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HMGB1 Neutralization Attenuates Hippocampal Neuronal Death and Cognitive Impairment in Rats with Chronic Cerebral Hypoperfusion via Suppressing Inflammatory Responses and Oxidative Stress

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Abstract—High-mobility group box-1 (HMGB1) acts as a proinflammatory molecule once released into the extracellular space and inhibition of HMGB1 signaling has been reported be neuroprotective in neurodegenerative diseases. Besides, chronic cerebral hypoperfusion (CCH) causes cognitive impairment in neurodegenerative diseases. Here we tested the protective role of HMGB1 inhibition using anti-HMGB1 neutralizing antibody (Ab) against CCH in rats after bilateral common carotid artery occlusion (2VO). 169 male Sprague–Dawley rats underwent 2VO or sham operation. PBS, anti-HMGB1 Ab (1 mg/kg), or control IgG Ab (1 mg/kg) was intravenously administered post-operation. HMGB1 translocation, blood–brain barrier (BBB) permeability and glial activation were evaluated at 3 d, as well as the levels of inflammatory cytokines and oxidative stress. NeuN immunostaining and Morris Water Maze (MWM) were performed at 3 d, 4 w and 12 w. We found that anti-HMGB1 neutralizing Ab inhibited HMGB1 translocation in hippocampal CA1 subarea and improved hippocampal HMGB1 level. Besides, anti-HMGB1 Ab preserved BBB integrity and reduced glial activation, in association with the related changes in oxidative stress (increased activities of superoxide dismutase (SOD) and catalase (CAT), and decreased malondialdehyde (MDA) production) and inflammatory cytokines (increased gene expression of IL-1β, IL-6 and TNF) at 3 d. Additionally, anti-HMGB1 neutralizing Ab improved hippocampal CA1 neuronal survival and behavioral outcomes in the chronic phase (4 w and 12 w). Taken together, these findings suggest that HMGB1 neutralization suppresses hippocampal inflammatory responses and oxidative stress in the acute phase, and these changes exert long-lasting beneficial effects in the chronic phase of CCH.

Key words: HMGB1 neutralization, chronic cerebral hypoperfusion, cognitive impairment, inflammation, oxidative stress.

INTRODUCTION

Chronic cerebral hypoperfusion (CCH) causes persistent reduction in cerebral blood flow (CBF) and cognitive impairment in both human and experimental models with neurodegenerative diseases (Akinyemi et al., 2013). Bilateral occlusion of the common carotid arteries (2VO) in rats has been widely used as an animal model of CCH, which could be divided into three successive phases including the acute phase (2–3 days post-operation), the chronic phase (maximum of 3 months) and the restitution phase with regard to the CBF changes and associated metabolic state (Farkas et al., 2007; Soria et al., 2013). Main pathological findings associated with cognitive impairments in this model include white matter lesions, hippocampal neuronal loss, neuroinflammation, glial activation (Du et al., 2017), etc. In particular, previous studies have suggested that neuronal loss, especially in hippocampal CA1 subarea, may be a key factor associated with cognitive impairment in 2VO rats (Bennett et al., 1998; Long et al., 2015). A few studies even presented direct correlation between cognitive function and hippocampal neuronal survival (Bennett et al., 1998; Xi et al., 2014). Therefore, hippocampal neuroprotection probably leads to positive outcomes in cognitive abilities in CCH, and the underlying mechanisms should be further investigated.

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Abbreviations: AD, Alzheimer's disease; BBB, blood–brain barrier; CAT, catalase; CBF, cerebral blood flow; CCH, chronic cerebral hypoperfusion; DAMPs, damage-associated molecular patterns; EB, Evans Blue; HMGB1, High-mobility group box-1; MDA, decreased malondialdehyde; MWM, Morris Water Maze; RAGE, receptor for advanced glycation end products; RT-PCR, reverse transcription-polymerase chain reaction; SO, stratum oriens; SOD, superoxide dismutase; SP, pyramidale; SR, radiatum; TLRs, toll-like receptors; VaD, vascular dementia.

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It is established that inflammation-related mechanisms are involved in the initiation and progression of CCH. Inflammatory responses occurred during CCH results in early blood–brain barrier (BBB) dysfunction and activation of resident glial cells (microglia and astrocytes), as well as subsequent up-regulated expression of proinflammatory cytokines including IL-1β, IL-6 and TNF (Cechetti et al., 2012; Du et al., 2017). The regulation of these inflammatory factors presents improved functional outcomes in rat models of CCH (Kim et al., 2017). On the other hand, it has been reported that the proinflammatory cytokines lead to cognitive deficits with collaborative involvement from oxidative stress (Fu et al., 2014). Increased levels of oxidative stress have been found in patients with Alzheimer's disease (AD) and vascular dementia (VaD) (Gustaw-Rothenberg et al., 2010), and in experimental models of CCH oxidative stress seems to further participate in the progression of neuronal loss and cognitive decline (Luca et al., 2015). More specifically, enhanced oxidative stress causes remarkable damage to lipids, proteins and DNA, and could be measured using markers of antioxidants (such as superoxide dismutase (SOD) and catalase (CAT)) and lipid peroxidation (such as malondialdehyde (MDA)), all of which presented temporal and spatial changes during CCH (Du et al., 2017; Institoris et al., 2007). But therapeutic effects on hippocampal neuronal survival and cognitive function could be made via suppressing oxidative stress in the early period of CCH (Institoris et al., 2007; Zhang et al., 2011). Taken together, these findings indicated that inflammatory responses and oxidative stress in early period of CCH plays an essential role in the progressive neuronal loss and cognitive impairment.

