NEURAL REGENERATION RESEARCH
May 2016, Volume 11, Issue 5
www.nrronline.org

RESEARCH ARTICLE

Methylprednisolone exerts neuroprotective effects by regulating autophagy and apoptosis

Wei Gao[#], Shu-rui Chen[#], Meng-yao Wu, Kai Gao, Yuan-long Li, Hong-yu Wang, Chen-yuan Li, Hong Li[*]

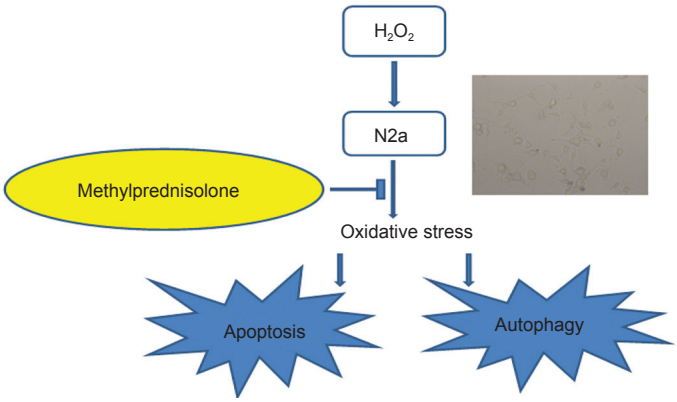
Department of Biochemistry, Liaoning Medical University, Jinzhou, Liaoning Province, China

How to cite this article: *Gao W, Chen SR, Wu MY, Gao K, Li YL, Wang HY, Li CY, Li H (2016) Methylprednisolone exerts neuroprotective effects by regulating autophagy and apoptosis. Neural Regen Res 11(5):823-828.*

Funding: *This research was supported by the National Natural Science Foundation of China, No. 81171799, 81471854; the Science and Technology Research Project of Education Department of Liaoning Province of China, No. L2013333; the "College Students' Science and Technology Innovation Project" of Liaoning Medical University Principal Fund-Aohong Boze Fund of China, No. 2014D08; the Liaoning Medical University Principal Fund-Aohong Boze Graduate Student Science Research Innovation Fund, No. AH2014017.*

Graphical Abstract

Methylprednisolone protects nerve cells against oxidative damage by suppressing autophagy and apoptosis



Correspondence to:
Hong Li, M.D.,
jzmu_lihong@sina.com.

#These authors contributed equally to this study.

orcid:
0000-0002-5446-6717
(Hong Li)

doi: *10.4103/1673-5374.182711*
http:*//www.nrronline.org/*

Accepted: *2015-12-22*

Abstract

Methylprednisolone markedly reduces autophagy and apoptosis after secondary spinal cord injury. Here, we investigated whether pretreatment of cells with methylprednisolone would protect neuron-like cells from subsequent oxidative damage *via* suppression of autophagy and apoptosis. Cultured N2a cells were pretreated with 10 μM methylprednisolone for 30 minutes, then exposed to 100 μM H_2O_2 for 24 hours. Inverted phase contrast microscope images, MTT assay, flow cytometry and western blot results showed that, compared to cells exposed to 100 μM H_2O_2 alone, cells pretreated with methylprednisolone had a significantly lower percentage of apoptotic cells, maintained a healthy morphology, and showed downregulation of autophagic protein light chain 3B and Beclin-1 protein expression. These findings indicate that methylprednisolone exerted neuroprotective effects against oxidative damage by suppressing autophagy and apoptosis.

Key Words: *nerve regeneration; spinal cord injury; methylprednisolone; oxidative stress; N2a cells; autophagy; light chain 3B; Beclin-1; apoptosis; neuroprotection; H_2O_2; neural regeneration*

Introduction

The pathophysiology of spinal cord injury (SCI) involves primary and secondary mechanisms (Silva et al., 2014; Gao et al., 2015b). Secondary SCI is caused by a series of biochemical events triggered by the primary injury. The pathology of secondary SCI involves microcirculatory changes, oxidative stress, ischemia, apoptosis, necrosis, and edema. These secondary effects further aggravate the primary injury and cause destructive lesions in the region surrounding the injury site (Webb et al., 2010; Gao et al., 2015a). Following acute SCI, an increase in free radical production leads to lipid peroxidation damage; decreasing oxidative stress would therefore greatly reduce secondary injury following trauma (Juurlink and Paterson, 1998).

