Brain Research 1678 (2018) 322–329

Contents lists available at ScienceDirect

Brain Research

journal homepage: www.elsevier.com/locate/bres

Research report

Chronic oral methylene blue treatment in a rat model of focal cerebral ischemia/reperfusion

Lei Huang [1], Jianfei Lu [1], Bianca Cerqueira, Yichu Liu, Zhao Jiang, Timothy Q. Duong *

Radiology and Preclinical Imaging Center, Stony Brook Medicine, Stony Brook, NY, USA

ARTICLE INFO

Article history:
Received 11 July 2017
Received in revised form 25 October 2017
Accepted 28 October 2017

Keywords:
Methylene blue
Chronic stroke
MRI
Lesion volume
White matter injury

ABSTRACT

A single acute low-dose methylene blue (MB), an FDA-grandfathered drug, has been shown to ameliorate behavioral deficits and reduces MRI-defined infarct volume in experimental ischemic stroke when administered intravenously or intraperitoneally. The efficacy of chronic MB treatment in ischemic stroke remains unknown. In a randomized, double-blinded and vehicle-controlled design, we investigated the efficacy of chronic oral MB administration in ischemic stroke longitudinally up to 60 days post injury using MRI and behavioral tests, with end-point histology. The major findings were chronic oral MB treatment, compared to vehicle, i) improves functional behavioral outcomes starting on day 7 and up to 60 days, ii) reduces MRI-defined total lesion volumes from day 14 and up to 60 days where some initial abnormal MRI-defined core and perfusion-diffusion mismatch were salvaged, iii) reduces white-matter damage, iv) gray matter and white matter damages are consistent with Nissl stains and Black Gold stain histology. These findings provide further evidence that long-term oral administration of low-dose MB is safe and has positive therapeutic effects in chronic ischemic stroke.

1. Introduction

Stroke is a leading cause of death and chronic disability worldwide (Benjamin et al., 2017). The available treatments for ischemic stroke remain very limited. Recombinant tissue plasminogen activator (rt-PA) is effective, but it benefits only 3–5% of patients due to the risk of fatal hemorrhagic transformation and limited treatment window (within 4.5 h after stroke onset) (Adeoye et al., 2011; Hacke et al., 2008). More recently, mechanical clot extraction has recently shown to be efficacious in clinical trials (Badhiwala et al., 2015; Goyal et al., 2016; Pereira et al., 2013). Mechanical thrombolytic devices can remove a clot in minutes, whereas pharmaceutical thrombolytics, even those delivered intra-arterially, may take as long as 2 h to dissolve a thrombus. There are currently no clinically approved neuroprotective drug for treatment of acute stroke.

Methylene blue (MB), an FDA-grandfathered drug, has been used to treat methemoglobinemia and cyanide poisoning (Schirmer et al., 2011). Growing preclinical evidence has demonstrated that MB exhibits therapeutic potential in numerous neurological disorders, including but not limited to Alzheimer's disease (Mori et al., 2014), Parkinson's disease (Wen et al., 2011) and traumatic brain injury model (Talley Watts et al., 2014). Moreover, MB can readily cross the blood-brain barrier and distributes in the central nervous system after been intravenously or orally administrated (Walter-Sack et al., 2009). Clinical safety study has shown long-term intake of low-dose of MB has an excellent safety profile (Naylor et al., 1986).

We have previously demonstrated that acute treatment with a single-dose MB ameliorates behavioral deficits and reduces MRI-defined infarct volume in a transient (Shen et al., 2013) and permanent (Rodriguez et al., 2014) ischemic stroke models. Although these studies have shown that MB is neuroprotective for *acute* ischemic stroke, the efficacy of chronic MB treatment on stroke recovery in the chronic phase remains unknown.

The goal of the current study is to test the long-term effects of chronic MB oral treatment on ischemic stroke in rats. A randomized, double-blinded and vehicle-controlled design was used to avoid bias. MRI was used to select animals by excluding incomplete occlusion based on hyperacute MRI before enrolling animals into the study. MRI was used to longitudinally evaluate the effect of chronic MB treatment up to 60 days post injury along with behavioral tests, with corroboration by end-point histology.

