Intrathecal Injection of 3-Methyladenine Reduces Neuronal Damage and Promotes Functional Recovery via Autophagy Attenuation after Spinal Cord Ischemia/Reperfusion Injury in Rats

Xing Wei, Zhentao Zhou, Lingyun Li, Jun Gu, Chen Wang, Fuqi Xu, Qirong Dong, and Xiaozhong Zhou*

Department of Anesthesiology, the Second Affiliated Hospital of Soochow University; Suzhou 215006, China; Department of Orthopaedics, the Second Affiliated Hospital of Soochow University; Suzhou 215006, China; and Experimental Center, the Second Affiliated Hospital of Soochow University; Suzhou 215006, China.

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The present study aimed to determine the occurrence of autophagy following ischemia/reperfusion (I/R) injury in the rat spinal cord and whether autophagy inhibition contributes to neural tissue damage and locomotor impairment. A spinal cord I/R model was induced via descending thoracic aorta occlusion for 10 min using systemic hypotension (40 mmHg) in adult male Sprague-Dawley rats. Then, 600 nmol 3-methyladenine (3-MA) or vehicle was intrathecally administered. Ultrastructural spinal cord changes were observed via transmission electron microscopy (TEM) and immunofluorescent double-labeling. Western blots were used to determine the protein expression of microtubule-associated protein light chain 3 (LC3) and Beclin 1. Autophagy was activated after spinal cord I/R injury as demonstrated by significantly increased LC3 and Beclin 1 expression at 3–48 h after injury. Furthermore, TEM images indicated the presence of autophagosomes and autolysosomes in the injured spinal cord. 3-MA significantly decreased LC3 and Beclin 1 expression and the number of LC3-positive cells in spinal cord of I/R versus vehicle groups. Moreover, the 3-MA-treated rats exhibited better neurobehavioral scores compared with control rats. These findings suggest activation of autophagy leading to neuronal cell death in the I/R injured spinal cord. These effects were significantly inhibited by intrathecal 3-MA administration. Thus intrathecal 3-MA administration may represent a novel treatment target following spinal cord I/R injury.

Key words: spinal cord ischemia/reperfusion (I/R) injury; autophagy; 3-methyladenine (3-MA); neuroprotection; Beclin 1; microtubule-associated protein light chain 3 (LC3)

Spinal cord ischemia/reperfusion (I/R) injury may induce paralysis, which represents the underlying pathological cause of patient mortality and morbidity.1 Spinal cord I/R injury triggers pathophysiological changes that involve excitotoxicity, free radical production, inflammation, and apoptosis.2–4 In recent years, evidence has indicated that autophagy may play an important role in various spinal disease models, including spinal cord contusion injury, spinal cord hemisection injury, and cervical spondylotic myelopathy.5–7 However, the occurrence and role of autophagy in neural tissues following spinal cord I/R injury have not been clearly illustrated.

Autophagy comprises an evolutionarily conserved process wherein damaged or dysfunctional cytoplasmic components undergo autophagosomal–lysosomal pathway clearance/degredation to facilitate the maintenance of homeostasis.8,9 Microtubule-associated protein light chain 3 (LC3) and Beclin 1 are two key proteins involved in the autphagic cascade. LC3 occurs in cytosolic (LC3-I) and membrane-bound (LC3-II) forms, and the extent of autophagosome formation is correlated with the ratio of LC3-I to LC3-II conversion.10 Beclin 1 is a mammalian homolog of yeast Atg6 that was initially described as a Bcl-2-interacting protein and has been demonstrated to promote autophagy.11 Autophagy is activated during different stress responses, including starvation, oxidative stress, and hypoxia.12,13 Abnormal autophagy has been linked to many pathophysiological events and is involved in disease development.14 The presence of autophagosomes in dying cells indicates that autophagy may play a role in cell death (i.e., “autophagic cell death”).15 Autophagy is a critical regulator of cell death and survival and interacts with necrosis and apoptosis in determining the outcome of injured cells. In recent years, the role of autophagy in the pathogenesis of cerebral ischemia had been clearly demonstrated.16–18 Investigations of autophagy-related cures to alleviate the hardships of spinal I/R injury include interventions that attenuate the secondary injury cascade, enhance endogenous repair mechanisms, and replace lost cells.