Methylprednisolone (MP), a synthetic glucocorticoid hormone, is the most commonly used anti-inflammatory and antioxidant drug in the treatment of acute SCI (Lee et al., 2008; Bains and Hall, 2012). However, the effects of MP on SCI have been questioned in recent studies, and the mechanisms underlying its preventive effects on secondary pathological damage after SCI remain poorly understood (Liu et al., 2009; Mazzocca et al., 2013; Fehlings et al., 2014;

Harrop, 2014). Following SCI, MP inhibits inflammation and promotes recovery of neurological function. To date, the main focus of research into the neuroprotective effect of MP has been from the aspect of glucocorticoid receptor and anti-inflammatory mechanisms, and studies of its antioxidant mechanism remain scarce (Xu et al., 1998; Mazzocca et al., 2013; Boyaci et al., 2014).

MP has been shown to regulate autophagy, but findings have been inconsistent. In a study using osteoblasts, application of MP markedly increased autophagic activity (Yao et al., 2015). However, Chen et al. (2012) demonstrated that MP suppressed autophagy. Furthermore, it remains unclear whether MP exerts its neuroprotective effect by reducing light chain 3B (LC3B) and Beclin-1 expression. LC3B and Beclin-1 are two key markers for autophagy. Apoptosis and autophagy are extensive following secondary SCI. Autophagy might be induced and activated by primary SCI (Kanno et al., 2009a, b, 2011; Walker et al., 2012; Hou et al., 2014). However, several studies have shown that apoptosis is also an important cell death mechanism after SCI (Crowe et al., 1997; Springer et al., 1999), and Lee et al. (2008) confirmed that MP selectively inhibits microglial apoptosis, which may be associated with its neuroprotective effect.

Here, we established an *in vitro* model of oxidative damage in N2a cells, using H_2O_2, and explored the effects of MP on autophagy and apoptosis after exposure to oxidative stress.

Materials and Methods

Cell culture

Frozen mouse neuroblastoma cells (N2a cells; Guangzhou Jiniou Co., Ltd., Guangzhou, China) were resuscitated in a water bath at 37°C for 3 minutes, placed in a 15 mL centrifuge tube, incubated with Dulbecco's modified Eagle's medium/F12 containing 10% fetal bovine serum (twice the volume of frozen liquid) at room temperature, and centrifuged. After removal of the supernatant, cells were incubated with Dulbecco's modified Eagle's medium (DMEM)/F12 containing 10% fetal bovine serum, at 37°C, 5% CO_2 and saturated humidity. Cells were passaged at approximately 80% confluence, and collected for use at passages 3–6. The medium was replaced every other day.

3-(4,5-Cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) cell proliferation rate assay

Logarithmic-phase N2a cells were seeded on a 96-well plate at 1×10^5 cells per well in 200 μL cell suspension. Phosphate buffered saline (PBS) was added to the surrounding wells. The plate was incubated at 37°C and 5% CO_2 for 24 hours, to allow the cells to adhere. The cells were then allocated to four groups: cells in the control group were incubated in DMEM containing 10% fetal bovine serum; in the H_2O_2 group, 0, 20, 40, 60, 80, 100, 120, 140, 160 or 200 μM H_2O_2 (Bori Site Trading Co., Ltd., Shenyang, China) was added to the culture medium; cells in the MP group were treated with 0, 0.5, 1, 2, 5, 10, 20, 50, 100 or 200 μM MP (Melonepharma, Dalian, China); and in the H_2O_2 + MP group, cells were pretreated with 0.1, 1, 5, 10, 50 or 100 μM MP for 30 minutes, then 100 μM H_2O_2 was added. Each group contained four parallel wells. Cells were observed 24 hours later under an inverted phase contrast microscope (Olympus, Tokyo, Japan).

Subsequently, 20 μL MTT (Sigma, St. Louis, MO, USA) was added to each well for 4 hours. The medium was removed and the cells were incubated with 150 μL of dimethyl sulfoxide for 10 minutes at 37°C. Optical density (OD) values were measured at 570 nm with a microplate reader (Bio-Rad, Hercules, CA, USA). Cell proliferation rate was calculated as $[(1-OD_{\text{experimental group}})/OD_{\text{control group}}] \times 100\%$.

