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BRAIN RESEARCH

Research Report

# Electroacupuncture pretreatment prevents cognitive impairment induced by limb ischemia–reperfusion via inhibition of microglial activation and attenuation of oxidative stress in rats☆

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ABSTRACT

Limb ischemia–reperfusion (LI/R) is associated with high morbidity and mortality. Furthermore, critical trauma survivors can present cognitive impairment. Cognitive function, survival rate, oxidative stress and neuronal health were examined to elucidate (1) the magnitude of cognitive effects of prolonged reperfusion, (2) potential players in the mechanistic pathway mediating such effects, and (3) possible benefits of electroacupuncture (EA) pretreatment at Baihui (GV20), Yanglingquan (GB34), Taichong (LR3), Zusanli (ST36) and Xuehai (SP10) acupoints. LI/R was induced in rats by placing a rubber tourniquet on each hind limb for 3 h, and the animals were evaluated periodically for 7 d after LI/R. Rats subjected to LI/R had significantly lower survival rates, and displayed evidence of brain injury and cognitive dysfunction (as determined by the Morris water maze test) 1 d and 3 d after reperfusion compared to sham-operated controls. LI/R also resulted in higher levels of reactive oxygen species (ROS) and malondialdehyde (MDA), microglial activation, and decreased superoxide dismutase (SOD) activity within Cornu Ammonis area 1 (CA1) of the hippocampus. Depressed survival rates, microglial activation, oxidative damage, and histological changes, as well as cognitive dysfunction were partially or fully attenuated in rats that received 14 d of EA prior to LI/R. These findings indicate that LI/R can result in cognitive dysfunction related to activated microglia and elevated oxidative stress, and that EA has neuroprotective potential mediated, at least in part, by inhibition of microglial activation and attenuation of oxidative stress.



## 1. Introduction

Limb ischemia–reperfusion (LI/R) injury is a common but grave condition common in some critical clinical settings including severe crush injury, damage of the great vessels in the extremities, and some surgical procedures. LI/R is associated with serious local and systemic effects, including multiple organ failure and death. As a result, LI/R presents high morbidity and mortality

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*Abbreviations:* LI/R, limb ischemia–reperfusion; CA1, Cornu Ammonis area1; MWM, Morris water maze; ROS, reactive oxygen species; MDA, malondialdehyde; SOD, superoxide dismutase; DCF-DA, dichlorofluorescein diacetate; EA, electroacupuncture; Iba 1, ionized calcium binding adaptor molecule 1

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rates (Yassin et al., 2002). Several studies have demonstrated that LI/R is capable of inducing further damage to skeletal muscle (Tran et al., 2011) as well as in such distant organs as the lung (Peng et al., 2011), heart (Lu et al., 2006), liver (Nie et al., 2002) and small intestine (Zhang et al., 2011), thereby potentially leading to multiple organ failure (Yassin et al., 1996). The brain has recently been identified as a LI/R vulnerable organ. While reported effects of LI/R injury include microglial activation and apoptosis in the brain (Bianco-Batlles et al., 2008), long-term neurological outcome and potential cognitive deficits have not been investigated.

Importantly, the length and frequency of the ischemia event are crucial in determining the magnitude of effect. Brief and intermittent limb ischemia can have a protective effect on multiple organs, including brain, when faced with a subsequent ischemic event (Jensen et al., 2011; Kanoria et al., 2007a,b), a benefit that may be mediated via a neurogenic pathway (Malhotra et al., 2011). Limb ischemia preconditioning has actually become a predominant form of therapy; however, ischemia durations are short (5–15 min) (Koch et al., 2011) and frequent (2–3 cycles) (Ren et al., 2008) in comparison to ischemia durations that elicit damage to underlying skeletal muscle and distant organs (≥2 h) (Whetzel et al., 1997).

Tragically, survivors of critical illness are frequently left with a legacy of cognitive impairment (Iwashyna et al., 2010; Jones et al., 2006; Rothenhausler et al., 2001; Sukantarat et al., 2005). Cognitive dysfunction is associated with both poor short-term and long-term outcomes including early retirement due to disability and an increased risk of mortality (Moller et al., 1998; Newman et al., 2001a,b; Steinmetz et al., 2009). As a principal part of the limbic system, the hippocampus plays crucial roles in learning and memory (Gilbert and Kesner, 2002); and hippocampal CA1 pyramidal neurons are highly vulnerable to hazardous insults (Nunn and Hodges, 1994) and their destruction and dysfunction has been linked to cognitive deficits (Suzuki and Clayton, 2000).