3-Methyladenine (3-MA) is a pharmacological inhibitor of autophagy. It blocks class III phosphoinositide 3-kinase (PI3K) to inhibit autophagosome formation, which subsequently inhibits autophagy. 3-MA exhibits neuroprotective effects. For example, intracerebral ventricular injection of 3-MA following global ischemia in rats significantly decreased the infarct volume, brain edema, and motor deficits.19 Similar 3-MA-induced effects were identified in oxygen and glucose-deficient PC-12 neuronal cells, whereas 3-MA significantly increased cortical PC-12 cell viability.20 Thus, the present study aimed to investigate whether autophagy activation occurs in a spinal cord I/R injury rat model and whether intrathecal 3-MA administration (not intraperitoneal administration) can inhibit autophagy as a neuroprotective intervention, which should thus reduce neuronal damage and promote functional recovery in this translational model.

These authors contributed equally to this work.

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MATERIALS AND METHODS

Animals Adult male Sprague-Dawley rats that weighed 375–400 g were provided by the Experimental Animals Center of Soochow University (certificate No. 20020008, Grade II). All experimental procedures were in compliance with the National Institutes of Health for the Care and Use of Laboratory Animals. The protocol was approved by the Institutional Animal Care and Use Committee of Soochow University. Anesthesia was induced via an intraperitoneal injection of 4% choral hydrate (0.1 mg/kg). The animals were orally intubated and mechanically ventilated (ALC-V9; Alcott Biotech, Shanghai, China). A heating pad was used to maintain the rectal temperature at 37.5°C. The animals were divided into one sham-operated and five I/R injury groups according to when assessments were performed after I/R 3, 6, 12, 24, and 48 h (I/R 3 h, I/R 6 h, I/R 12 h, I/R 24 h, I/R 48 h).

Spinal Ischemia The tail artery was cannulated with a polytetrafluoroethylene catheter (22 gauge) to monitor the mean distal arterial pressure (MDAP), as well as to enable the intraarterial infusion of heparin. A polytetrafluoroethylene catheter (22 gauge), which was connected to an external blood reservoir (37.5°C), was inserted into the left carotid artery to monitor the mean proximal arterial blood pressure (MPAP) and control the MPAP to 40 mmHg during aortic occlusion. The left femoral artery was exposed, and a 2 F Fogarty catheter (Edwards Life Sciences, LLC, Berkeley, U.S.A.) was passed to the thoracic aorta to enable the catheter tip to reach the left subclavian artery. Immediately following cannulation completion, heparin (200 U) was injected into the tail artery. Spinal cord ischemia was induced via balloon inflation with saline (0.05 mL), followed by blood flow to the external reservoir. The success of the occlusion was determined by an immediate and sustained loss of detectable pulse pressure and a decrease in the MDAP assessed via the tail artery. Following 10 min of ischemia, the balloon was deflated, and blood was re-infused over a period of 60 s. Protamine sulfate (4 mg) was subsequently administered via intraperitoneal injection. The arterial lines were removed, followed by incision closure. The endotracheal catheter was extubated until spontaneous respiration recovery. The sham-operated rats underwent the same surgical procedures, with the exception of balloon catheter inflation.

Western Blot Analysis To identify changes in LC3 and Beclin 1 expression, Western blots were conducted. The rats were euthanized at different time points (3, 6, 12, 24, 48 h) following ischemia and immediately following the sham operation (n=6 per time point). The fourth to sixth lumbar segments were removed and homogenized, and the cells were lysed using Western blot lysis buffer (Bio-Rad Laboratories, MA, U.S.A.). The protein concentration was determined via a Bio-Rad protein assay (Bio-Rad Laboratories). Proteins (30 μg) were separated using 12% polyacrylamide gel electrophoresis (PAGE), followed by transfer to nitrocellulose membranes. The membranes were incubated with rabbit anti-LC3 monoclonal antibodies (1:1000; Abcam, Cambridge, MA, U.S.A.) and goat anti-Beclin 1 monoclonal antibodies (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.). The membranes were subsequently incubated in a 1:3000 dilution of secondary antibody (anti-goat or anti-rabbit immunoglobulin G (IgG)) conjugated to horseradish peroxidase (1:5000; Sigma-Aldrich, St. Louis, MO, U.S.A.) for 1 h. Visualization of the bands occurred via a VersaDoc 4000 Imagine System (Bio-Rad Laboratories). The band densities were quantified via scanned densitometric analysis and the Image J 1.37v software program (National Institutes of Health, Bethesda, MD, U.S.A.). The band densities were normalized to β-actin and subsequently compared between the ischemic and sham groups.