High-mobility group Box-1 (HMGB1) is a nonhistone DNA-binding protein that is known to be damage-associated molecular patterns (DAMPs), and it could activate proinflammatory molecular cascades after translocation to the extracellular space by both active secretion during brain infection and passive leakage in brain injuries (Tang et al., 2011). Although HMGB1 could be released from innate immune cells, previous studies have shown that HMGB1 is massively released by neurons in brain ischemia, and the extracellular HMGB1 in turn causes inflammatory responses, leading to delayed neuronal death (Kikuchi et al., 2009; Lee et al., 2016). Moreover, recombinant HMGB1 alone can induce acute inflammation and anti-HMGB1 reagents (mainly HMGB1 antagonists and neutralizing antibodies), on the contrary, has been reported to suppress inflammatory responses and oxidative stress, leading to behavioral and biochemical improvement under inflammatory conditions (Gong et al., 2014; Kikuchi et al., 2009; Liu et al., 2007). Besides, recent studies have found that HMGB1 expression also increases in neurodegenerative diseases and HMGB1 neutralization successfully exert neuroprotective effects in the experimental models including aging, Parkinson's disease, AD (Festoff et al., 2016; Sasaki et al., 2016; Terrando et al., 2016), etc. Taken these findings into consideration, HMGB1-related mechanisms should be further investigated in CCH.

As it remains unclear whether HMGB1 could be used as an efficient molecular target in CCH treatment, here we aim to present evidence in a rat model of CCH that anti-HMGB1 neutralizing Ab improves long-term neuronal survival and cognitive function by modulating inflammatory responses and suppressing oxidative stress.

EXPERIMENTAL PROCEDURES

Animals and groups

All 169 male Sprague–Dawley rats (ages of 4–6 weeks) from the Experimental Animal Center of Fourth Military Medical University were raised in a vivarium with regular 12:12-h light–dark cycle. Food and water were applied ad libitum. The protocol of the animal studies was approved by the Institutional Animal Care and Use Committee and complied with National Institutes of Health guidelines. Apart from 30 rats in the sham group (injected with PBS only), 139 rats underwent bilateral common carotid artery occlusion (2VO) surgery and were randomly divided into the anti-HMGB1 group (intravenously injected with anti-HMGB1 Ab dissolved in PBS), PBS group (injected with PBS) and the control IgG group (injected with control IgG Ab dissolved in PBS)

Model establishment of CCH and anti-HMGB1 neutralizing Ab treatment

As described previously (Long et al., 2015), chronic cerebral hypoperfusion of the rats were induced by permanent bilateral common carotid artery occlusion (2VO). Anesthesia was performed using chloral hydrate (10%, 3 ml/kg, i.p.). For the surgery, firstly, a 1.5-cm midline incision was done on the ventral side of the cervical region. After separation of the adipose tissue and subsequent bilateral nerve fibers, bilateral common carotid arteries were exposed and permanently ligated with silk sutures. The arteries in the sham group were exposed without ligation. During the whole operation, the temperature was maintained at around 37.5 °C to reduce death rate. However, 25 rats died within the first day after 2VO surgery (10 in the PBS group, eight in the anti-HMGB1 group and seven in the control IgG group). The anti-HMGB1 neutralizing Ab (Shino-test, Kanagawa, Japan) and paired control IgG Ab (Sigma–Aldrich, MO, USA) were used here. The neutralizing Ab could be detected using western blotting and targets both HMGB1-induced cytokine/chemokine release and chemotactic activities (Abeyama et al., 2005; Wang et al., 2014) and the its therapeutic effects have been tested in other animal models (Degos et al., 2013; Miura et al., 2014). The dose and the treatment schedule were rationally designed based on previous evidence of the drug's therapeutic effects in rodent models of acute brain injury and chronic neurodegenerative diseases (usually multiple injections with 1 mg/kg i.v.) (Sasaki et al., 2016; Terrando et al., 2016). Rats were injected with anti-HMGB1 neutralizing Ab (1 mg/kg, dissolved in 0.9% PBS), PBS or control IgG Ab (1 mg/kg, dissolved in 0.9% PBS) via the tail vein. To investigate the therapeutic effects of anti-HMGB1, the

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