Moreover, increasing evidence (Cheng et al., 2008; Feng et al., 2010; Liu et al., 2006) has shown that acupuncture can attenuate cognitive deficits, maintain oxidant–antioxidant balance, inhibit apoptosis, and regulate cell proliferation in the hippocampus. On the other hand, some conflicting studies have reported that treatment with electroacupuncture (EA) did not affect cognitive functioning (Rorsman and Johansson, 2006; Yang et al., 2009). Hence, more research is needed to clarify whether EA can ameliorate cognitive dysfunction and, if so, what mechanism mediates this neuroprotective effect.

Based on the existing literature, we hypothesized that LI/R is capable of inducing cognitive dysfunction in rats, and that this damage that may be averted by EA pretreatment via prevention of development of oxidative stress. The results from this study should provide a better understanding of long-term detriments associated with LI/R and the neuroprotective potential and mechanism of EA pretreatment.

## 2. Results

### 2.1. EA pretreatment improves survival rate after LI/R

Survival rate after 7 d was significantly reduced in rats subjected to a LI/R event (40%, 8/20 rats) compared to sham controls (100%, 20/20 rats) ($p<0.05$). In contrast, the 7-d survival rate among rats that received EA for 14 d prior to the LI/R event was significantly higher (80%, 16/20 rats) than that of rats those that received no pretreatment (40%) ($p<0.05$). Survival rate did not differ significantly between Sham and EA groups (Fig. 1).

### 2.2. EA pretreatment reduces pathological damage induced by LI/R

Representative hematoxylin and eosin (H&E) stained micrographs (Fig. 2) illustrated the normal nerve cell bodies within the hippocampal CA1 region, their tight arrangement, clear structure and abundant cytoplasm, that were observed among individuals of the Sham group (Fig. 2A). In contrast, the majority of neurons within the hippocampal CA1 region of the LI/R group were shrunken and darkly stained with enlarged intracellular spaces (Fig. 2B). Neurons within the same region in EA individuals were well preserved and appeared normal, indicative of eumorphism (Fig. 2C). Morphometric analysis revealed that the number of normal cells within the hippocampal CA1 region was significantly lower in the LI/R group compared to either Sham or EA groups at 1 d, 3 d and 7 d post-reperfusion ($p<0.01$). The number of normal cells within the same region in the EA group was significantly greater than that in the LI/R group ($p<0.01$) but lower than that in the Sham group ($p<0.05$ or 0.01) (Fig. 2D).

### 2.3. EA pretreatment attenuates LI/R stimulated neuron apoptosis

Representative micrographs of terminal deoxynucleotidyl transferase-mediated dUDP-biotin nick end labeling (TUNEL)-positive apoptotic cells, which contain nuclei that are large and stained a dark brown are shown in Figs. 3A–C. Differences in the percentage of TUNEL-positive cells within the hippocampal CA1 region 1 d, 3 d, and 7 d after the LI/R event were significant among all three experimental groups ($p<0.01$) (Fig. 3C). Whereas no TUNEL-positive cells were detected in the hippocampal CA1 region in the Sham group, TUNEL-positive cells comprised 50.3–65.1% of the total cell population in the LI/R group and comprised 25.6–35.7% of the total cell population in the EA group 1–7 d after reperfusion.

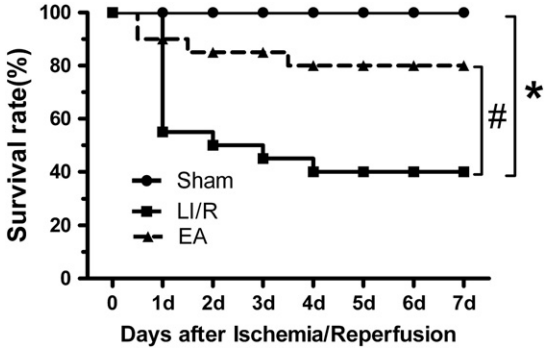


**Fig. 1 – Survival rates among the three experimental groups up to 7 d after the LI/R event. Data are expressed percentages. Each datapoint represents the group mean ($n=20$; $^{*}p<0.05$ vs. Sham; $^{\#}p<0.05$ vs. EA).**