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Research report

Electroacupuncture pretreatment attenuates spinal cord ischemia-reperfusion injury via inhibition of high-mobility group box 1 production in a LXA_4 receptor-dependent manner

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ABSTRACT

Paraplegia caused by spinal cord ischemia is a severe complication following surgeries in the thoracic aneurysm. HMGB1 has been recognized as a key mediator in spinal inflammatory response after spinal cord injury. Electroacupuncture (EA) pretreatment could provide neuroprotection against cerebral ischemic injury through inhibition of HMGB1 release. Therefore, the present study aims to test the hypothesis that EA pretreatment protects against spinal cord ischemia-reperfusion (I/R) injury via inhibition of HMGB1 release. Animals were pre-treated with EA stimulations 30 min daily for 4 successive days, followed by 20-min spinal cord ischemia induced by using a balloon catheter placed into the aorta. We found that spinal I/R significantly increased mRNA and cytosolic protein levels of HMGB1 after reperfusion in the spinal cord. The EA-pretreated animals displayed better motor performance after reperfusion along with the decrease of apoptosis, HMGB1, TNF-α and IL-1β expressions in the spinal cord, whereas these effects by EA pretreatment was reversed by rHMGB1 administration. Furthermore, EA pretreatment attenuated the down-regulation of LXA_4 receptor (ALX) expression induced by I/R injury, while the decrease of HMGB1 release in EA-pretreated rats was reversed by the combined BOC-2 (an inhibitor of LXA_4 receptor) treatment. In conclusion, EA pretreatment may promote spinal I/R injury through the inhibition of HMGB1 release in a LXA_4 receptor-dependent manner. Our data may represent a new therapeutic technique for treating spinal cord ischemia-reperfusion injury.

1. Introduction

Paraplegia resulting from spinal cord ischemia is a severe complication of surgeries in the thoracic and thoraco-abdominal aneurysm with an incidence of 3–33% (Drinkwater et al., 2010; MacArthur et al., 2005; Zoli et al., 2010). During the surgery, the aortic cross-clamping can lead to episodes of perfusion absence or impairment, which directly induce ischemia and metabolic neuronal injury in the spinal cord. In addition, the following reperfusion also exaggerates the inflammation to the metabolic injury, leading to irreversible and lethal injury to spinal cord neurons (Bell et al., 2012). Although considerable progress has been made concerning the alleviation of spinal cord ischemia injury (Grabenwöger et al., 2012; Khan and Stansby, 2012; Sinha and Cheung, 2010), there is still few effective non-invasive technique for treating neurological dysfunctions after spinal cord ischemia and reperfusion (I/R).

Inflammatory response has been recognized as an important contributor to neuronal damages in spinal cord injury (Donnelly and Popovich, 2008). Indeed, the inhibition of various inflammatory cascades could alleviate damages in experimental spinal cord injury (Fan et al., 2011). Recent studies has identified

Abbreviations: ALX, LXA_4 receptor; BOC-2, butoxy carbonyl-Phe-Leu-Phe-Leu-Phe; EA, electroacupuncture pretreatment; HMGB1, high mobility group box 1; I/R, ischemia and reperfusion; IL-1β, interleukin-1β; rHMGB1, recombinant HMGB1; TNF-α, tumor necrosis factor α.

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the high-mobility group box 1 (HMGB1), a kind of DNA-binding protein ubiquitously expressed in the central nervous system as a key mediator in inflammation and neuroinflammation (Fang et al., 2012; Martinotti et al., 2015; Yang et al., 2013). Upon ischemia or injury stimulation, HMGB1 translocates from the nucleus to the cytoplasm, thereby leading to the activation of inflammatory-related transcription factors such as nuclear factor-κB (NF-κB) (Chen et al., 2004). In the model of compression-induced spinal injury, the release of HMGB 1 was associated with neuronal damages, which may subsequently lead to neurologic dysfunctions (Kawabata et al., 2010). Therefore, HMGB1 may be a promising therapeutic target for neuroprotection against neurological dysfunctions after spinal cord ischemia.

Accumulative evidence has demonstrated that electroacupuncture (EA) pretreatment is an effective technique for protecting against cerebral I/R injury (Wang et al., 2005; Zhu et al., 2013). Electroacupuncture is a modified technique that stimulates specific acupoints with electrical impulses. Importantly, it is also noted that the protection of EA pretreatment against cerebral ischemic injury is related to the inhibition of HMGB1 release (Wang et al., 2012). Therefore, we hypothesized that EA pretreatment may protect against spinal cord ischemia-reperfusion injury through inhibition of HMGB1 release.

Another interesting issue is the mechanism of EA-mediated neuroprotection against inflammatory responses and HMGB1 release after spinal cord ischemic injury. A large number of studies have highlighted lipoxins as endogenously produced anti-inflammatory molecules in various disease models, including cerebral (Ye et al., 2010a,b) or renal (Kieran et al., 2003) ischemia/reperfusion injury and lipopolysaccharide (LPS)-induced acute lung injury (Gong et al., 2012a,b). Lipoxin A_4 (LXA_4) is the major physiological lipoxin during inflammation, and has been demonstrated with an anti-inflammatory action via inhibition of chemotaxis (Waechter et al., 2012) and pro-inflammatory cytokines production (Chen et al., 2013) through coupling with its receptor (lipoxin A_4 receptor, ALX). Recently, the anti-inflammatory role of LXA_4/ALX signaling in the spinal cord is revealed in the models of spinal cord hemisection (Martini et al., 2016) and post-injury neuropathic pain (Miao et al., 2015; Wang et al., 2014a,b,c). However, the links between ALX and EA-mediated down-regulation HMGB1 production during I/R injury has not been clarified.

