Effects of Mitochondrial-Targeted Antioxidants on Real-Time Blood Nitric Oxide and Hydrogen Peroxide Release in Acute Hyperglycemic Rats

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Introduction

Diabetes and prediabetes are major public concerns worldwide due to the high risk of developing micro- and macro-vascular complications. Hyperglycemia, the major criteria for diabetes diagnosis, is causally related to pathogenesis of vascular complications in diabetic patients. One of early events in hyperglycemia is vascular endothelial dysfunction. Normally, vascular endothelium facilitates blood flow principally by releasing endothelial-derived nitric oxide (NO) via vascular endothelial NO synthase (eNOS) in the presence of tetrahydrobiopterin (BH_4). By contrast, acute and chronic hyperglycemia increase oxidative stress and reduces NO bioavailability [1, 2]. The reduced endothelial-derived NO bioavailability promotes vasoconstrictive, pro-inflammatory, and pro-thrombotic events, initiating inflammation and eventually resulting in tissue/organ damage (Figure 1). Therefore, reduction of oxidative stress under hyperglycemia will mitigate vascular endothelial dysfunction and organ damage. Crabtree et al found that mitochondria-derived superoxide (SO) contributes to hyperglycemia-induced oxidative stress in cultured vascular endothelial cells. Subsequently, the overproduction of SO promotes the eNOS to shift its product from NO to SO due to oxidation of BH_4 to dihydrobiopterin (BH_2) [1] (Figure 1). However, the role of mitochondria in acute hyperglycemia-induced oxidative stress and blood NO reduction has not been evaluated in vivo. Recently, our lab showed that mitoquinone (MitoQ) and SS-31 (Szeto-Schiller, D-Arg-Dmt-Lys-Phe-Amide) peptide (Figure 2), mitochondria-targeted antioxidants, significantly reduced blood H_2O_2 (an index of oxidative stress) and increased blood NO in a hind limb ischemia/reperfusion (I/R) animal model [3]. Oxidative stress is also an important cause of reperfusion injury during I/R. Thus, we hypothesize that MitoQ and SS-31 will reduce blood oxidative stress and increase blood NO under acute hyperglycemic conditions.

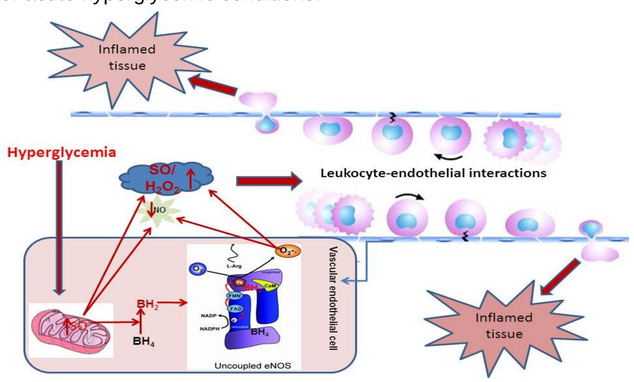

Figure 1. Possible role of mitochondrial-derived SO in hyperglycemia-induced oxidative stress, vascular endothelial dysfunction and tissue inflammation.

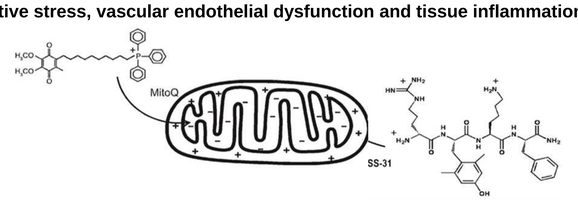

Figure 2. Chemical structure of MitoQ and SS-31 Adapted from Szeto 2008 [4].

Hypothesis

We hypothesized that acute hyperglycemia (200 mg/dL) would increase H_2O_2 and decrease NO levels in blood relative to saline group. By contrast, MitoQ and the SS-31 peptide would attenuate acute hyperglycemia-induced oxidative stress (e.g., H_2O_2) and improve vascular function (e.g., increase NO production) under acute hyperglycemia.

Methods

Male Sprague-Dawley rats (275 to 325g, Charles River, Springfield, MA) were anesthetized with 60 mg/kg of pentobarbital sodium with 1000 unit heparin via intraperitoneal (i.p.) injections. The jugular vein was catheterized to allow for the infusion of saline, 20% D-glucose, or 20% D-glucose with 1.86 mg/kg MitoQ (MW=600 g/mol; complexed with cyclodextrin to improve water solubility, total MW=1714 g/mol) or with 2.7 mg/kg SS-31 (MW=640 g/mol, Genemed Synthesis, Inc., San Antonio, TX). The continuous infusion of 20% D-glucose solution was to maintain hyperglycemia around 200 mg/dL for about 180 min. MitoQ or SS-31 was added to 20% glucose to reach approximately 13 μM and 50 μM in blood, respectively. Both femoral veins will be exposed and catheterized in order to place the calibrated NO and H_2O_2 microsensors (100 μm, WPI Inc., Sarasota, FL) at random into each femoral vein. These microsensors were then connected to the Apollo 4000 free radical analyzer (WPI Inc., Sarasota, FL) to measure for blood NO and H_2O_2 levels in real-time. NO, H_2O_2, and glucose levels will then be recorded at baseline and at 20 minute intervals throughout the 180 minute infusion period [2].

