Methylene blue treatment delays the progression of ischemic penumbra into infarct

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Target Audience Researchers in stroke and neuroprotection

INTRODUCTION Methylene blue (MB) – clinically used as an antidote for methemoglobinemia and cyanide poisoning [1] – has recently been shown to reduce neurobehavioral impairment in animal models of Parkinson's Disease [2] and cognitive decline in Alzheimer's disease [3]. These effects are likely because MB has unique energy enhancing and antioxidant properties [1-3]. The auto-oxidizing property of MB allows it to act as an electron cycler and permits the redirection of electrons to the mitochondrial electron transport chain, thereby enhancing ATP production and promoting cell survival under stress conditions. MB has been shown to enhance ATP production and oxygen consumption in vitro. Our group recently demonstrated that MB has significant effect on hemodynamics, metabolism, and fMRI responses in vivo [4-5]. In bypassing complex I-III, MB also reduces reactive oxygen species production from the mitochondrial electron transport chain, which has antioxidant effect. Thus, MB has the potential to minimize ischemic injury and functional deficit. The goal of this study was to evaluate the neuroprotective effect of MB treatment in ischemic stroke. Specifically, we hypothesized that MB treatment delays infarct growth in a permanent MCAO model in rats.

METHODS Male SD rats (250-350g) were subjected to permanent MCAO using a randomized and double-blinded experimental design, either vehicle (n=12) or MB (n=12) was administered (1 mg/kg, i.v. infusion over 30 mins) starting 30 mins after MCAO (after the MRI data were acquired at the first time point). Oximetry and rectal temperature were maintained within normal physiological ranges. MRI experiments were performed on a 7-T/30-cm magnet. Quantitative CBF and ADC were measured using continuous arterial spin labeling and diffusion-weighted EPI. T2 maps were acquired using fast spin echo sequence on day-1. Based on 30-min ADC and CBF maps, ischemic core, perfusion/diffusion mismatch and normal tissues were classified.

Initial lesion volumes were defined by the core tissue volumes using threshold mean ADC value of normal hemisphere minus three times of standard deviation. Infarct volumes were derived from day-1 T2 maps using threshold of mean T2 value of normal hemisphere plus two times of standard deviation. Edema correction was applied. T-test was used for statistical comparison with a p value < 0.05 taken to be statistically significant. Data showed in figures and texts are mean±SEM.

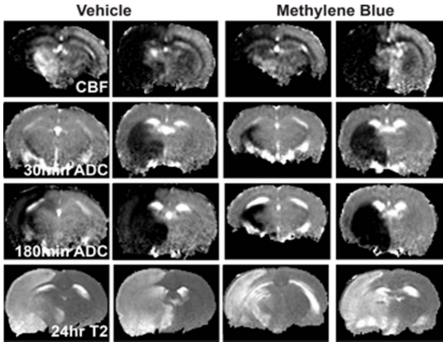

Figure 1. CBF, ADC & T2 maps.

RESULTS AND DISCUSSION Figure 1 shows representative CBF and ADC images of the vehicle and MB treatment group at different time points. **Figure 2** showed the lesion volumes defined by abnormal CBF and ADC for the vehicle and MB group. CBF defined lesion volume in the vehicle was relatively constant across all time points as expected due to permanent MCAO. Careful inspection showed that, in the MB group, CBF defined lesion volume was slightly lower than the vehicle group, suggesting that MB improved tissue perfusion slightly (p=0.081), consistent with a previous study that MB elevates CBF [5].

ADC and CBF initial lesion volumes at 30 mins post MCAO were not statistically different from each other (P>0.05), confirming the starting lesion before treatment were similar between the two groups. For both groups, ADC defined lesion at 30 mins post MCAO was small and grew with time. In the MB group, ADC defined lesion volume grew at a significantly slower pace compared with the vehicle group, and was statistically different for the 60, 90, 120min time points (p=0.029, 0.044, 0.031), respectively. These results indicate that MB slows lesion growth.

At 24hrs (end point), infarct volumes by T2 were 236.8±9.3 mm^3 and 235.0±8.1 mm^3 for the vehicle and MB group, respectively (P>0.05), indicating that without reperfusion the tissue will eventually infarct as expected.

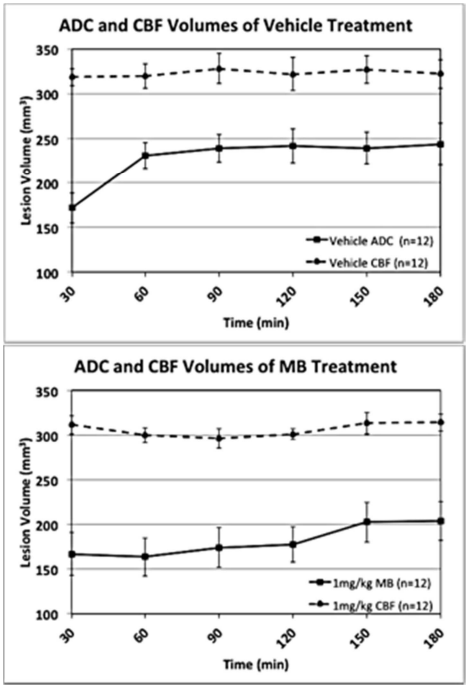

Figure 2. Lesion volume evolution of the (top) vehicle and (bottom) MB treated group (N =12 each)

CONCLUSIONS MB slightly improved perfusion and significantly delayed the progression of the perfusion-diffusion mismatch into infarct. This neuroprotective effect is consistent with MB's unique properties as a metabolic enhancer and an antioxidant. Future studies will investigate different occlusion durations, chronic stroke, embolic stroke model with combination therapy with rtPA, as well as incorporate other MRI measurements. The novelties of this study included randomized double-blinded experimental design and subject selection to ensure similar initial lesion volumes between groups in the same animals using non-invasive MRI. Since MB is an FDA-grandfathered drug currently used in the clinics with an excellent safety profile at low dose, human stroke trials can be readily explored.

REFERENCES: [1] Zhang X, et al, Neurotox Res 2006;9:47. [2] Rojas JC, et al, Progress in Neurobiology 2012;96:32. [3] Oz M, el al, Biochem Pharmacol 2009;78(8):927. [4] Huang et al, Neuroimage 2013 72: 237. [5] Lin et al, PlosOne 7:e46585