fig.2).

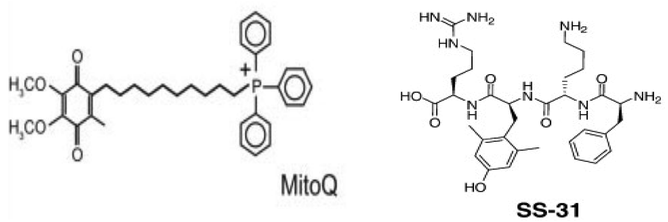

Figure 1. Mitoquinone (mitoQ; 600g/mol) and SS-31 (642g/mol) molecular structure.

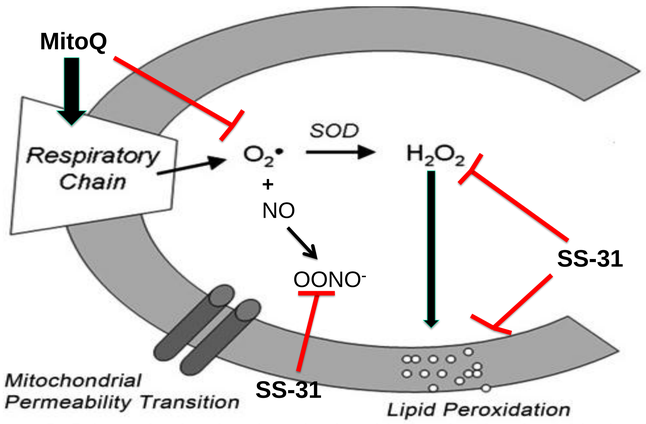

Figure 2. Schematic showing the mitochondrial mode of action of both mitoQ and SS-31 peptide. Red-lines denote areas of inhibition. Adapted from Szeto 2006.

Hypothesis

We hypothesized that the femoral I/R vein will exhibit increased levels of H_2O_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_2O_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.

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 30mg/kg of sodium pentobarbital intraperitoneally (i.p.). The rats also received sodium heparin (1000 USP units/mL) i.p. to act as an anticoagulant. We measured blood H_2O_2 or NO release from femoral veins in real-time: one vein was subjected to I/R while the other was used as a non-ischemic sham control. The H_2O_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 non-drug control group) was administered as a bolus injection via the jugular vein at the beginning of reperfusion. We continuously recorded the H_2O_2 or NO release and collected the data at 5 min intervals during a 15 min baseline period, 30 min ischemia and 45 min reperfusion. The changes in H_2O_2 or NO release during reperfusion (in picoamps) are expressed as relative change to baseline after correction to the calibration curve of H_2O_2 (µM) 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.

Figure 3. The experimental preparation for measuring blood H_2O_2 or NO release from I/R and sham femoral veins in the male SD rats.