2. Results

2.1. Chronic MB treatment improved behavioral recovery

Prior to MCAO, foot fault scores were not significantly different between the vehicle and MB-treated groups (4.0 ± 2.8 vs. 5.0 ± 1.4%,

* Corresponding author at: Radiology, Stony Brook Medicine, 101 Nicolls Road, Stony Brook, NY 11794-8434, USA.
E-mail address: tim.duong@stonybrookmedicine.edu (T.Q. Duong).
[1] Lei Huang and Jianfei Lu contributed equally to this work.

https://doi.org/10.1016/j.brainres.2017.10.033
0006-8993/

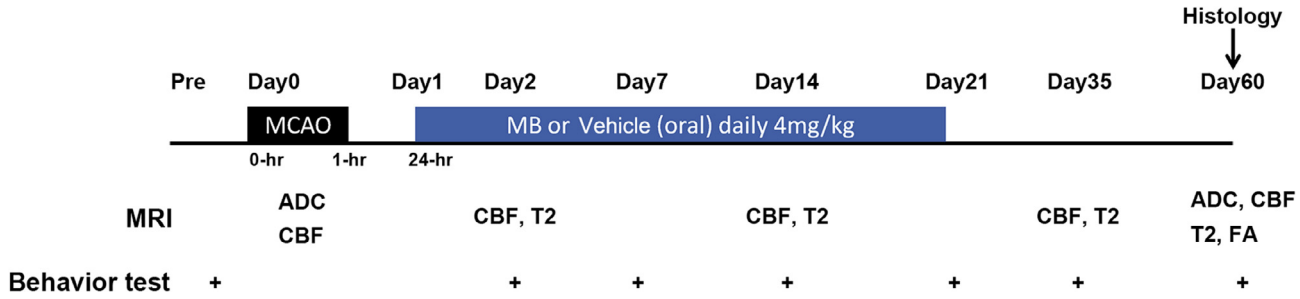

Fig. 1. Experimental design.

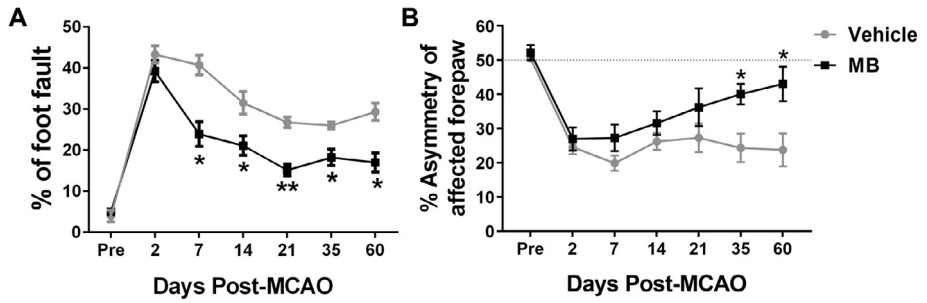

Fig. 2. Foot fault test (A) and cylinder test (B) at pre-MCAO and day 2, 7, 14, 21, 35, 60 post-MCAO for vehicle and methylene blue (MB) treated groups (mean ± SEM, n = 5 per group, $^{*}P < .05$, $^{**}P < .01$ compared to the vehicle-treated animals).