Cell counting and morphology

Cells were seeded in the culture dish and the medium was replaced every 3–4 days for approximately 1 week. After digestion with trypsin (Gibco, Carlsbad, CA, USA), cells were incubated in six-well plates for 24 hours. Cells were allocated to three groups: in the H_2O_2 group, cells were exposed to 100 μM H_2O_2 for 24 hours; cells in the H_2O_2 + MP group were pretreated with 10 μM MP for 30 minutes, before exposure to 100 μM H_2O_2; control cells were in DMEM containing 10% fetal bovine serum. Cell morphology was observed at 24 hours under an inverted phase contrast microscope (Olympus).

Flow cytometry

Cells were incubated in 6-well plates for 24 hours, then grouped and treated as described above (see Cell counting and morphology). A single-cell suspension was made using trypsin without ethylenediamine tetraacetic acid, and centrifuged at 300 × *g* for 3 minutes. Following removal of the supernatant, cells were washed twice with precooled PBS, and centrifuged in 1 mL annexin V (Tianjin Sungene Biotech Co., Ltd., Tianjin, China) for 10 minutes. Cells were adjusted to 10^6/mL. Cell suspension was centrifuged and washed three times with PBS. Samples (100 μL) were added to Eppendorf tubes with 5 μL annexin V-APC (Tianjin Sungene Biotech Co., Ltd.) and 7-AAD (Tianjin Sungene Biotech Co., Ltd.), and mixed. The volume was made up to 500 μL with PBS and the tubes were incubated at room temperature for 15 minutes in the dark. Apoptosis was quantified by flow cytometry (BD FACSCanto II, BD Becton Dickinson, San Jose, CA, USA). Cell apoptosis rate was calculated as follows: number of apoptotic cells/total number of cells × 100%. Experiments were conducted twice.

Immunofluorescence staining

Cells were plated onto 24-well plates for 24 hours, then the medium was discarded and the cells were grouped and treated as described above (see Cell counting and morphology). The cells were then fixed in 4% paraformaldehyde at 4°C overnight. The next day, samples were washed three times with PBS, for 5 minutes each time. Triton X-100 (1%) was added for 15 minutes, and the samples were blocked with goat serum for 1 hour at 4°C. Samples were then incubated overnight with rabbit polyclonal LC3B antibody (1:500 dilution in goat serum; Abcam, Cambridge, MA, USA). The following day, the samples were washed with PBS as before,

and incubated with goat anti-rabbit IgG/Cy3 in the dark for 2 hours at room temperature, followed by three 5-minute washes with PBS. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole for 15 minutes, followed by three more 5-minute washes with PBS. Protein localization was observed under a fluorescence microscope (Olympus).

Western blot assay

Cells were assigned to control, H_2O_2 and H_2O_2 + MP groups as described above. After lysis, protein concentrations were measured using the bicinchoninic acid method. Lysates were electrophoresed on 5% stacking gel at 90 V, and 10% separating gel at 120 V, then wet-transferred onto polyvinylidene fluoride membranes at 350 mA for 1 hour 35 minutes, after immersing the dry membranes in methanol for 30 seconds and transfer buffer for 10 minutes. Membranes were blocked with 1% bovine serum albumin for 2 hours, and incubated with rabbit anti-LC3B polyclonal antibody (1:1,000; Cell Signaling, China), mouse anti-β-actin polyclonal antibody (1:1,000; Abcam), and rabbit anti-Beclin-1 polyclonal antibody (1:1,000; Abcam) at 4°C overnight. The next day, the membranes were rinsed in Tris-buffered saline with Tween-20 (TBST), three times for 10 minutes each time, incubated with goat anti-rabbit or anti-mouse secondary antibody (1:1,000; Bioss, Beijing, China) at room temperature for 2 hours, then washed with TBST as before. Proteins were visualized using enhanced chemiluminescence. Gray values of the protein bands (target protein/β-actin) were calculated using ImageJ2x software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

Data are expressed as the mean ± SD, and were analyzed using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). Groups were compared using one-way analysis of variance and the least significant difference test. $P < 0.05$ was considered statistically significant.

