Effects of NOX1 Inhibition on Real-Time Blood Nitric Oxide and Hydrogen Peroxide Levels in Acute Hyperglycemia

Ashley E. Mawhinney, Matthew Bertolet, Michael Minni, Robert Barsotti, Lindon Young, Qian Chen

Department of Bio-Medical Sciences, Philadelphia College of Osteopathic Medicine
4170 City Avenue, Philadelphia, PA 19131

Introduction

Chronic hyperglycemia is a major condition of diabetes and it leads to vascular complications in diabetic patients. In non-diabetic patients, acute hyperglycemia also results in decreased wound healing and immune function, increased myocardial infarction size post myocardial infarction, and mortality. These effects of hyperglycemia are initiated by vascular endothelial dysfunction which is characterized by increased levels of reactive oxygen species (ROS) and decreased levels of endothelial-derived nitric oxide (NO). Endothelial NO synthase (eNOS) produces NO and is responsible for maintaining an anti-inflammatory surface to facilitate blood flow. NADPH oxidases are a principle source of superoxide (SO) during inflammation [1]. SO can directly quench NO by forming peroxynitrite which induces eNOS uncoupling and additional ROS release (see figure 1).

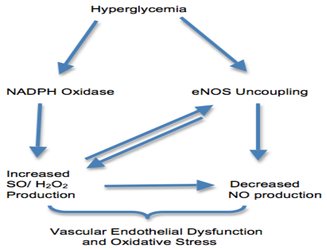

Figure 1. The possible roles of NADPH oxidase and eNOS uncoupling in hyperglycemia-induced vascular endothelial dysfunction.

SO is rapidly converted to H_2O_2, which can be measured in blood due to its relatively longer half-life (i.e., minutes vs seconds). There are 7 isoforms of NADPH oxidase (NOX) in mammals. Of these isoforms, NOX1 and NOX2 are mainly expressed in vascular endothelial cells and smooth muscle cells [2]. Previously, we have shown that gp91ds-tat, a NOX2 inhibitor, partially reduced blood H_2O_2 levels induced by hyperglycemic conditions [1]. However, the role of NOX1 in hyperglycemia induced oxidative stress and vascular endothelial dysfunction is still unclear. In this study, 2-acetylphenothiazine (ML171), a specific inhibitor for NOX1, was used to determine the role of NOX1 in hyperglycemia-induced vascular endothelial dysfunction by measuring blood NO and H_2O_2 levels in real-time (see figure 2) [3].

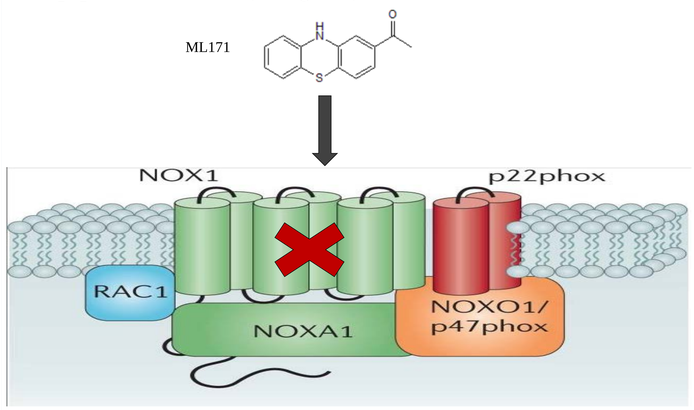

Figure 2. Structure of NADPH Oxidase Isoform 1 (NOX1) and its selective inhibitor, ML171.

Hypothesis

We hypothesized that acute hyperglycemia (200 mg/dL) would increase blood H_2O_2 levels and decrease blood NO levels compared to saline control. By inhibiting NOX1 using ML171(2-acetylphenothiazine, MW=241.31 g/mol, Tocris Bioscience), acute hyperglycemia-induced vascular dysfunction would be attenuated. This will be noted by decreased blood H_2O_2 levels and increased blood NO levels compared to acute hyperglycemia control.