The changes of blood NO (nM) and H_2O_2 (μM) levels were expressed as the relative change to the baseline or to saline group, respectively. All the data was represented as a mean ± SEM. The data were then analyzed by ANOVA using post hoc analysis with the Student Newman Keuls. $p<0.05$ was considered as significant.

Results

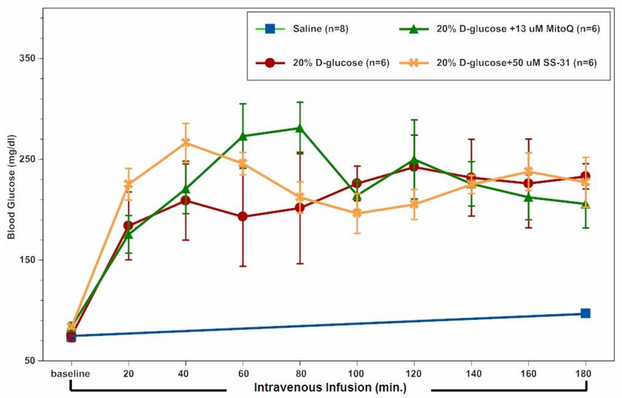

Figure 3. Blood glucose level among saline and 20% glucose infusion groups.

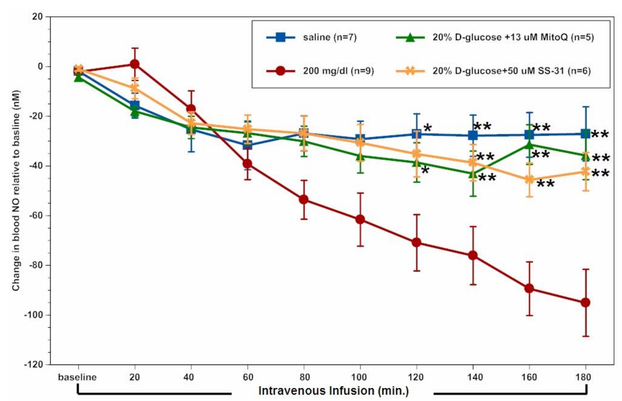

Figure 4. The comparison of change in blood NO levels relative to baseline among saline, 20% D-glucose, 20% D-glucose with MitoQ (13 μM), and 20% D-glucose with SS-31 (50 μM) groups (*$p<0.05$, **$p<0.01$ vs D-glucose).

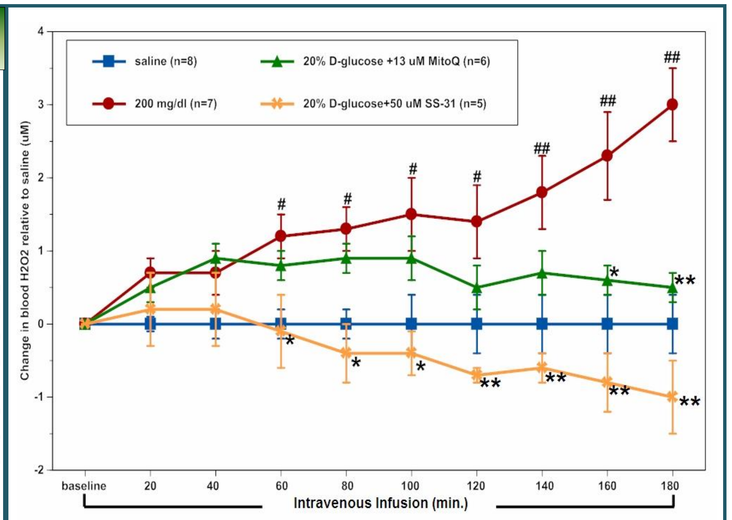

Figure 5. The comparison of change in blood H_2O_2 levels relative to saline group among 20% D-glucose, 20% D-glucose with MitoQ (13 μM), and 20% D-glucose with SS-31 (50 μM) (#$p<0.05$, ##$p<0.01$ vs saline; *$p<0.05$, **$p<0.01$ vs D-glucose).

Conclusions

We found that acute hyperglycemia significantly reduced blood NO levels compared to saline group. The administration of MitoQ or SS-31 during hyperglycemia significantly improved blood NO levels, similar to saline control. Meanwhile we found acute hyperglycemia maintained a higher level of H_2O_2 in blood compared to saline group. By contrast, MitoQ or SS-31 during hyperglycemia significantly reduced blood H_2O_2 levels compared to those under hyperglycemia. Moreover, SS-31 treatment showed a trend to reduce blood H_2O_2 levels more than those in MitoQ treatment, but was not significant. These results suggest that mitochondrial derived SO is a significant source of oxidative stress and vascular endothelial dysfunction under acute hyperglycemic conditions. Moreover, treatment with mitochondrial-targeted antioxidants, MitoQ or SS-31, may be beneficial to attenuate hyperglycemia induced oxidative stress and vascular endothelial dysfunction.

References

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