Results

MP prevented H_2O_2-induced reduction in cell survival

N2a cells were exposed to different concentrations of H_2O_2 for 24 hours. MTT assay showed that cell survival rate decreased with increasing concentrations of H_2O_2. At 100 μM H_2O_2, cell viability was approximately 50% of the control group value (0 μM H_2O_2; $P < 0.001$; **Figure 1A**).

To exclude the effects of MP on cell viability, N2a cells were exposed to different concentrations of MP for 24 hours. MP had no observable effect on cell viability (**Figure 1B**), confirming that it neither improved cell survival nor was cytotoxic.

After pretreatment of N2a cells with different concentrations of MP for 30 minutes, followed by 100 μM H_2O_2 for 24 hours, MP markedly suppressed the reduction in cell viability induced by H_2O_2 ($P < 0.001$; **Figure 1C**).

MP prevented H_2O_2-induced damage to cell morphology and number

Control cells were plump, with long dendrites and axons, indicating good growth. After exposure to H_2O_2, cell bodies floated and appeared round, and some processes retracted and were noticeably damaged, indicating that H_2O_2 was cytotoxic. In the H_2O_2 + MP group there were significantly fewer damaged and floating cells than in the H_2O_2 group (**Figure 2**).

MP prevented H_2O_2-induced apoptosis

Flow cytometry showed that there were significantly more apoptotic cells in the H_2O_2 group than in the control group ($P < 0.001$), but fewer in the MP + H_2O_2 group than in the H_2O_2 group ($P < 0.01$; **Figure 3**).

MP prevented H_2O_2-induced upregulation of LC3B and Beclin-1

Immunofluorescence staining was used to detect LC3B expression and localization. LC3B was mainly expressed in the cytoplasm. Western blot assay revealed that expression levels of LC3B and Beclin-1 protein were significantly higher in the H_2O_2 group than in the control group ($P < 0.01$), and significantly lower in the H_2O_2 + MP group than in the H_2O_2 group ($P < 0.05$ or $P < 0.001$; **Figure 4**).

Discussion

Cytosolic LC3A fractions convert to autophagic membrane-form (phosphatidyl ethanolamine-conjugated) LC3B. When autophagy occurs, LC3B expression increases, and LC3B becomes selectively incorporated into the autophagic membrane. Therefore, LC3B expression is directly proportional to the number of autophagosomes, making LC3B a unique autophagosomal marker. The gene that encodes Beclin-1 is the first gene involved in a series of events that activate autophagy in mammals, and as such is a key marker of autophagy initiation (Walker et al., 2012; Fitzwalter and Thorburn, 2015). Cells undergoing autophagy, a self-degradative process, deliver cytoplasmic material and organelles to lysosomes for degradation (Magraoui et al., 2015). Apoptosis is another cell death mechanism, also under genetic control (Edinger and Thompson, 2004). Initial studies suggested that there were considerable differences between autophagy and apoptosis in terms of morphology, biochemistry, and molecular mechanism, but recent evidence indicates that the two processes can occur successively or simultaneously in the same cell (Marino et al., 2014; Mukhopadhyay et al., 2014; Fitzwalter and Thorburn, 2015). Our flow cytometry results showed that the proportion of apoptotic cells was markedly lower in the H_2O_2 + MP group than in the group exposed to H_2O_2 alone. Furthermore, the immunofluorescence and western blot experiments showed that MP prevented the H_2O_2-induced upregulation of Beclin-1 and LC3B protein expression.

Our results clearly show that H_2O_2 is cytotoxic in N2a cells, and autophagy and apoptosis were observed. This result supports the notion that autophagy and apoptosis exist simultaneously and interact with each other during periods of oxidative damage. However, pretreatment with MP decreased the rate of apoptosis, and inhibited autophagy, in N2a cells