Methods

Male Sprague-Dawley rats (275-325g; Charles River, Springfield, MA) were anesthetized and subsequently infused with saline, 30% glucose, or 30% glucose with 20 µg/Kg or 100 µg/Kg ML171 (approximately 1 or 5 µM in blood) via the cannulation of jugular vein. Hyperglycemic conditions (≥200 mg/dL) were induced, maintained, and monitored as previously described [4]. Mean arterial blood pressure (MABP) was monitored through catheterization of the carotid artery. Blood NO and H_2O_2 levels were measured in real time by NO or H_2O_2 microsensors as previously described (see figure 3) [4]. All data in the figures are presented as means ± S.E.M. The data were analyzed by ANOVA using the Student- Newman- Keuls post hoc test, $p<0.05$ were considered to be statistically significant.

Figure 3. Nitric oxide and hydrogen peroxide sensors in the femoral veins.

Results

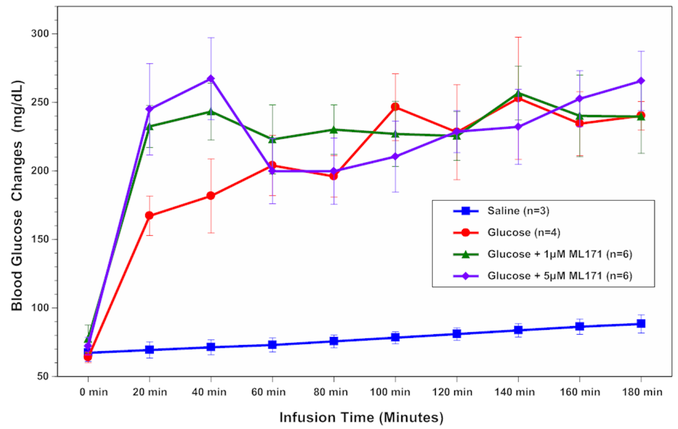

Figure 4. Blood glucose changes throughout 180 minute experiment with 30% D-glucose. Blood glucose levels were maintained at ~200 mg/dL in all study groups except for saline.

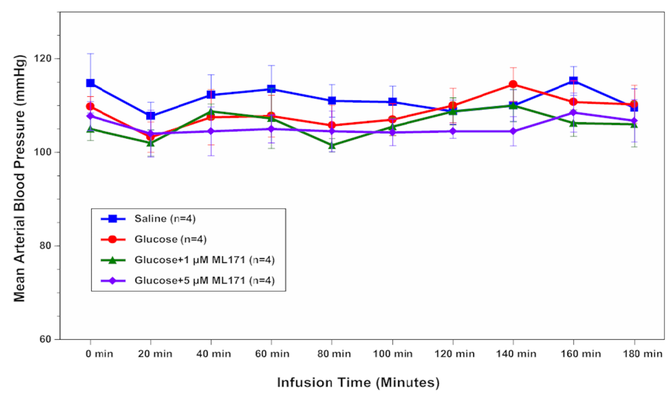

Figure 5. Mean Arterial Blood Pressure among groups taken every 20 minutes for 180 minute experiment. MABP was maintained between 100-120 mmHg in all study groups.

