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Research Report

Methylene blue treatment delays progression of perfusion–diffusion mismatch to infarct in permanent ischemic stroke

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ABSTRACT

Stroke is a leading cause of morbidity and mortality in the world. Low-dose methylene blue (MB), which has been used safely to treat methemoglobinemia and cyanide poisoning in humans, has energy enhancing and antioxidant properties. We tested the hypothesis that methylene blue treatment delays progression of at-risk tissue (*ca.* perfusion–diffusion mismatch) to infarct in permanent middle cerebral artery occlusion in rats at two MB treatment doses. Serial MRI was used to evaluate MB treatment efficacy. The major findings were: (i) MB significantly prolonged the perfusion–diffusion mismatch, (ii) MB mildly increased the CBF in the hypoperfused tissue, (iii) MB did not change the final infarct volume in permanent ischemic stroke, and (iv) there were no dose-dependent effects on mismatch progression for the 1 and 3 mg/kg doses studied. This neuroprotective effect is likely the result of sustained ATP production and increased CBF to tissue at risk. This work has the potential to readily lead to clinical stroke trials given MB's excellent safety profile.

1. Introduction

Stroke is the second leading cause of death and the leading cause of disability in the world (World Health Organization, 2011). Recombinant tissue plasminogen activator (rt-PA) remains the only approved ischemic stroke therapy available to the masses. Moreover, rt-PA treatment only reaches less than 5% of patients due to its narrow therapeutic window and its risk of intraparenchymal hemorrhage. There is an urgent need for new neuroprotective therapies that can extend the thrombolytic therapeutic window.

Methylene blue (MB) is a grandfathered FDA drug that was first synthesized at the end of the 19th century and has been used to treat methemoglobinemia and cyanide poisoning. Low-dose MB has an excellent safety profile. MB has redox recycling properties in that it acts as an electron cycler and facilitates electron transfer in the mitochondrial electron transport chain from NADH to cytochrome c with resultant

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effect of ATP production. By rerouting electrons directly to cytochrome c, and bypassing complexes I–III, MB also minimizes oxygen free radical production in the mitochondrial electron transport chain, especially under oxidative stress conditions. The energy-enhancing and antioxidant properties of MB could have potential neuroprotective effects.

Recently, low dose MB has been shown to exhibit therapeutic effects in a number of neurological disorders. MB reduces neurobehavioral impairment in optic neuropathy (Rojas et al., 2009; Zhang et al., 2006), Parkinson's disease (Rojas et al., 2012) and Alzheimer's disease (Congdon et al., 2012; O'Leary et al., 2010) in animal models. Clinical studies have shown that MB can slow the progression of Alzheimer's disease (Wischik and Staff, 2009; Wischik et al., 2008). MB was also shown to have neuroprotective effect in transient ischemic stroke models (Shen et al., 2013; Wen et al., 2011) and in a traumatic brain injury model in rats (Watts et al., 2014).

Magnetic resonance imaging offers a non-invasive means to track the progression of ischemic brain injury in a longitudinal fashion. T2-weighted MRI is widely used to visualize edema and define final infarct volume (Shen et al., 2005). Perfusion-weighted MRI can measure cerebral blood flow (CBF) at the tissue level, allowing for the detection of tissue with reduced perfusion that is at risk of ischemic brain injury. Diffusion-weighted MRI (DWI), which measures water motion, is very sensitive to early ischemic brain injury in contrast to computed tomography and T2 MRI (Moseley et al., 1990). As such DWI has become the method of choice for early detection of ischemic brain injury. Although the underlying biophysical mechanisms of DWI signal contrast is not fully understood (Duong et al., 1998), the combined use of perfusion and diffusion MRI are now widely used to distinguish reversible from irreversibly ischemic brain injury, and to guide acute stroke treatment in preclinical and clinical settings (Astrup et al., 1981; Schlaug et al., 1999).

The goals of the current study were: (i) to test the hypothesis that methylene blue treatment delays progression of perfusion–diffusion mismatch to infarct in permanent ischemic stroke in rats, and (ii) to evaluate the effects of two treatment doses. A randomized, double-blind and placebo controlled design was used to avoid bias. MRI was used to verify the presence of mismatch at the hyperacute phase, to exclude incomplete occlusion, and ensure similar initial lesion sizes between the two groups before treatment. Such subject selection, which has been demonstrated to be critical in clinical stroke treatment trials, would not have been possible with terminal histological measurements.

2. Results

2.1. *Physiological parameters and mortality rates*

For all three (vehicle group, 1 and the 3 mg/kg MB) groups, the baseline heart rate (350–450 bpm), arterial oxygen saturation (94–96%), end tidal expiratory CO_2 (35–45 mmHg) were within normal physiological ranges and were not statistically different from each other, except for the arterial oxygen saturation of the 3 mg/kg MB group which showed a transient and mild reduction at the 60 min time point (from 94.9 to 91.0%, $p=0.03$) and returned to pre-MB value after the 90 min time point. This was likely due to light absorption of MB, which interfered with pulse oximetry. The mortality rates of the vehicle, 1 and 3 mg/kg MB groups were 1 out of 12, 3 out of 12 and 2 out of 7 at 24 h after stroke, respectively.

2.2. *Lesion volume evolutions*

2.2.1. *Overview*

Representative CBF maps at 30 min, ADC maps at 30 and 180 min, and T2 maps at 24 h are shown in Fig. 1 for the vehicle, 1 and 3 mg/kg MB groups. At 30 min after MCAO, abnormal CBF and ADC were detected and a perfusion and diffusion mismatch was present. At 30 min after MCAO, the ADC lesion volumes after MCAO were similar for all three groups. At 180 min after MCAO, the ADC lesion volumes of the vehicle group were larger than those of the MB treated groups at 180 min. At 24 h after MCAO, T2 infarct volumes were similar amongst the groups.

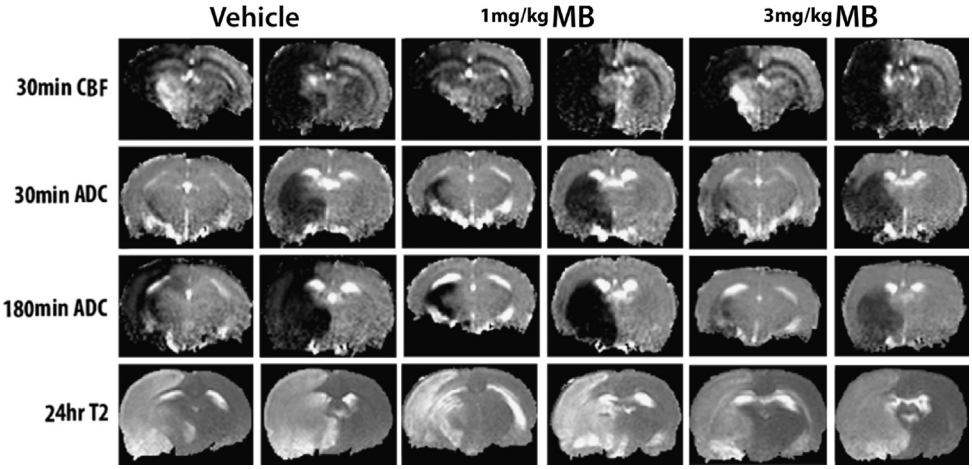

Fig. 1 – Representative baseline cerebral blood flow (CBF) and apparent diffusion coefficient (ADC) lesion volumes at 30 min, ADC volumes at 180 min and T2 volumes at 24 h for the vehicle, 1 and 3 mg/kg methylene blue (MB) groups.