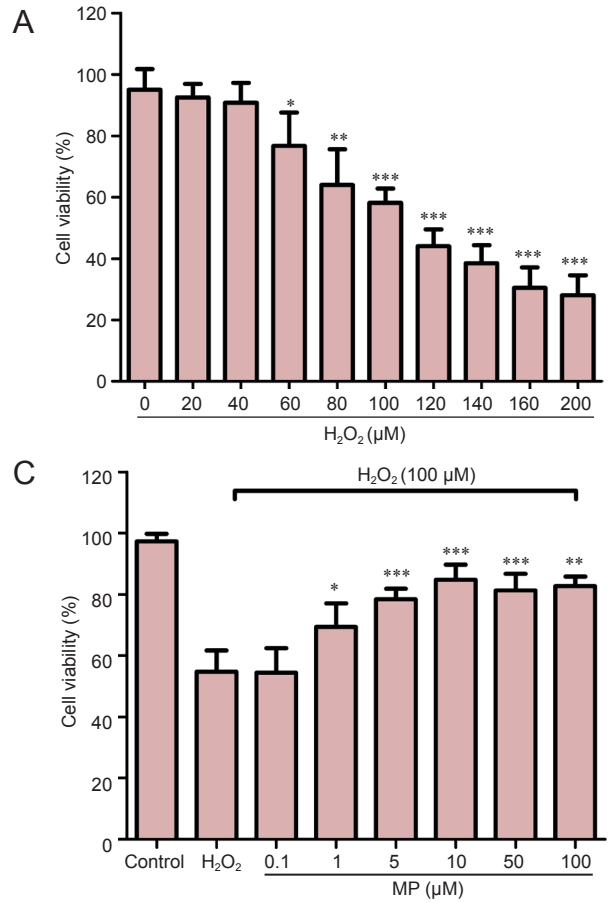


Figure 1 Neuroprotective effect of MP against oxidative damage induced by H_2O_2 (100 μM) in N2a cells.
(A) Viability of N2a cells decreased with exposure to increasing concentrations of H_2O_2 for 24 hours. *P < 0.05, **P < 0.01, ***P < 0.001, *vs.* 0 μM H_2O_2 (one-way analysis of variance and the least significant difference test). (B) Treatment with MP alone for 24 hours had no effect on N2a cell viability at any concentration tested. (C) Protective effects of different concentrations of MP on N2a cells exposed to 100 μM H_2O_2. Mean ± SD of four experiments; *P < 0.05, **P < 0.01, ***P < 0.001, *vs.* H_2O_2 group (one-way analysis of variance and least significant difference test). MP: Methylprednisolone.

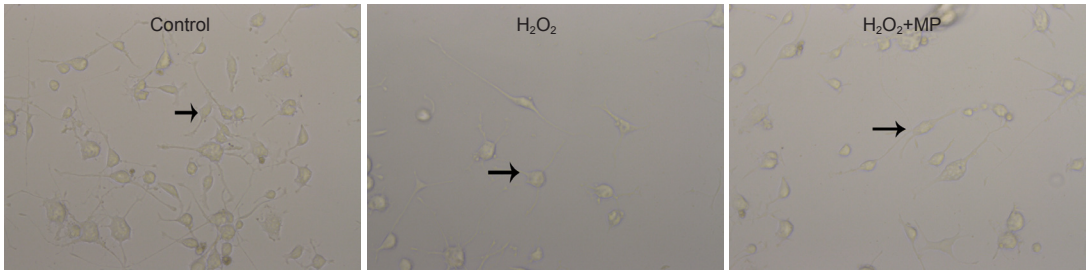


Figure 2 Effects of 10 μM MP on the morphology and number of N2a cells (inverted phase contrast microscope, × 400).
Control (no H_2O_2 or MP): cells showed good growth. H_2O_2 (100 μM): cell bodies were rounded, with few processes. H_2O_2 (100 μM) + MP (10 μM): cell shape appeared normal, and processes were observed. MP: Methylprednisolone.

subsequently exposed to H_2O_2-induced oxidative stress. We also investigated whether the neuroprotective effect of MP depended on the downregulation of key proteins in the autophagy and apoptosis signaling pathways. Our results show that MP pretreatment diminished oxidative stress-induced cell damage and protected cells *via* inhibition of autophagy and apoptosis.

In conclusion, MP has anti-apoptotic and anti-autophagic effects in neuron-like cells exposed to oxidative damage. We are the first to show that MP downregulates LC3B and Beclin-1 protein expression. However, this is only a preliminary investigation of the effects of MP on autophagic and apoptotic signaling pathways, and further experiments are needed to determine whether our results can be extrapolated to the whole organism. Nevertheless, the present results provide strong and novel evidence elucidating the neuroprotective mechanism of MP against oxidization.