Results

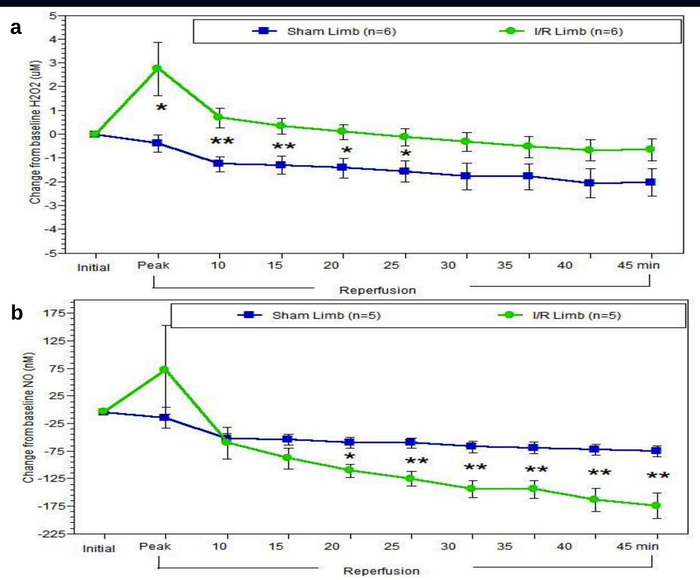

Figure 4. The time course of change in H_2O_2 (µM) or NO (nM) release from anesthetized rat femoral veins in the saline control group. **(a)** There was a significant increase in H_2O_2 release during the first 25 min of reperfusion from I/R veins compared to sham veins in saline controls (* p<0.05, **p<0.01 from sham).
(b) There was a significant decrease in NO release from I/R veins compared to sham veins from 20-45 min reperfusion in saline controls. I/R and sham limbs were compared using Student's t-test (* p<0.05, **p<0.01 from sham).

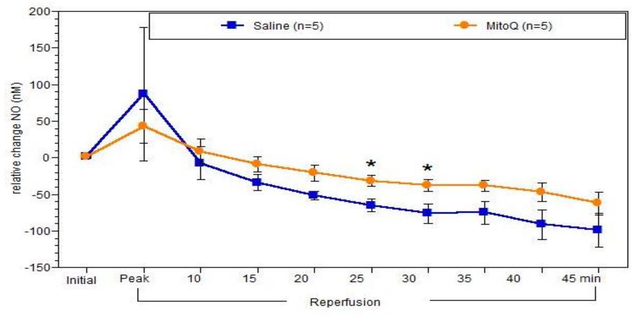

Figure 5. Comparison of relative difference in NO release between I/R and sham femoral veins during reperfusion. There was a significant increase in NO release in the mitoQ-treated group at 25 min and 30 min of reperfusion (* p<0.05 from saline control using Student's t-test).

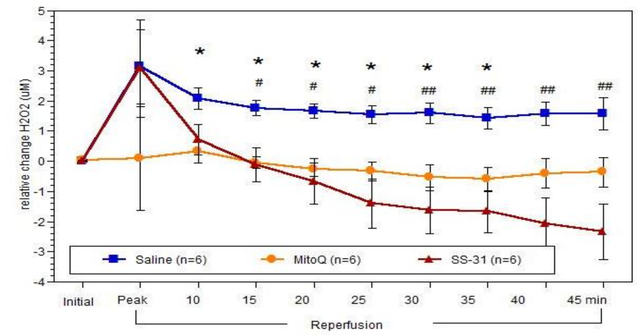

Figure 6. Comparison of relative difference in H_2O_2 release between I/R and sham femoral veins during reperfusion in saline, mitoQ, or SS-31 groups. There was a significant decrease in H_2O_2 release in the mitoQ-treated and SS-31 treated groups from 10-35 min of reperfusion and 15-45 min respectively (MitoQ * p<0.05, SS-31 # p<0.05, ## p<0.01 from saline controls using ANOVA).

Conclusions

When mitoQ or SS-31 is given at the beginning of reperfusion, there is a significant reduction of blood H_2O_2 and a significant increase in endothelial-derived NO bioavailablity 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_2O_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_2O_2 and NO levels suggesting that mitochondrial derived ROS are major contributors to oxidative stress in I/R injury.

References

1. Perkins K, Pershad S, Chen Q, et al. The effects of modulating eNOS activity and coupling in ischemia/reperfusion (I/R). 2012;385(1):27-38.
2. Adlam VJ, Harrison JC, Porteous CM, et al. Targeting an antioxidant to mitochondria decreases cardiac ischemia-reperfusion injury. *The FASEB Journal*. 2005;19(9):1088-1095.
3. Szeto HH. Cell-permeable, mitochondrial-targeted, peptide antioxidants. *The American Association of Pharmaceutical Scientists*. 2006;8(2):E277-E283.
4. Szeto HH. Mitochondria-targeted cytoprotective peptides for ischemia-reperfusion injury. *Antioxidants & redox signaling*. 2008;10(3):601-620.