Metabolic and hemodynamic effects of methylene blue

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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 mitochondrial-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 (CMRO2), cerebral metabolic rate of glucose (CMRGlu), 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. CMRglu was determined using 18FDG positron emission tomography (PET) (Focus 220 MicroPET scanner). 18FDG 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 (SUVmean) 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 mm², 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 CMRO2, blood samples were taken to measure oxygenation from jugular vein (Yv) and from femoral artery (Ya) using a blood gas analyzer (RadiometerABL5, Copenhagen). Oxygen extraction fraction (OEF) was determined with (Ya-Yv)/Ya and CMRO2 equals OEF × CBF × CaO2 (CaO2 is the oxygen content from the blood gas measurements).

For in vitro experiments, HT-22 murine hippocampal cells were plated at 9000 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 10⁶ 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 PO2, PCO2 and pH were not statistically different between pre and post MB injection. MB increased CMRglu (p<0.05, Fig. 1A), CBF (p<0.05, Fig. 1B), and CMRO2 (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 showed 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 CMRO2, CMRglu 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 CMRGlu, (B) CBF, (C) OEF, (D) CMRO2 before and after MB (±SE, whole brain ROI). * p < 0.05.

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