Acknowledgments: *We are very grateful to Xi-fan Mei from the Department of Orthopedics, the First Affiliated Hospital of Liaoning Medical University of China for selfless guidance and assistance. We would like to thank Jing Bi and Dong-he Han from the Liaoning Province Key Laboratory for Neurodegenerative Diseases of China for their valuable advice and technical support.*

Author contributions: *WG conceived and designed the study, participated in cell culture, MTT, flow cytometry, immunofluorescence and western blot assay, analyzed data and wrote the paper. SRC provided data support, retrieved references, and ensured the integrity of the data. MYW participated in cell cul-*

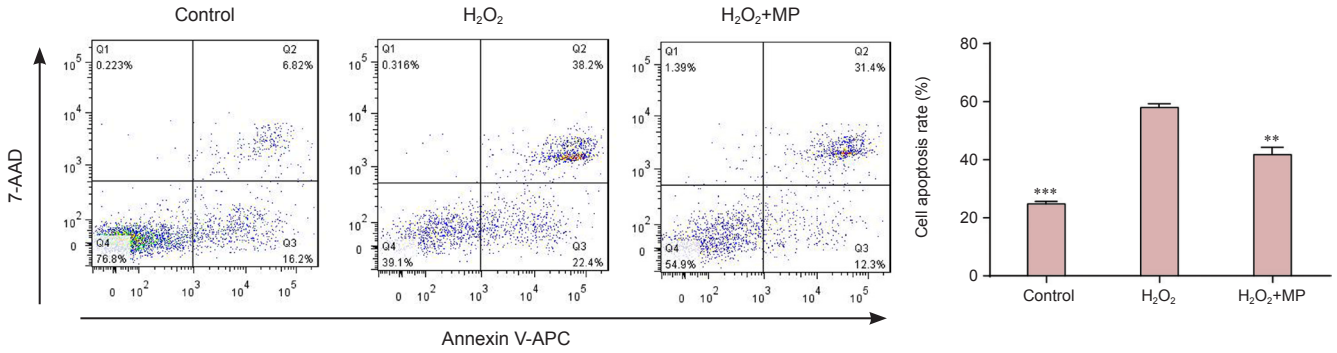


Figure 3 Pretreatment with MP protected against H_2O_2-induced apoptosis (flow cytometry).
Cells pretreated with 10 μM MP showed less apoptosis after exposure to 100 μM H_2O_2 than those exposed to 100 μM H_2O_2 alone. $^{**}P < 0.01$, $^{***}P < 0.001$, *vs.* H_2O_2 group (mean ± SD of three experiments; one-way analysis of variance and the least significant difference test). MP: Methylprednisolone.