t-test, $P > .05$. Fig. 2A). The foot fault percentage increased dramatically on day 2 followed by a reduction from day 7 to 60 for both groups. However, compared to vehicle group, the foot faults in the MB-treated group were significantly lower on day 7 through day 60 (23.9 ± 6.8% vs. 40.7 ± 4.8%, $P < .0001$ on day 7; 21.1 ± 5.3% vs. 31.5 ± 5.5%, $P = .007$ on day 14; 15.2 ± 3.2% vs. 26.8 ± 2.6%, $P = .002$ on day 21; 18.2 ± 4.4% vs. 26.0 ± 1.6%, $P = .039$ on day 35; 18.0 ± 4.1% vs. 28.0 ± 4.3%, $P = .001$ on day 60; Two-way ANOVA). Similarly, the forelimb asymmetry scores were not statistically different between groups before the stroke and dramatically decreased 2 days after stroke (Fig. 2B). No obvious improvement in the score was observed in the vehicle group from day 2 to day 60. However, the MB treatment group showed progressive improvement from day 2 to day 28, reaching significance on day 35 and day 60 compared to vehicle group (40.1 ± 7.0% vs. 24.3 ± 10.2% on day 35, $P = .01$; on day 60, 43.6 ± 8.7% vs. 23.8 ± 11.8%, $P = .02$, two-way ANOVA with Bonferroni post hoc test).

2.2. Chronic MB treatment reduced lesion volume

The MRI-defined lesion volumes before treatment and day 2 after stroke were not significantly different between groups (Fig. 3). By contrast, lesion volumes of the MB-treated group were significantly lower than the vehicle group at day 14–60. (100.5 ± 12.5 vs. 184.0 ± 26.3, $P = .022$ on day 14; 96.6 ± 5.8 vs. 177.3 ± 22.1, $P = .016$ on day 35; 97.7 ± 14.7 vs. 172.4 ± 17.2 $P = .049$ on day 60; Two-way ANOVA).

We further identified that MB treatment, compared to vehicle treatment, salvaged significantly more MRI-defined core tissue (4 0.9 ± 4.3 vs. 26.1 ± 4.2%, $P = .036$, *t*-test, Fig. 4) as well as mismatch tissue albeit not significantly (68.1 ± 2.0% vs. 72.1 ± 3.1%, $P > .05$).

Nissl staining was performed to verify MRI findings at the end of the study (day 60 after stroke) (Fig. 5). In both groups, Nissl stain indicated the apparent tissue loss or neuronal damage caused by stroke, which was consistent with MRI. Compared vehicle group, tissue loss was less in the MB-treated group.

2.3. Chronic MB treatment attenuated post-stroke hyperperfusion

CBF after stroke was measured by arterial spin labeling MRI. Representative CBF maps are shown in Fig. 6A. Two ROIs (initial core and mismatch) were used to analyze CBF changes separately. CBF was markedly reduced following ischemic stroke in both groups on day 0. From day 2 to day 14, hyperperfusion was observed in the two ROIs of both groups. However, CBF was significantly lower in both ROIs of MB-treated group on day 14, compared to the vehicle group (1.07 ± 0.3X vs. 1.77 ± 0.3X of normal CBF, $P = .021$ on core ROI; 0.64 ± 0.2X vs. 0.92 ± 0.1X of normal CBF, $P = .016$ on mismatch ROI, ANOVA). From day 35 to day 60, CBF of mismatch region was close to the CBF of contralateral side, while CBF of core area persisted low. Moreover, CBF was significantly higher in initial mismatch area of MB treated group on day 35 and day 60, compared to the vehicle group (0.84 ± 0.03X vs. 0.62 ± 0.03X of normal CBF, $P = .024$ on day 35; 0.82 ± 0.05X vs. 0.59 ± 0.03X of normal CBF, $P = .04$ on day 60; ANOVA).

2.4. Chronic MB treatment decrease white matter injury

FA was analyzed to evaluate white-matter changes after stroke (Fig. 7). The corpus callosum (CC) volume was higher after MB-treatment compared to vehicle in the ipsilesional hemisphere (50.14 ± 3.6 vs. 36.2 ± 3.6, $P = .028$, *t*-test), but not in the contralesional hemisphere (57.5 ± 1.8 vs. 57.6 ± 3.8, $P = .983$, *t*-test) at day 60 after stroke.

Black-Gold II staining was performed at the final time point of the study to corroborate the MRI findings (Fig. 8). FA map was consistent with Black-Gold II staining. CC of the contralateral hemisphere was intact in both groups. However, CC of ipsilateral