Neuroprotective effects of Delayed Methylene Blue in Mild Traumatic Brain Injury

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Introduction: Methylene blue (MB) is a FDA grandfathered drug and is clinically used to treat malaria, methemoglobinemia, and cyanide poisoning (1). MB acts as an electron cycler that allows MB to redirect electrons to the mitochondrial electron transport chain, enhancing ATP production and promoting cell survival. In bypassing complex I-III activity, MB reduces reactive oxygen species production, minimizing oxidative stress and ischemic injury. Previous MB studies reported neuroprotective effects of MB in animal models of Parkinson's disease, Alzheimer's disease, and ischemic stroke (1,2). In a double-blinded, randomized, vehicle-control study, we previously demonstrated that MB given one-hour post injury had positive neuroprotective effects in a rat model of Traumatic Brain Injury (TBI) (3). The goal of this study was to investigate whether delayed MB treatment would provide a neuroprotective effect on TBI measured by MRI (T_2, ADC, CBF and FA) and functional outcomes.

Methods: Anesthetized rats were placed in a stereotaxic frame and a 6mm craniotomy over the left primary motor/somatosensory cortex region exposed the intact dura matter. The dura was impacted directly using a pneumatic cortical impactor with an impact velocity of 5.0m/s, a 250**μ**s dwell time, and 1mm depth to mimic a moderate TBI. A double-blinded randomized design was utilized. Vehicle (n=6) or MB (n=6) was administered (1mg/kg acutely at 1 and 3 hours or delayed at 24 post TBI). Longitudinal T_2, CBF, ADC and FA MRI were performed on the day of the TBI, and again on days 2, 7 and 14 after TBI onset. Comparison of MRI scans was made with the evolution of lesion volume and behavioral analysis (cylinder test and foot fault test).

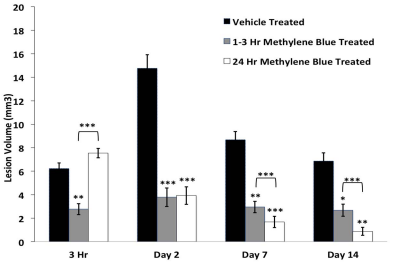

Figure 1: T_2 calculated lesion volumes for vehicle and MB treated (acute or delayed) animals are shown at 3 hours, 2, 7, and 14 days post TBI (*p<0.05, **p<0.01, ***p<0.001; n=8 per group).

Results & Discussion: **Fig 1** displays T_2 lesion volumes for vehicle, acute MB, and delayed MB treated animals at 3 hrs and 2, 7 and 14 days post TBI. MB treatment significantly reduced lesion volumes with delayed treatment, resulting in the reduction on day 14 compared to vehicle treatment. Note the delayed MB group had similar lesion volumes at the three-hour time point (before MB) compared to vehicle treated group as expected while the acute MB group (after MB) had reduced T_2 at this time point.

T_2, ADC, FA and CBF percent differences from the contralesional cortex were also analyzed for the vehicle, acute MB, and delayed MB treated groups at 3 hrs and 2, 7 and 14 days post TBI (**Fig 2**). At 3 hrs after, the delayed MB group did not showed significance difference from the vehicle group in T_2, ADC and FA at 3 hrs post TBI as expected, whereas the acute MB group showed significance difference from the vehicle group as expected.

T_2, ADC, and FA differences were generally worst on day 2 and returned toward normal in all groups (**Fig 2A-C**). However, the delayed MB group showed small differences in T_2, ADC, and FA (less severe) compared to the vehicle group on day 2, 7 and 14. There were no statistically differences between acute and delayed MB group on day 2, 7 and 14. No significant differences in CBF amongst vehicle, acute and delayed MB groups at all time points (**Fig 2D**).

In the vehicle group, forelimb asymmetry scores worsened on days 1 and 2 after TBI, indicating decreased utilization of the affected forelimb. In the acute MB group forelimb asymmetry scores did not change substantially with time and remained close to pre-TBI values at all time points studied. In the delayed MB group forelimb asymmetry scores peaked on day 2 and returned toward pre-TBI values by day 7 and 14 (**Fig 3A**). In the vehicle group, foot fault scores of the affected forelimb worsened dramatically on day 1, persisted on days 2 and 7, and improved on day 14. In the acute MB group, by contrast, foot faults were only slightly elevated post-TBI but did not reached the severity observed in the vehicle group. There were significantly lower numbers of foot faults in the MB-treated group compared to the vehicle-treated group on days 1, 2 and 7 post TBI (P=0.043, 0.018 and 0.0058, respectively). In the delayed MB group, foot faults followed the same pattern as the vehicle treated animals but were lower on each time point studied (**Fig 3B**). Together, these data indicated that acute or delayed MB treatment reduces sensorimotor deficits following TBI.

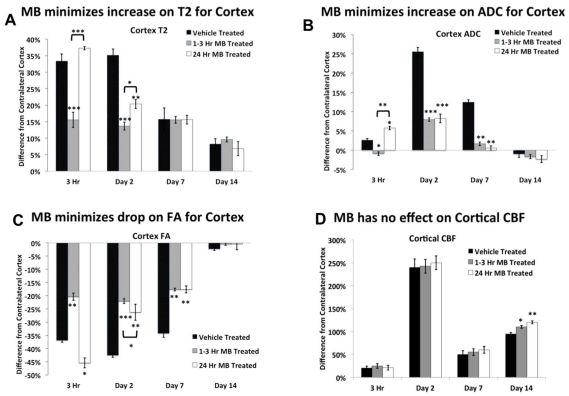

Figure 2: The percent difference in T_2, ADC, CBF and FA values for vehicle and MB treated (acute or delayed) animals are shown at 3 hours, 2, 7, and 14 days post TBI (*p<0.05; n=8 per group).

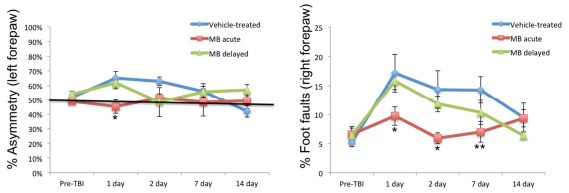

Figure 3: Line graphs demonstrate the group-averaged % asymmetry (A) or foot fault (B) for vehicle and MB treated (acutely or delayed) animals prior to injury and post injury on days 1, 2, 7 and 14 (*p<0.05; ** p<0.01 n=8 per group).

Conclusion: This study demonstrated that MB is effective in reducing lesion volume and functional deficits when given 24 hrs after mild TBI. This finding supports the notion that MB's energy-enhancing and antioxidant properties have therapeutic effects in a number of neurological injuries and disorders. Future studies will investigate chronic MB treatment in following TBI and additional combination therapies. MB has a good safety profile and is clinically approved for other indications and thus MB clinical trials on TBI can be readily explored.

References: 1) Rojas JC et al., 2012. Prog Neurobiol 96: 32-45. 2) Oz et al., 2009. Biochemical Pharmacology. 78(8): 927-32. 3) Watts LT et al., 2014. J Neurotrauma. 31(11): 1063-71.