Metabolic and hemodynamic effects of methylene blue

Ai-Ling Lin[1], Ethan Poteet[2], Fang Du[1], Andrew Bresnen[1], Shiliang Huang[1], Yi Wen[2], Shao-Hua Yang[2], and Timothy Q Duong[1]

[1]*Research Imaging Institute, University of Texas Health Science Center at San Anotnio, San Antonio, TX, United States,* [2]*, Institute for Alzheimer's Disease and Aging Research, University of North Texas Health Science Center at Fort Worth, Fort Worth, TX, United States*

Introduction: Mitochondria are the power houses and the major source of free radicals in mammalian species. Mitochondrial dysfunction is implicated in many neuropathological diseases, including Alzheimer's disease and stroke. Neuroprotective strategies, including free radical scavengers, ion channel modulators, and inflammatory agents, have been extensively explored for the treatment for mitochondria-related neurological diseases.

Methylene blue (MB) can help sustain ATP production by acting as an electron donor in the mitochondrial electron transport chain and reduces free radical production under metabolically stressed conditions because it by-passes the free radical production in the electron transport chain (1). MB has long been used to treat methemoglobinemia and cyanide poisoning. More recently, it has been shown to have neuroprotective in a number of neurological diseases, including stroke and Alzheimer diseases (2). In the study, we evaluated, for the first time, the metabolic and hemodynamic effects of MB in vivo. Cerebral metabolic rate of oxygen ($CMRO_2$), cerebral metabolic rate of glucose (CMR_{Glc}), oxygen extraction fraction (OEF) and cerebral blood flow (CBF) were measured in rats. Comparisons were made with in vitro measurements of glucose and oxygen consumption.

Material and Methods: Male Sprague-Dawley rats (250-300g, N =6) were studied under 1.2% isoflurane. Respiration rate (90-130 bpm) and rectal temperature (37±0.5°C) were continuously monitored. CMR_{Glc} was determined using ^{18}FDG positron emission tomography (PET) (Focus 220 MicroPET scanner). ^{18}FDG of 0.5 mCi was injected through the tail vein. Emission data was acquired for 20 min after 40 min of injection. Glucose uptake was determined using the mean standardized uptake value (SUV_{mean}) equation. CBF MRI was acquired using the arterial spin labeling (ASL) technique (3) at a horizontal 7T Bruker Biospec system. Paired images were acquired in an interleaved fashion with FOV = 12.8×12.8 mm^2, matrix = 64×64, slice thickness = 1 mm, 9 slices, labeling duration = 2100 ms, TR = 3000 ms per segment, and TE = 15 ms. ASL image analysis employed codes written in Matlab and STIMULATE software (University of Minnesota) (5). To determine global $CMRO_2$, blood samples were taken to measure oxygenation from jugular vein (Yv) and from femoral artery (Ya) using a blood gas analyzer (Radiometer ABL5, Copenhagen). Oxygen extraction fraction (OEF) was determined with (Ya-Yv)/Ya and $CMRO_2$ equals OEF x CBF x CaO_2 (CaO_2 is the oxygen content from the blood gas measurements).

For in vitro experiments, HT-22 murine hippocampal cells were plated at 8000 cells/well and cultured on Seahorse XF-24 plates. Oxygen consumption rate (OCR) was analyzed by a Seahorse XF-24 extracellular flux analyzer (2). For glucose uptake analysis, HT-22 cells were seeded overnight at a density of 1 x 10^5 cells on 25 mm glass coverslips in DMEM (+10 % FBS). Cells were incubated in 100 µM 2-NBDG with or without 50 µM methylene Blue for 5 minutes at 37 °C. The coverslips were washed twice in PBS and then mounted and imaged on a Zeiss S1 microscope.

Results: Oxygen saturation, hematocrit, blood-gas pO_2, pCO_2 and pH were not statistically different between pre and post MB injection. MB increased CMR_{Glc} ($p< 0.05$, Fig. 1A), CBF ($P<0.05$, Fig. 1B), and $CMRO_2$ ($P<0.05$, Fig. 1D), but not OEF ($P>0.05$, Fig. 1C). To corroborate these findings, similar in vitro studies were performed. The results show MB increased cellular oxygen consumption rate and glucose uptake in vitro (Figs. 2 & 3).