Immunohistochemical Staining of LC3 Immunohistochemical staining was conducted to investigate LC3 expression at 3, 6, 12, 24, and 48 h following reperfusion in the ischemia group and immediately in the sham group (n=3 rats per time point). The rats were anesthetized and received a direct left ventricular bolus of 0.5 mL heparin. Perfusions were performed with 100 mL of saline (0.9%; 37°C) followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH 7.4). Afterward, the fourth to sixth lumbar segments were dissected, fixed in the same fixative overnight at 4°C, and embedded in paraffin. The sections were deparaffinized, hydrated in distilled water, placed in 3% H2O2 (10 min) to remove the residual peroxidase, and subsequently rinsed with PBS. The sections were then permeabilized with 0.1% TritonX-100 (10 min), blocked with 10% normal goat serum (2 h; room temperature), and incubated in anti-LC3 antibody (1:400; Sigma-Aldrich; 4°C for 24 h). The sections were rinsed with PBS and incubated with goat anti-rabbit IgG Alexa Fluor 594 secondary antibodies (1:500; Molecular Probes, Eugene, OR, U.S.A.) in a humidified container (1 h; 37°C), followed by incubation with 4,6-diamidino-2-phenylindole (DAPI; 0.5 mg/ mL; 10 min). The sections were subsequently washed with PBS and cover slipped. In each experiment, the sections were stained under the equivalent conditions.

Counting of LC3-Positive Cells Following LC3 immunohistochemical staining, each section was examined via a confocal microscope (BX51; Olympus, Tokyo, Japan). To quantify spinal cord LC3 expression, the LC3-positive cells were counted in the spinal cord dorsal horn in transverse sections. Thirteen sequential sections were recorded at 250 μm intervals, which spanned 3000 μm in spinal cord length centered from the fourth to sixth lumbar segments. The LC3-positive cells were counted per section. Sections from each animal with the maximum number of LC3 positive cells, as well as 250 μm rostral and caudal sections, were analyzed. The LC3-positive cells were counted in three sequential sections at 250 μm intervals for each animal. The total numbers were compared among the I/R and sham groups.

Transmission Electron Microscopy (TEM) To investigate the autophagy process, the spinal cords were examined via TEM. At 3, 12, and 24 h following I/R injury and immediately after sham operation (n=3), the rats were deeply anesthetized and transcardially perfused with 0.1× PBS, followed by 4% paraformaldehyde and 1% glutaraldehyde. The fourth to sixth lumbar segments were cut into 1 mm transverse sections and maintained in the same fixative overnight. The sections were subsequently immersed in 1% osmium tetroxide (2 h), dehydrated in graded ethanol, and embedded in epoxy resin. Ultrathin sections (60–70 nm) were obtained using an ultramicrotome; these sections were then post-stained with uranyl acetate and lead citrate and examined using a TEM (Tenai G2 Spirit; Hillsboro, OR, U.S.A.).

Intrathecal Administration of 3-MA To determine the
influence of 3-MA on autophagy after spinal I/R injury, rats were administered an intrathecal injection of 3-MA (600 nmol; Sigma-Aldrich) 1 h after spinal ischemia/reperfusion ($n=6$ for 3, 12, 24, 48 h). 3-MA was dissolved in normal saline via heating to 60–70°C immediately prior to the injection. 3-MA was administered in 20 µL volumes via direct lumbar puncture between the L5 and L6 vertebrae. The rats in the vehicle group received 20 µL intrathecal saline injections.

Assessment of Neurological Function The effects of 3-MA on functional recovery were assessed via neurobehavioral scores. At 24 and 48 h after I/R injury, the 3-MA treatment and vehicle groups were subjected to neurological assessment. Four neurological tests were performed, including the Tarlov scale, righting, placing, and inclined plane tests. The tests and scores are provided in Appendix 1. A total score of 0 indicated normal locomotion, whereas a score of 10 indicated no spontaneous movement.

Statistical Analysis Data represent the mean ± standard deviation (S.D.). The data were analyzed by one-way ANOVA, followed by Scheffe’s F-test using SPSS (SPSS Inc., Chicago, IL, U.S.A.). Significance was defined as $p<0.05$.

