Chronic methylene blue treatment decreases ischemic stroke volume and improves functional behavioral recovery

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INTRODUCTION We previously reported that methylene blue (MB), a mitochondria energy enhancing drug, reduced infarct volume in permanent and transient MCAO rat models up to 2 days.[1, 2] The goal of this study was to investigate the efficacy of chronic MB treatment (up to 14 days) in transient ischemic stroke. MRI and behavioral tests were performed longitudinally up to 28 days following stroke. We hypothesized that chronic MB treatment yielded better outcome than vehicle treatment in ischemic stroke.

METHODS Male SD rats (250-350g, N=16) were subjected to 60-min MCAO. Two treatment groups were studied using a randomized and double-blinded experimental design: MB (n=8) and Vehicle (n=8). 1mg/kg MB or vehicle was administered at 0.5hr, 1.2hrs (immediately post-reperfusion), 2 days, 7 days, and 14 days after stroke (1 mg/kg i.p. at 2, 7 and 14 days). Animals were ventilated with room air and maintained under 1.2-1.5% isoflurane. Oximetry and rectal temperature were maintained within normal physiological ranges. Behavioral testing (forelimb asymmetry and foot fault scores) was also acquired at 2, 7, 14 and 28 days after stroke.

A pretreatment MRI data set was acquired 25-30min after stroke and then at 55-60min prior to reperfusion on a 7T scanner. ADC and CBF MRI measurements were acquired from 25 mins to 3 hrs, again on 2, 7, 14 and 28 days after stroke.

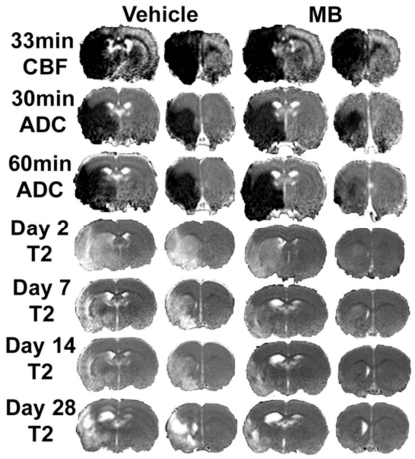

Figure 1. Representative CBF, ADC and T2 measurements vehicle and MB

T-test was used for comparison between initial lesion and final infarct or between treated and control groups. Edema correction was applied for the day 2 T2 measurements.[3] A p value of 0.05 was taken to be statistically significant. Data showed in figures and texts are mean ± SEM.

RESULTS Figure 1 shows representative CBF, ADC and T2 images of the MB and vehicle treatment groups at different time points. The initial (30min) CBF lesion volume was 224.5 ± 21.9 mm^3 for vehicle and 226.0 ± 16.7 mm^3 for MB, not statistically different from each other. Reperfusion was successful in all animals as indicated by restoration of CBF. **Figure 2** shows the group-averaged lesion volumes defined by abnormal CBF, ADC and T2. ADC and CBF initial lesion volumes at 30 min post MCAO before treatment were not statistically different among groups ($p>0.05$), confirming the starting lesion before treatment were similar between groups. For both groups, ADC defined lesion decreased after reperfusion and remained smaller than the initial 30 mins time points. The ADC defined lesion volume of the MB group was smaller than that of vehicle group prior to reperfusion but not statistically significant ($p > 0.05$). The T2 infarct volumes of the MB group were smaller than those of the Vehicle group for Day 2, 7, 14 and 28 ($P<0.05$).

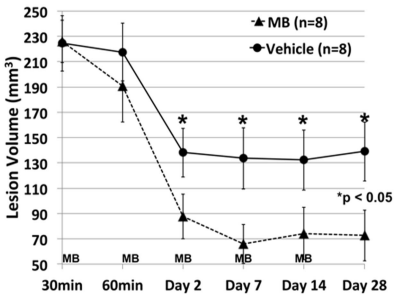

Figure 2. ADC and T2 longitudinal infarction volume measurement in vehicle and MB groups

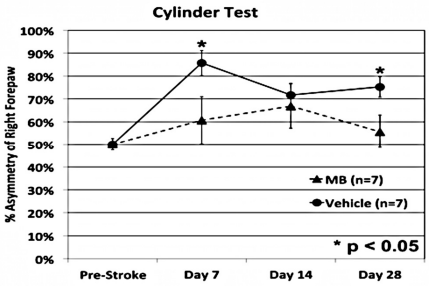

Figure 3. Ipsilateral forepaw overutilization at 7, 14 and 28 days decreases faster in the MB group

Figure 3 shows the (unaffected) forepaw asymmetry score. Day 2 could not get scored due to lack of vertical exploration in most animals at this acute time point. One animal was also excluded here from each group due to inactivity. There was a statistically significant difference in asymmetric use of the forepaw in the MB group on 7 and 28 days. **Figure 4** shows the percentage of (affected) foot faults at day 2, 7, 14 and 28. There was a statistically significant difference in foot fault scores in the MB group on 2, 7, 14 and 28 days. Animals with MB treatment showed less motor behavioral deficits compared to vehicle.

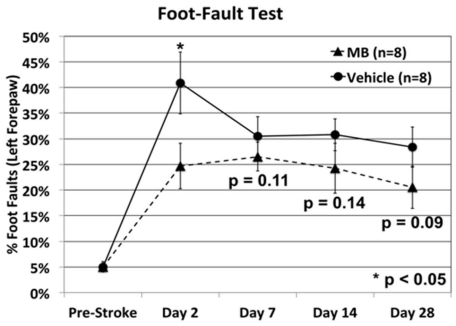

Figure 4. Percentage of left forepaw foot-faults at 2, 7, 14, and 28 days decreases faster in the MB group

DISCUSSION AND CONCLUSIONS Chronic MB treatment reduced the stroke volume and sensory-motor deficits compared to control up to 28 days after stroke. The improved efficacy compared to the prior study resulted from giving an *earlier* and *higher* total MB dose (2mg/kg vs. 1.5mg/kg). This suggests that MB can potentially act as a neuroprotectant from hyperacute to chronic phases of ischemic stroke and expedites functional recovery. It is also important to note that the accelerated behavioral recovery occurs without significant change in the stroke volume over 28 days.

REFERENCES (1) Rodriguez et al. Brain Res. 2014; 1588:144. (2), Shen et al. PloS One 2013; 8:11. (3) Gerriets et al. Stroke 2004; 35:566-71.