In the present study, we report that, after spinal cord I/R injury, EA pretreatment reduces apoptotic pathway activation and pro-inflammatory cytokines production by inhibiting HMGB1 release in a LXA_4 receptor-dependent manner, resulting in the improvement of post-ischemia neurological functions.

2. Results

2.1. HMGB1 increases after spinal I/R injury

To determine the effects of I/R injury on HMGB1 expressions in the spinal cord, we first detected the mRNA and cytosolic protein levels of HMGB1 by real-time PCR or western blot at 1, 3 and 7 days after reperfusion (n = 4 per group for each time point). As shown in Fig. 1A and B, spinal mRNA and cytosolic protein expressions of HMGB1 increased significantly 1 day after reperfusion ($P < 0.05$ and $P < 0.01$, respectively), peaked at 3 d ($P < 0.01$ and $P < 0.01$, respectively), and then began to decrease (n = 4 per group for each time point).

We next determined the time course of tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) by Enzyme-Linked ImmunoSorbent Assay (ELISA) assays. Similar with the results of HMGB1, we found that the spinal expressions of TNF-α and IL-1β also increased after ischemia and peaked at 3 d after reperfusion ($P < 0.01$ and $P < 0.01$, respectively).

2.2. EA pretreatment attenuates the up-regulation of HMGB1 and improves neurologic function after spinal I/R injury

The motor function scale (Tarlov scale) in I/R, EA + I/R and EA + I/R + rHMGB1 groups declined at 1 days after I/R injury (n = 6 per group, Fig. 2A). Importantly, the animals in EA + I/R group displayed better motor performance at 12, 24 and 72 h after reperfusion when compared with I/R group ($P < 0.05$ or $P < 0.01$ by Mann-Whitney test, Fig. 2A). However, the improvement of neurologic function after EA pretreatment was reversed in the presence of rHMGB1 (recombinant HMGB1, an antagonist of HMGB1) administration ($P < 0.01$ by Mann-Whitney test, Fig. 2A).

Next, we sought to investigate the effects of EA pretreatment on the cytosolic HMGB1 protein expression and the production of TNF-α and IL-1β after ischemia of the spinal cord. Our results showed that the up-regulation of cytosolic HMGB1 protein expression as well as the overproduction of TNF-α and IL-1β induced by I/R injury was alleviated after EA pretreatment ($P < 0.05$, n = 5 per group, Fig. 2B and C), whereas these effects of EA pretreatment were abrogated by the combined rHMGB1 treatment ($P < 0.01$, Fig. 2B and C).

2.3. EA pretreatment alleviates spinal apoptosis after ischemia and reperfusion

In the spinal cord injury, the up-regulation of HMGB1 has been associated with increased apoptosis (Kawabata et al., 2010). Therefore, we next detected the effects of EA pretreatment on spinal apoptosis after I/R injury. Our results showed that the active caspase-3 expressions was markedly decreased in EA + I/R group compared with I/R group ($P < 0.05$, Fig. 3A), whereas these effects of EA pretreatment was blocked in the presence of intrathecal rHMGB1 treatment ($P < 0.01$, Fig. 3A).

Furthermore, we also determine the spinal expression of apoptosis regulatory proteins, such as Bcl-2 (anti-apoptotic) and Bax (pro-apoptotic). A significant increase in Bcl-2 expression along with a decrease in Bax expression were observed in EA + I/R group compared with I/R group ($P < 0.01$, n = 5 per group, Fig. 3B), whereas these effects of EA pretreatment was reversed by the combined intrathecal rHMGB1 treatment ($P < 0.01$, Fig. 3C).

2.4. The recovery of LXA_4 receptor signaling is involved in EA pretreatment-mediated attenuation of HMGB1 release and apoptosis

Recently, it is reported that the activation of lipoxin A_4 (LXA_4) receptor (ALX) is capable of providing anti-inflammatory actions, resulting in improvement of brain ischemia injury (Wu et al., 2012). Therefore, we investigated the role of ALX signaling in EA-mediated neuroprotective and anti-inflammatory effects.

Firstly, the effects of EA pretreatment or combined rHMGB1 on the spinal ALX expressions were examined by western blot at 72 h after ischemia and reperfusion. Our results showed that the I/R injury resulted in a significant decrease in the ALX expressions ($P < 0.01$, n = 5 per group, Fig. 4A), whereas the down-regulation of ALX expression was attenuated by the EA pretreatment ($P < 0.01$, Fig. 4A). Of note, the statistical differences in spinal ALX expression were not observed between EA + I/R and EA + I/R + rHMGB1 groups ($P > 0.05$, Fig. 4A).

Next, the role of LXA_4 receptor signaling in EA pretreatment-mediated neuroprotection was further investigated by intrathecal administration of its specific inhibitor butoxy carbonyl-Phe-Leu-Phe-Leu-Phe (BOC-2) (Wang et al., 2014a,b,c). Our results showed that EA pretreatment-mediated reduction in the protein