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Effects of beta-hydroxybutyrate on brain vascular permeability in rats with traumatic brain injury

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ABSTRACT

This study investigates the effect of beta-hydroxybutyrate (BHB) on blood–brain barrier (BBB) integrity during traumatic brain injury (TBI) in rats. Evans blue (EB) and horseradish peroxidase (HRP) were used as determinants of BBB permeability. Glutathione (GSH) and malondialdehyde (MDA) levels were estimated in the right (injury side) cerebral cortex of animals. The gene expression levels for occludin, glucose transporter (Glut)-1, aquaporin4 (AQP4) and nuclear factor-kappaB (NF-κB) were performed, and Glut-1 and NF-κB activities were analyzed. BHB treatment decreased GSH and MDA levels in intact animals and in those exposed to TBI ($P<0.05$). Glut-1 protein levels decreased in sham, BHB and TBI plus BHB groups ($P<0.05$). NF-κB protein levels increased in animals treated with BHB and/or exposed to TBI ($P<0.05$). The expression levels of occludin and AQP4 did not significantly change among experimental groups. Glut-1 expression levels increased in BHB treated and untreated animals exposed to TBI ($P<0.05$). While NF-κB expression levels increased in animals in TBI ($P<0.01$), a decrease was noticed in these animals upon BHB treatment ($P<0.01$). In animals exposed to TBI, EB extravasation was observed in the ipsilateral cortex regardless of BHB treatment. Ultrastructurally, BHB attenuated but did not prevent the presence of HRP in brain capillary endothelial cells of animals with TBI; moreover, the drug also led to the observation of the tracer when used in intact rats ($P<0.01$). Altogether, these results showed that BHB not only failed to provide overall protective effects on BBB in TBI but also led to BBB disruption in healthy animals.

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1. Introduction

Traumatic brain injury (TBI) is a major risk factor for death and disability, mainly in childhood and older age. In addition, there are consequential economic costs of TBI for health care systems worldwide. In the posttraumatic period as well as in the early stages of TBI, the components of the neurovascular unit, including the capillary endothelial cells and neurons, are affected to various extents depending on the severity of the trauma. Barrier type-endothelial cells of brain microvessels protect the brain from harmful substances in the circulation and control neuronal microenvironment. Impairment of the blood–brain barrier (BBB) integrity contributes to the loss of neuronal homeostasis causing secondary pathophysiological processes within the brain (Zlokovic, 2008; Abbott et al., 2010). Alterations in neurovascular unit leading to the loss of BBB integrity and brain edema have been described during the course of TBI in a large number of studies that have been performed over the last four decades (Povlishock et al., 1978; Tanno et al., 1992; Unterberg et al., 2004; Alluri et al., 2015). Following BBB disruption, circulatory substances, which under normal conditions cannot extravasate into the brain, gain access to and accumulate in brain parenchyma, and interfere with the neuronal homeostasis (Beaumont et al., 2000; Alves, 2014).

