Methylene blue enhances cerebral glucose and oxygen consumption under hypoxia

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Introduction: Methylene blue (MB) can sustain ATP production by acting as an electron donor in the mitochondrial electron transport chain (1), which may be helpful under metabolically stressed conditions. MB has long been used to treat methemoglobinemia and cyanide poisoning, and more recently, MB has been shown to have neuroprotective effects in a number of neurological diseases, including stroke and Alzheimer disease (2). MB is relatively safe and it has therapeutic effects at nanomolar dose. It can cross the blood-brain barrier. A possible mechanism of action is that MB provides a source for energy to generate ATP under metabolically stress conditions.

We recently showed that, under normal conditions in the rat brain, MB increased cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO2), and cerebral metabolic rate of glucose (CMR_{Glc}), with corroboration by parallel in vitro experiments to measure glucose and oxygen consumption (3). In this study, we asked the question whether MB could help sustain hemodynamic and metabolic status under hypoxic conditions in vivo.

Material and Methods: Male Sprague-Dawley rats (250-300g, N =6 for each group) were studied under 1.2% isoflurane in air and 10% O2 inhalation. 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). ^{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 at a horizontal 7T Bruker Biospec system. Paired images were acquired in an interleaved fashion with FOV = 12.8×12.8 mm, 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, also determined by the blood gas measurements).

Results & Discussion:

Before MB treatment: Relative to air, hypoxia decreased CMR_{Glc} by 39 ± 12%, decreased CBF by 24 ± 9%, increased OEF by 28 ± 6%, and decreased CMRO2 by 20 ± 4% ($P<0.05$ for all). Under these experimental conditions, hypoxia herein did not evoke CBF increases, consistent with those reported previously under similar conditions (4). Reduced glucose and CBF are consistent given that these two parameters are tightly coupled. With reduced O2 content and reduced CBF, OEF increased as expected. CMRO2 and glucose reduction suggest that the overall energy metabolism of the animal under hypoxia was reduced.

After MB treatment: During hypoxia, MB increased CMR_{Glc} by 45 ± 18% (Figure A), CBF by 25 ± 10% (Figure B), OEF by 75 ± 19% (Figure C), and oxygen consumption by 117 ± 29% (Figure D) ($P<0.05$ for all). These findings suggest that MB rescued much of the cerebral energy metabolism, which was reduced by hypoxia. These results are consistent with previous in vitro studies that showed MB acts as an alternative electron carrier in the mitochondrial electron transport chain (2). We speculate that MB increases mitochondrial phosphorylation efficiency, hence, ameliorates cerebral energy metabolic crisis during hypoxic challenge.

Conclusion: Hypoxia markedly reduced energy metabolism and MB rescued much of the metabolic depression observed under hypoxia. These findings indicated that MB can preserve mitochondrial oxidative metabolism capacity and maintain basal neurometabolic and neurovascular coupling under hypoxia. Future studies will measure ATP and lactate production in vivo and investigate MB effects on stroke and Alzheimer disease. Non-invasive imaging approach may prove useful in future MB studies of preclinical disease models and clinical trials aimed at further testing neuroprotective effects of MB treatments.

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) Lin et al, ISMRM 2012, submitted. (4) Sicard et al. NI 25:850 (2005). Duong et al. MRM 45:61 (2001).

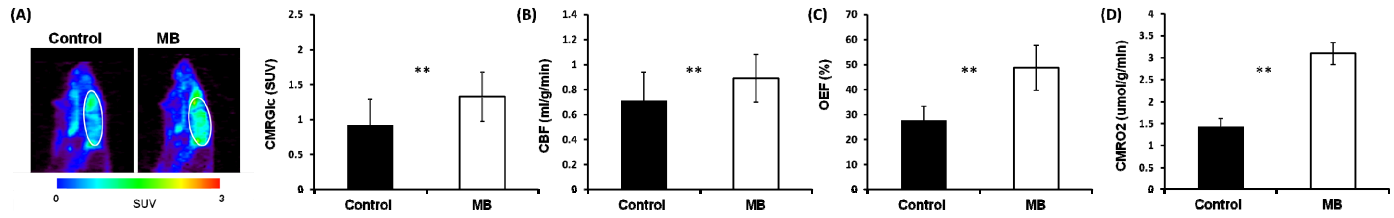

Figure (A) Glucose uptake maps and averaged CMR_{Glc}, (B) CBF, (C) OEF, (D) $CMRO_2$ before and after MB (±SE, whole brain ROI). ** $p < 0.05$.