Neuroprotective effects of Chronic Oral Methylene Blue Treatment in Mild Traumatic Brain Injury

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Target audience: Researchers in Traumatic Brain Injury

Introduction: Methylene blue (MB), a FDA grandfathered drug, is clinically used to treat methemoglobinemia, carbon-monoxide poisoning and cyanide poisoning.[1] MB has established energy-enhancing and antioxidant properties.[1] Recently, MB has been shown to reduce neurobehavioral impairment in animal models of Parkinson's Disease and cognitive decline in Alzheimer's. Furthermore, we have recently demonstrated that a single intravenous MB dose reduces lesion volume, behavioral deficits and neurodegeneration in an animal model of traumatic brain injury (TBI).[2] The goal of this study was to evaluate chronic oral MB administration in the same TBI model.

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. MB (1mg/kg each day) was placed in Nutra Gel (flavored) food. Vehicle (n=6) or oral MB (n=4) was given daily to 14 days. Daily 4 mg/kg oral MB has been used safely for one year in clinical trials (1). Longitudinal T_2 MRI was performed 3 hrs after TBI, and again on days 2, 7 and 14 after TBI onset. Comparison of MRI scans was made with the evolution of lesion volume, behavioral analysis (cylinder test and foot fault test), and immunohistochemistry (day 14).

Results & Discussion: **Figure 1A** shows typical T_2 maps at 0, 2, 7 and 14 days post TBI for both vehicle and oral MB treated animals. The hyperintense area in the S1 cortex indicates the lesion. T_2 MRI lesions were apparent 3 hours after TBI in both treatment groups. In the vehicle-treated group, the lesion volume peaked at 2 days, and decreased by 14 days post TBI (**Figure 1B**). In the oral MB-treated group, by contrast, the lesion volumes were significantly smaller on day 2 (p=0.002) than those of the vehicle-treated group. These results indicate that oral MB treatment reduces TBI lesion volume and vasogenic edema.

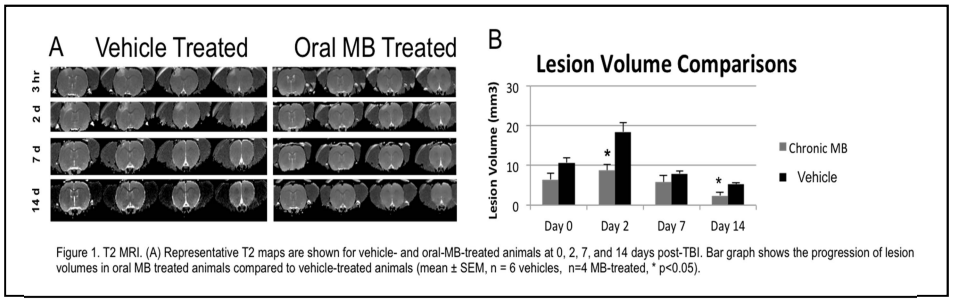

Figure 1. T2 MRI. (A) Representative T2 maps are shown for vehicle- and oral-MB-treated animals at 0, 2, 7, and 14 days post-TBI. Bar graph shows the progression of lesion volumes in oral MB treated animals compared to vehicle-treated animals (mean ± SEM, n = 6 vehicles, n=4 MB-treated, * p<0.05).

In the vehicle group, forelimb asymmetry scores worsened on days0 and 2 after TBI, indicating increased utilization of the left (unaffected) forelimb in the vehicle-treated animals. In the oral MB group left forelimb asymmetry significantly worsened on day 2 (p=0.0132) and remained close to pre-TBI values across all other time points studied (**Figure 2A**). Right forelimb asymmetry was also significant with decreased utilization at p=0.0206. All other time points were non-significant (data not shown). In the vehicle group, foot fault scores worsened in the right forelimb dramatically on day 2, persisted on day 7 after TBI, and improved slightly on day 14. In the oral MB group, by contrast, right 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 0, 7 and 14 post TBI (P=0.0001, 0.0033 and 0.0067, respectively) (**Figure 2B**). We detected no significant increases in foot faults of the left forepaw at all time points (data not shown). Together, these data indicated that daily oral MB administration markedly reduced sensorimotor deficits following TBI. These data also suggest that there were functional compensations in both groups and the extent of functional compensation differed between groups.

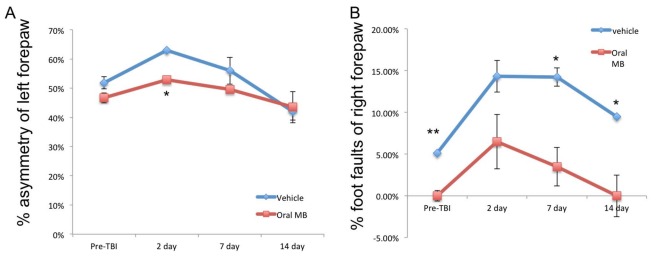

Figure 2: Preliminary data demonstrates a reduction in functional deficits with oral MB Treatment compared to vehicle treated animals using the cylinder and foot fault tests. N=4; * p<0.05 **p<0.001

Conclusion: This study demonstrates a neuroprotective effect of oral administration of MB in a rat model of mild TBI, as demonstrated by reduced lesion volume and functional deficits compared to vehicle-treated animals. Oral administration is more practical and non-invasive (as well as less labor intensive compared to iv injection), and thus should be have more applications. Further our data suggest that oral MB administration is as effective as intravenous administration. MB has a good safety profile and is clinically approved for other indications and thus MB clinical trials on TBI can be readily expedited.

References: 1) Rojas, *Prog Neurobiolo* 2014. 2) Watts, *Neurotrauma* 2014.