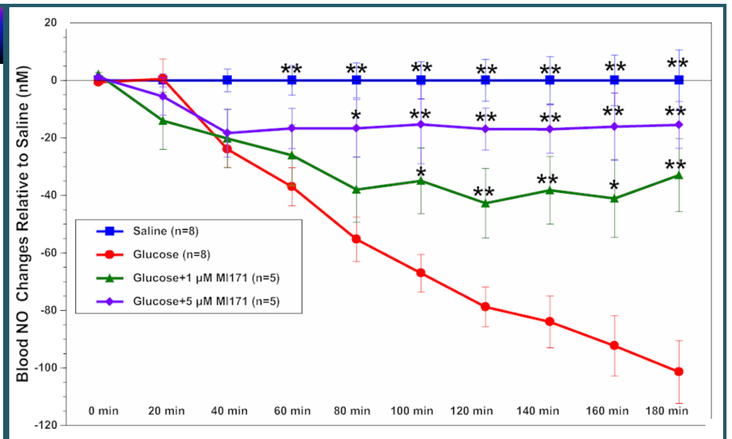

Figure 6. Comparison of blood NO levels relative to saline among 30% D-glucose, 30% D-glucose with 1µM ML171, and 30% D-glucose with 5µM ML171(*p<0.05, **p<0.01 vs Glucose). Hyperglycemia significantly reduced blood NO levels (101.41±10.91 nM, n=8). ML171 (1 and 5 µM) attenuated the hyperglycemia induced decrease in blood NO levels and increased blood NO levels by 68.48±12.67 nM (P<0.01, n=5) and 85.95±8.13 nM (P<0.01, n=5) respectively at 180 min.. Saline showed no change throughout the experiment.

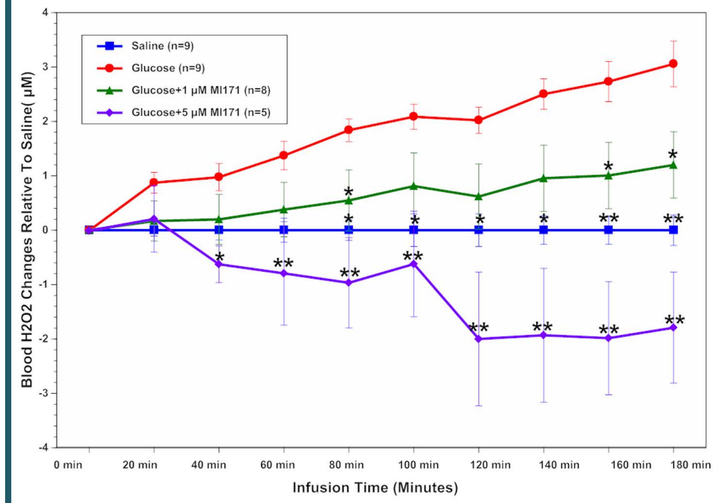

Figure 7. Comparison of blood H_2O_2 levels relative to saline among 30% D-glucose, 30% D-glucose with 1µM ML171, and 30% D-glucose with 5µM ML171(*p<0.05, **p<0.01 vs Glucose). Hyperglycemia significantly increased blood H_2O_2 levels by 3 ± 0.42 µM (n =9). ML171 (1 and 5 µM) significantly decreased blood H_2O_2 levels by 1.86 ± 0.61 µM (n=8) and 4.85 ± 1.02 µM (n=5) respectively at 180 min. Saline showed no change throughout the experiment.

Conclusions

Our results indicate that NOX1 activation is a key mechanism contributing to acute hyperglycemia-induced oxidative stress and NO reduction in vascular tissue. Furthermore, inhibition of NOX1 may mitigate the deleterious effects of acute hyperglycemia. The outcomes from this study suggest that ML171 may be a therapeutic tool to attenuate vascular dysfunction associated with diabetic patients.

References

1. Bertolet M, Minni M, Galbreath T, Barsotti R, Young L, Chen Q. Effects of Selective NADPH Oxidase Inhibitors on Real-Time Blood Nitric Oxide and Hydrogen Peroxide Release in Acute Hyperglycemia. *Proceedings of the 23rd American Peptide Symposium.* 2013: 34-35.
2. Drummond G, Selemidis S, Griendling K, Sobey C. Combating Oxidative stress in vascular disease: NADPH oxidases as therapeutic targets. *Nat Rev Drug Discov.* 2011;10(6):453-471.
3. Gianni, D. Taulet N, Zhang H, DerMardirossian C, Kister J, Martinez L, Roush W, Brown S, Bokoch G, Rosen H. A novel and specific NADPH oxidase-1 (Nox1) small-molecule inhibitor blocks the formation of functional invadopodia in human colon cancer cells. *ACS Chem Biol.* 2010;5(10): 981-993.
4. Minni M, Chen Q, Bartol K, Iames E, Kern M, Perkins K, Rueter B, Young L. Effects of Protein Kinase C Selective Beta II Peptide Inhibitor on Real-time Blood Nitric Oxide and Hydrogen Peroxide Release Under Acute Hypergycemia. *Proceedings of the 22rd American Peptide Symposium.* 2011:280-281.