RESULTS

Spinal I/R Injury Induced Autophagy-Related Protein Expression LC3 and Beclin 1 protein expression at different time points following ischemia were determined and compared with the corresponding levels in the sham-operated rats via Western blot analysis. LC3 expression represents the conversion of LC3-I to LC3-II. The cytoplasmic form, LC3-I, is diffusely distributed in the cytoplasm. During autophagy, the processed form, LC3-II, is transported to the outer membrane of autophagosomes, which generates LC3 expression in a punctate pattern. Thus, an increase in the smaller molecular weight LC3-II protein can be identified, and an increased LC3-II represents a hallmark of up-regulated autophagy. Our results demonstrated that the expression of LC3-II was significantly increased in the ischemic vs. sham-operated groups ($p<0.05$). The expression of LC3-II increase, which was initiated after 3 h of reperfusion, peaked at 24 h and was maintained for at least 48 h (Fig. 1A), with levels equal to approximately 1.6-fold, 2.1-fold, and 1.8-fold those of the sham group, respectively. The expression of Beclin-1, another autophagy activation marker, exhibited a similar pattern compared with LC3-II and was significantly increased compared with the sham-operated group ($p<0.05$) after ischemia. The up-regulation of Beclin-1 was initiated after 3 h of reperfusion, peaked at 24 h, and then slightly decreased, with levels equal to approximately 1.7-fold, 2.0-fold, and 1.9-fold those of the sham group, respectively (Fig. 1B).

Morphological Changes in Neurons after Spinal Ischemia Morphological changes in the dorsal horn neurons were identified via TEM. In the sham group, typical neurons were identified, with relatively healthy nuclei, mitochondria, and endoplasmic reticulum. The formation of autophagic vacuoles was not identified in the sham group cells (Fig. 2A). In contrast, the damaged neurons in the ischemia groups (Figs. 2B–D) exhibited a dilated endoplasmic reticulum, swollen mitochondria, and autophagic vacuoles, which included autophagosomes with double-membrane structures and multilamellar bodies at various stages. Autophagosome formation was identified at 3 h after I/R injury. Twenty-four hours after I/R injury, several key features were identified, including a remarkably increased number of autophagosomes and autolysosomes, autophagosomes that contained damaged subcellular organelles and undigested membranous structures, and autolysosomes with partially digested membranous structures and protein aggregates.

Immunohistochemical staining images indicated that cells that expressed LC3 were present in both the gray and white matter of the ischemic spinal cord; however, not all ischemic
area cells expressed LC3. The number of LC3-positive cells was increased at each assessment in the ischemia rats compared with the sham rats \( (p<0.05; \text{Fig. 3}) \). The increase in the numbers of LC3-positive cells, which was initiated at 3 h and lasted for at least 48 h, was approximately 4.1-fold and 15.3-fold that in the sham group at these time points, respectively. The maximum number of LC3-positive cells was identified at 24 h after spinal I/R injury and was 19.4-fold that in the sham group (Fig. 4).

**3-MA Attenuated I/R Injury and Behavioral Deficits**

All vehicle-treated animals exhibited acute and persistent spastic paraplegia, which did not change during 48 h of ischemia. The animals treated with 3-MA exhibited substantially better neurological function outcomes at 24 and 48 h following spinal ischemia (Fig. 5). There were no significant differences in motor function between the 24 and 48 h groups. Western blot data indicated that 3-MA largely inhibited the increased conversion of LC3-I to LC3-II at 3, 12, 24, and 48 h compared with the vehicle groups after spinal ischemia injury. The LC3-II was significantly lower at 17.2±1.8% of that in the vehicle-treated group. Similar to the results at these time points, 3-MA attenuated ischemia-induced Beclin-1 upregulation by 19.3±4.1% \( (p<0.05) \) (Figs. 6A, B). The numbers of LC3-positive cells in the vehicle-treated group were significantly greater by approximately 1.9-fold, 1.5-fold, 2.6-fold, and 2.1-fold, respectively, than those in the 3-MA treated groups at the same time points \( (p<0.05; \text{Fig. 7}) \).