Discussion and Conclusion: As an electron carrier, MB can facilitate electron transfer in mitochondria by bypassing complex I-III via accepting electrons from NADH and transferring them to cytochrome c. MB-induced increases cellular oxygen consumption rates were observed in cell cultures. We used multi-metric neuroimaging to non-invasively investigate the *in vivo* neuroprotective effect of MB. Significant increases of $CMRO_2$, CMR_{Glc} and CBF were observed with MB administration. Our results suggested that MB can preserve mitochondrial oxidative metabolism capacity and maintain basal neurometabolic and neurovascular coupling. The present study supports the notion that rerouting mitochondrial electron transfer by MB provides a novel strategy for neuroprotection against acute insults. MB may have strong implications in many mitochondria-related neurological diseases (e.g, stroke, Alzheimer's disease and Parkinson's disease). Future studies will measure ATP and lactate production in vivo and investigate functional activations under metabolically stressed (such as hypoxic and ischemic) conditions associated with MB treatments. In conclusion, this study presents the first studies of hemodynamic and metabolic effects of MB in vivo. Non-invasive neuroimaging techniques offer a means to evaluate MB treatment efficacy longitudinally.

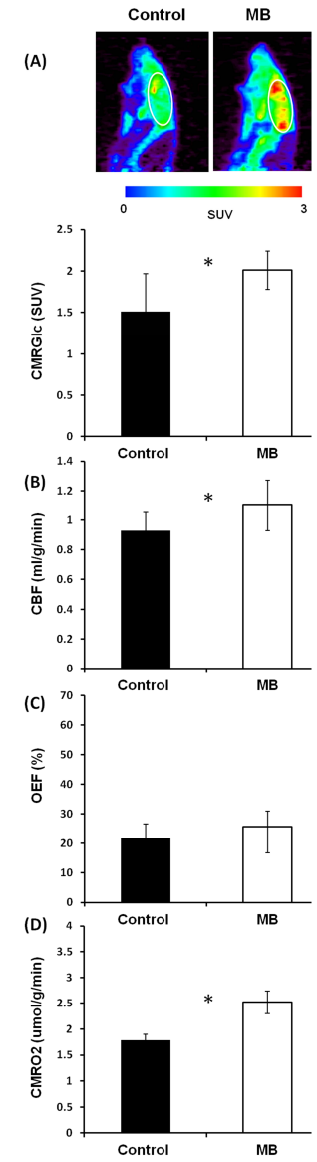

Figure 1. (A) Glucose uptake maps and CMR_{Glc}, (B) CBF, (C) OEF, (D) $CMRO_2$ before and after MB (±SE, whole brain ROI). * $p < 0.05$.

References (1) S Scheindlin, *Mol Interv* 8:268 (2008). (2) Wen et al. J Bio Chem. 286:16504 (2011). Medina et al., Brain Pathol: 21, 140-149. (3) Silva et al. JCBFM 19:871 (1999).

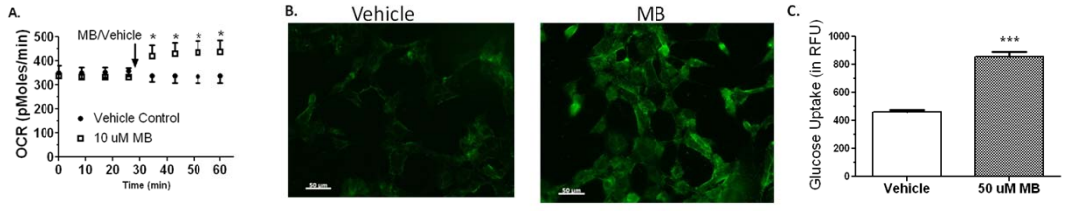

Figure 2. MB enhances oxygen consumption rate (OCR, A) and glucose uptake (B, C) in vitro. *p<0.05, ***p<0.01