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ROLE OF AUTOPHAGY IN THE BIMODAL STAGE AFTER SPINAL CORD ISCHEMIA REPERFUSION INJURY IN RATS

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Abstract—Autophagy plays an important role in spinal cord ischemia reperfusion (I/R) injury, but its neuroprotective or neurodegenerative role remains controversial. The extent and persistence of autophagy activation may be the critical factor to explain the opposing effects. In this study, the different roles and action mechanisms of autophagy in the early and later stages after I/R injury were investigated in rats. The spinal cord I/R injury was induced by 14-min occlusion of the aortic arch, after which rats were treated with autophagic inhibitor (3-methyladenine, 3-MA) or agonist (rapamycin) immediately or 48 h following the injury. Autophagy markers, microtubule-associated protein light chain 3-II (LC3-II) and Beclin 1 increased and peaked at the early stage (8 h) and the later stage (72 h) after spinal cord I/R injury. Beclin 1 was mostly expressed in neurons, but was also expressed to an extent in astrocytes, microglia and vascular endothelial cells. 8 h after injury, rats treated with 3-MA showed a decrease in the hind-limb Basso–Beattie–Bresnahan (BBB) motor function scores, surviving motor neurons, and B-cell lymphoma-2 (Bcl-2) expression, and increase in the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL)-positive cells, Bcl-2-associated X protein (Bax), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) expression, and activation of microglia, while those treated with rapamycin showed opposing effects. However, 72 h after injury, rats treated with 3-MA improved the BBB scores, and the surviving motor neurons, and reduced the autophagic cell death, while those treated with rapamycin had adverse effects. These findings provide the first evidence that early activated autophagy alleviates spinal cord I/R injury via inhibiting apoptosis and inflammation; however later excessively elevated autophagy aggravates I/R injury through inducing autophagic cell death. **© 2016 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: autophagy, spinal cord, ischemia reperfusion injury, apoptosis, microglia, autophagic cell death.

INTRODUCTION

Spinal cord ischemia reperfusion (I/R) can result in devastating paraplegia, which is a major complication of surgery for thoraco-abdominal aneurysms with a reported incidence of 3–18% (Coselli et al., 2000). Though many strategies have been attempted to mitigate spinal cord I/R injury, the protective effect is very limited and the incidence of paraplegia remains high (Bischoff et al., 2011).

The maintenance of the normal metabolic balance of eukaryotic cells involves two main pathways: ubiquitin proteasome pathway and autophagy lysosomal pathway (Klionsky and Emr, 2000). Autophagy is an intracellular catabolic mechanism that maintains a balance between protein synthesis and degradation, which can both promote cell survival and induce cell death (autophagic cell death) depending on the specific pathological event. Accumulating evidence has demonstrated that autophagy activation is elevated in the central nervous system under stressed conditions (Park et al., 2015; Li et al., 2015a; Villamil-Ortiz and Cardona-Gomez, 2015; Yang et al., 2015). As shown in the previous studies, autophagy plays an important role in spinal cord injury (Baba et al., 2009; Seo et al., 2015; Fujita et al., 2015); however, its expression change and mechanism after spinal cord I/R injury are largely unknown. Furthermore, the role of autophagy in ischemic injury remains controversial. Studies have shown that transient spinal cord ischemia-induced autophagy in motor neurons contributes to neuronal death (Baba et al., 2009; Fujita et al., 2015). However, Fan et al., have demonstrated that autophagy plays a protective role in that ischemia and ischemic preconditioning protects spinal neurons by sustaining autophagy (Fan et al., 2014).

The primary aim of this study was to investigate the expression change of autophagy in different neurocytes after spinal cord I/R injury. The secondary aim was to evaluate the role and action mechanism of autophagy in the early stage and later stage after injury, respectively.

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Abbreviations: 3-MA, 3-methyladenine; Bax, Bcl-2-associated X protein; BBB, Basso–Beattie–Bresnahan; Bcl-2, B-cell lymphoma-2; CD31, platelet endothelial cell adhesion molecule-1; ELISA, enzyme-linked immunosorbent assay; GFAP, glial fibrillary acidic protein; I/R, ischemia reperfusion; Iba-1, ionized calcium-binding adaptor molecule 1; IL-1β, interleukin-1β; LC3, microtubule-associated protein light chain 3; NeuN, neuronal nuclei; PBS, Phosphate-buffered saline; TNF-α, tumor necrosis factor-α; TUNEL, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling.