An understanding of the mechanism(s) underlying BBB disruption in the setting of TBI can introduce a major aspect of the therapeutic management of the patients. In this context, cellular excitotoxicity, increase in oxidant capacity, and brain edema leading to further tissue damage have been documented in the posttraumatic period of TBI (Cernak et al., 2000; Shellington et al., 2011). On the other hand, many investigators have attempted to modulate the secondary effects of TBI through the administration of several pharmacologic compounds (Prins et al., 2004, 2005; Deng-Bryant et al., 2008; Corrigan et al., 2014). One of these compounds is beta-hydroxybutyrate (BHB), a ketone body, which is transported from blood to brain parenchyma by monocarboxylic acid transporter located in capillary endothelium (Chowdhury et al., 2014). The systemic administration of BHB reduces production of oxidant species in distinct cortical areas and subregions of the hippocampus and efficiently prevents neuronal death in the cortex of hypoglycemic animals (Julio-Amilpas et al., 2015). Consuming medium chain triglycerides increased levels of BHB in patients with Alzheimer's disease or mild cognitive impairment and increased BHB levels were found to be associated with a greater cognitive improvement (Reger et al., 2004). A recent study reported improvement in behavior along with cognitive and daily activity performance by repeated diurnal elevations of circulating BHB levels (Newport et al., 2015). In addition, ketone bodies including BHB upregulate connectin 43, a gap junction protein, which plays an important role in the regulation of vascular permeability in bovine aortic endothelial cells (Ho et al., 2013). Importantly, several studies have shown neuroprotective properties of ketone bodies including BHB in experimental animal models of TBI (Prins et al., 2004, 2005; Yosunkaya et al., 2004). Preclinical studies employing both pre- and postinjury implementation of the ketogenic diet have demonstrated improved structural and functional outcome in severe or mild TBI models (Prins and Matsumoto, 2014). Ketone bodies can also exert effective neuroprotection in different models of neuronal excitotoxicity and ischemia (Suzuki et al., 2001, 2002; Noh et al., 2006).

Although most of the above-mentioned studies have attributed the beneficial effects of BHB to the action of the drug on oxidant/antioxidant status which is an important determinant of BBB integrity, the modulatory effects of BHB on oxidative stress are still in debate and the mechanisms of action of the drug have not been fully elucidated. Besides, the influence of BHB on the disrupted BBB following TBI is still unknown. Therefore, in the present study we intended to investigate the effects of systemically applied BHB on BBB damage in a rat model of TBI induced using a lateral fluid percussion device.

2. Results

In the ipsilateral cortex, glutathione (GSH) and malondialdehyde (MDA) levels significantly decreased upon BHB treatment to intact animals and to those exposed to TBI compared to the levels in animals in control and sham groups (Fig. 1A

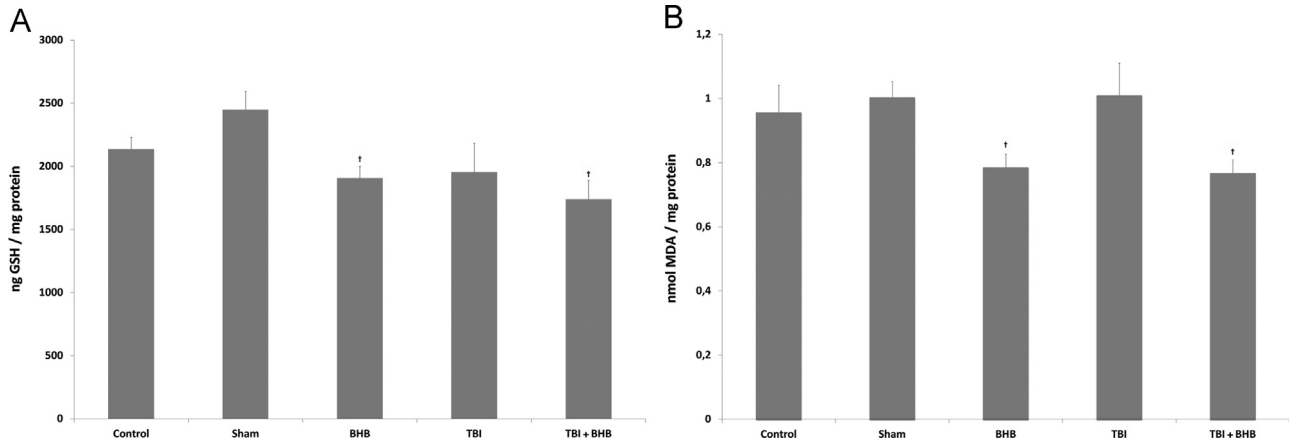

Fig. 1 – Glutathione (GSH; Fig. 1A) and malondialdehyde (MDA; Fig. 1B) levels in the right cerebral cortex of animals in experimental groups. Data represent mean ± SEM. $^{*}P < 0.05$ vs. control and sham values.