**DISCUSSION**

In the present study, autophagy was activated following spinal cord I/R injury. The LC3-II, Beclin 1 expression and number of LC3-positive cells were increased after spinal cord I/R injury. The molecular changes were initiated at 3 h, peaked at 24 h, and were maintained for at least 48 h after injury. TEM images further confirmed autophagy activation in the spinal cord neurons following I/R injury. Intrathecal 3-MA injection suppressed these molecular changes. Furthermore, the number of spinal cord LC3-positive neurons was downregulated by 3-MA. The 3-MA-treated rats exhibited significantly better lo-
Fig. 3. Immunohistochemical Staining of LC3 in the Ischemia Groups at Different Time Points

LC3 expression was increased at each time point (D–R) compared with the sham group (A–C). The LC3-expressing cell population was increased at 24 and 48 h (K, N) compared with the other time points. Scale bar=100 µm.
comotor functions compared with the vehicle-treated rats after spinal I/R injury. These findings demonstrated that 3-MA treatment inhibited autophagy; it also decreased neural tissue damage and locomotor impairment following spinal cord I/R injury.

Most animal models of contused, compression, or transection spinal cord injuries require the performance of a laminectomy. However, a laminectomy is associated with significant problems, including a longer surgery, neuropathic pain, spinal instabilities, deformities and biomechanical problems.23 Irreversible ischemic injury and neurological deficits may be induced via temporary interruption of the spinal cord blood supply. Descending aortic occlusion combined with systemic hypotension (40 mmHg) provides a reliable and reproducible spinal cord injury model of complete paraplegia in rats. In a rabbit model, Baba et al. reported autophagy was induced in motor neurons following spinal cord I/R injury.24 The rabbit differs from humans with regard to the infrarenal aorta25; in contrast, the arterial vascularization in the rat spinal cord is substantially more similar to humans.26 A rat model is also

Fig. 4. LC3-Positive Cells Were Counted in the Injured Groups at Different Time Points

The numbers of LC3-positive cells were significantly greater in the injured groups compared with the sham group at all time points. \( n = 3 \) per group, \( * p < 0.05, \) \( ** p < 0.01. \)

Fig. 5. Data from the Four Motor Neurological Tests Were Combined to Create a Composite Neurological Score

The vehicle-treated animals exhibited acute and persistent spastic paraplegia; treatment with 3-MA induced a significant decrease in the neurological function at 24 and 48 h after ischemia injury. \( n = 6 \) per group, \( * p < 0.05. \)

Fig. 6. LC3-II and Beclin 1 Expression Changes Following 3-MA Treatment

(A, B) LC3-II and Beclin 1 changes following 3-MA treatment. 3-MA treatment reduced the optical density (OD) ratio of LC3-II and Beclin 1 compared with the I/R+Veh groups. The \( \beta \)-actin protein levels represent the loading control. \( n = 6 \) per group, \( * p < 0.05, \) \( ** p < 0.01. \) I/R+3-MA vs. I/R+Veh group.
highly suitable for studies that aim to identify and develop pharmacological treatments against specific I/R-related dysfunctions in lumbosacral areas of the spinal cord. Therefore, for the first time, we employed a rat I/R model to assess the presence of autophagy activation following spinal cord injury.

LC3 is a mammalian homolog of the yeast Atg8. In the formation of the autophagy membrane, the LC3-I to LC3-II conversion is considered a marker of autophagic induction. Therefore, the detection of LC3 via immunoblotting or immunofluorescence represents a reliable approach used to monitor autophagosome formation and autophagy-related processes, such as autophagic cell death. Beclin 1 is a component of the Class III phosphatidylinositol-3-kinase complex, which is essential for autophagosome formation. Many studies have identified a robust increase in LC3/Beclin-1 expression and autophagosomes following spinal cord contusion and spinal cord hemisection injury in rat models. Our results demonstrated that autophagy was activated after 3 h and reached its peak level 24 h after spinal I/R injury, which was consistent with previous studies. However, the peak and duration of autophagy were different from previous studies, which may be a result of the use of different injury models.

TEM remains the gold standard for autophagy assessment. In the ultrastructural examination of I/R rats, numerous autophagosomes, C-shaped double-membrane structures, and cytoplasmatic material engulfment via autophagosomes were present in the damaged spinal cord cells. Autophagy activation was confirmed via TEM. Autophagic cell death is primarily a morphologic definition (i.e., autophagosome/autolysosome-associated cell death). In this study, the damaged cells displayed an intact nucleus without fragmentation, which was surrounded by lysosomes and autolysosomes. These morphological features suggested the induction of autophagic cell death.