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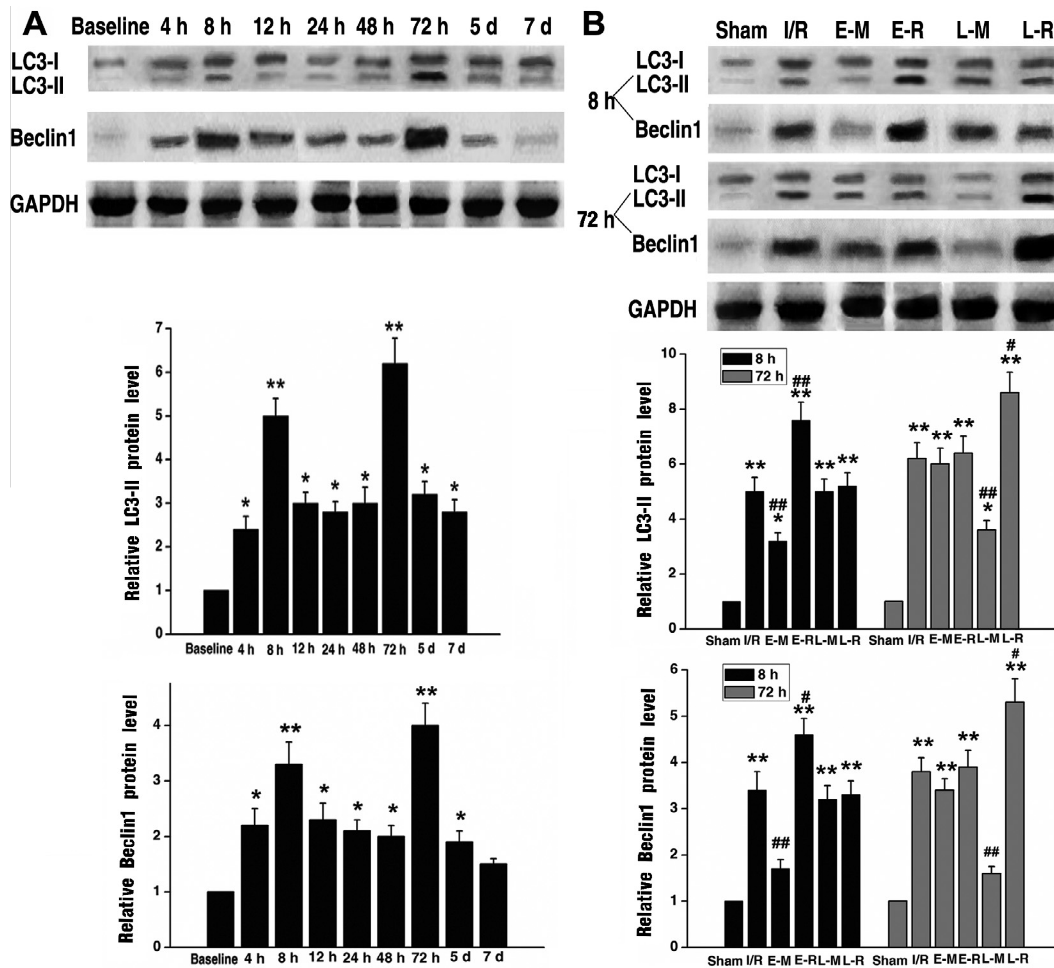

Fig. 1. Changes of LC3 and Beclin 1 protein expression after spinal cord I/R injury. (A) Representative western blot and quantitative protein analysis of LC3-II and Beclin 1 following varying time points. (B) Representative western blot and quantitative protein analysis of LC3-II and Beclin 1 at 7 d after I/R injury in each group. All data are represented as mean ± SEM (n = 6 per group or time point). $^{**}P$ < 0.01 versus sham, $^{*}P$ < 0.05 versus sham; $^{\#\#}P$ < 0.01 versus I/R, $^{\#}P$ < 0.05 versus I/R.

EXPERIMENTAL PROCEDURES

Experimental animals

All experimental procedures were conducted with the approval of the ethics committee of China Medical University and in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). Male Sprague–Dawley rats, weighing 200–250 g were used in this study. All rats were neurologically intact before anesthesia and housed in standard cages with free access to food and water. They were separately housed after surgery.

Experimental protocol

A total of 144 rats were randomly assigned to six groups, with six animals being used in each experiment group at each time point. The sham group (n = 24) and I/R group (n = 48) received aortic arch exposure or cross-clamping for 14 min following intraperitoneal equivalent volume of phosphate-buffered saline (PBS). The early treatment with 3-methyladenine (3-MA) (E–M) group (n = 18) and early treatment with rapamycin (E–R) group (n = 18) received intraperitoneal 3-MA or rapamycin immediately after injury. The later treatment with 3-MA (L–M) group (n = 18) and later treatment with rapamycin (L–R) group (n = 18) received intraperitoneal 3-MA or rapamycin at 48 h after injury.

In each group, six rats were anesthetized at each time point with an overdose of pentobarbital and the L4-6 segments of spinal cords were rapidly collected for histological study, immunofluorescence staining, terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-biotin nick end labeling (TUNEL) assay, western blot and enzyme-linked immunosorbent assay (ELISA).

Surgical procedure

The spinal cord I/R model was induced by occluding the aortic arch for 14 min through a left lateral thoracotomy approach, as previously reported (Awad et al., 2010). All rats were anesthetized with intraperitoneal injection of 4% sodium pentobarbital at an initial dose of 50 mg/kg. Lung ventilation was achieved using a small animal

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