Enhanced autophagy has been implicated in traumatic spinal injury28,27; however, whether autophagy promotes cell survival or cell death is controversial and dependent on the situation and time point.28 The autophagy inducer rapamycin induces autophagy via the inhibition of the mammalian target of rapamycin (mTOR).29 Chen et al. and Sekiguchi et al. demonstrated that an intraperitoneal injection of rapamycin enhanced LC3 and Beclin 1 expression, attenuated neuronal loss and cell death, and improved locomotor function in spinal cord contusion and hemisection injury models.30,31 Moreover, poor therapeutic value of 3-MA treatment has also been reported in traumatic spinal injury models.32,33 In cerebral ischemia studies, substantial evidence suggests 3-MA inhibits autophagy activation and maturation, reduces I/R injury, and improves cell survival in vivo and in vitro.34,35 An intrathecal injection of 3-MA was administered to enable the drug to enter the cerebrospinal fluid to directly act on the injured spinal nerves. Intrathecal injections of 3-MA at the time of reperfusion significantly reduced LC3/Beclin-1 expression levels and the number of LC3-positive cells and improved neurological function. These findings are consistent with reports regarding agents such as methylprednisolone, basic fibroblast growth factor, bisperoxovanadium, and valproic acid, which act by blocking the autophagy process in spinal cord injury.36–39

Autophagy represents a regulated process that is activated to remove cellular proteins and organelles. In theory, autophagy may facilitate the promotion of cell survival via removal of damaged organelles or toxic pathogens, as well as the regeneration of metabolites that can be used as energy in stress conditions. However, excessive or prolonged autophagy may also stimulate cell death via the excessive self-digestion and degradation of essential cellular constituents. In a similar model of ischemia reperfusion spinal cord injury induced by Fan et al.,40 it was demonstrated that ischemic preconditioning enhanced LC3 expression and suppressed Beclin-1 and Bcl-2/Beclin-1 complex expression, which suggested that autophagy promotes the survival of spinal neurons following ischemia. However, this model was induced via infrarenal cross clamping of the abdominal aorta, which caused a significant but incomplete reduction in the spinal cord blood flow. Moreover, moderate autophagy was activated, which eliminated superfluous organelles and aggregated proteins and provided metabolic substrates for survival. Our model was established via descending thoracic aorta occlusion with systemic hypotension, which induced complete spinal cord ischemia; thus, the lack of oxygen and glucose reached critical levels. The autophagy intensity was consistently strengthened, and consequently, extensive autophagy was accompanied by autophagic neuronal death. 3-MA inhibits autophagosome formation and thus functions as a specific inhibitor of autophagic/lysosomal protein degradation. 3-MA has facilitated the determination of the role of autophagy in physiological conditions, such as cerebral ischemia.16 In the present study, autophagy was activated in the spinal cord in the early stage following I/R in rats; the time–course investigation demonstrated autophagy peaked at 24 h and recovered by 48 h after spinal I/R injury. The I/R-induced increases in LC3-II/Beclin-1 expression and LC3-positive cells were significantly modulated by intrathecal 3-MA administration, which demonstrated that autophagy was regulated by PI3K blockade in this spinal I/R injury model. Furthermore, 3-MA remarkably ameliorated behavioral functions. These findings demonstrated that autophagy played a death-promoting role in neuronal death following spinal
I/R injury because administration of the autophagy inhibitor 3-MA induced neuroprotective effects after spinal I/R injury. Thus, to our knowledge, these findings provide novel evidence that autophagy pathway inhibition may represent a potential target for neuronal protection in spinal I/R injury rat model.

Despite these promising findings, several limitations must be considered in the interpretation of the current results. There are two major upstream signaling pathways, including mTOR and PI3K, that regulate autophagy in mammals. The current study did not investigate the effects of rapamycin, which inhibits mTOR and promotes autophagy by releasing this inhibition. In addition, we only investigated 3-MA, whereas other autophagy inhibitors affect different autophagy pathways. For example, bafilomycin A1 (BFA) inhibits autophagy via the inhibition of vacuolar H+–ATPase. Additional studies are needed to further determine how to regulate autophagy to protect neurons, as well as to clarify the relation between apoptosis and autophagy following spinal I/R injury.

CONCLUSION

In summary, spinal cord I/R injury led to activation of autophagy and behavioral deficits. Intrathecal administration of 3-MA attenuated spinal cord I/R injury-induced locomotor impairment. These findings indicate that autophagy prevention represents a potential therapeutic target for spinal cord I/R injury. Thus, 3-MA should be investigated as a neuronal protective agent spinal cord I/R injury treatment.

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Conflict of Interest The authors declare no conflict of interest.

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