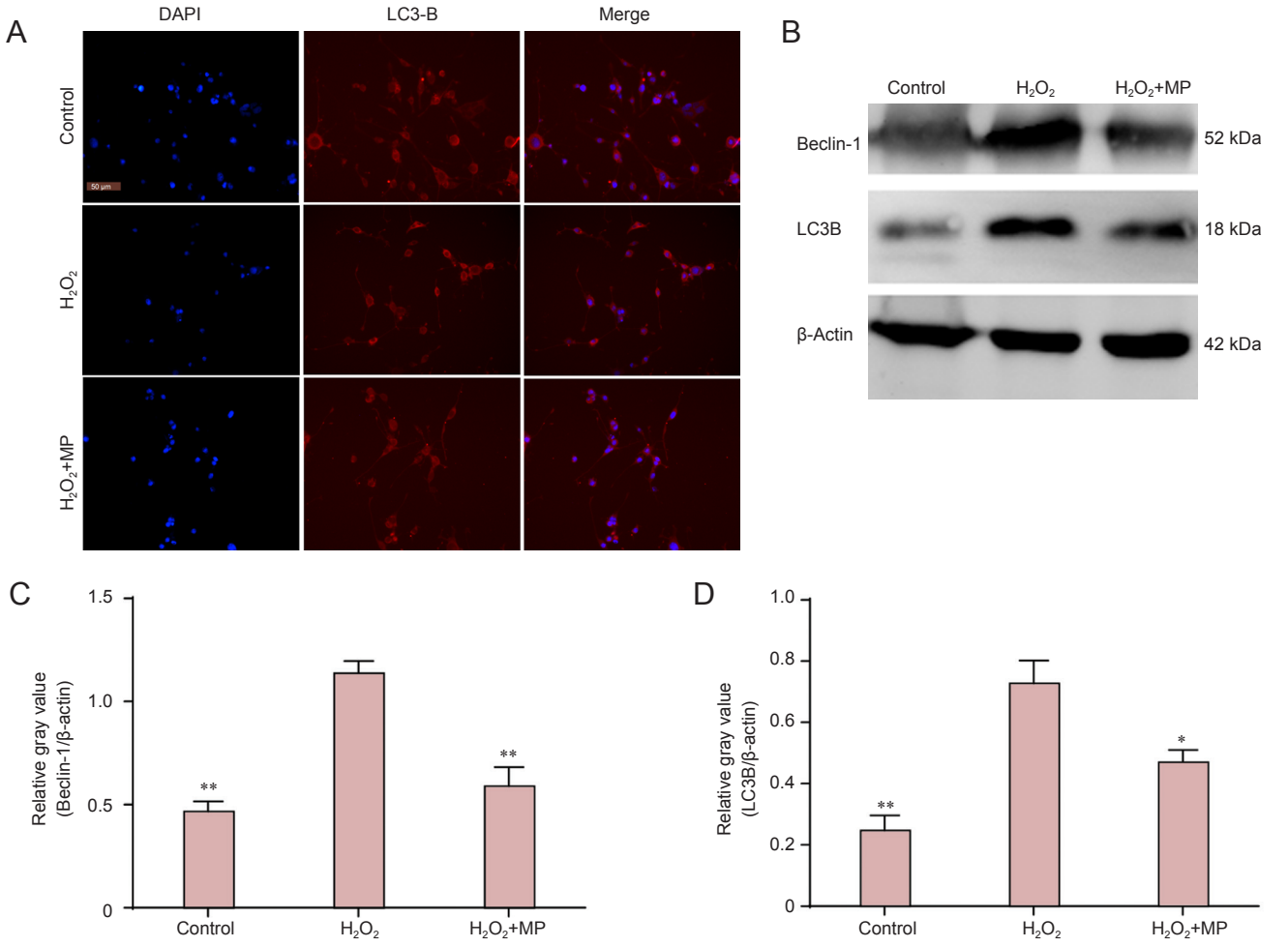


Figure 4 Pretreatment with MP protected against H_2O_2-induced LC3B and Beclin-1 protein upregulation in N2a cells.
(A) LC3B expression in control cells (top panel), N2a cells exposed to 100 μM H_2O_2 (middle panel), and N2a cells pretreated with 10 μM MP before H_2O_2 exposure (bottom panel) (immunofluorescence staining). Blue: DAPI-stained nuclei; red: Cy3-stained LC3B; scale bar: 50 μm. (B) Autophagic protein expression was upregulated in N2a cells exposed to 100 μM H_2O_2 compared to control cells, but this upregulation was markedly reduced in cells pretreated with 10 μM MP. (C, D) Quantification of Beclin-1 (C) and LC3B (D) protein expression. $^{*}P < 0.05$, $^{**}P < 0.01$, *vs.* H_2O_2 (mean ± SD of three experiments; one-way analysis of variance and the least significant difference test). DAPI: 4′,6-Diamidino-2-phenylindole; MP: methylprednisolone.

ture, passage, resuscitation and cryopreservation. KG analyzed data. YLL participated in flow cytometry. HYW provided reference data and technical support. CYL obtained the funding, conceived and designed the study. HL served as a principle investigator. All authors approved the final version of the paper.

Conflicts of interest: *None declared.*

Plagiarism check: *This paper was screened twice using Cross-Check to verify originality before publication.*

Peer review: *This paper was double-blinded and stringently reviewed by international expert reviewers.*

References

Bains M, Hall ED (2012) Antioxidant therapies in traumatic brain and spinal cord injury. Biochim Biophys Acta 1822:675-684.

Boyaci MG, Eser O, Kocogullari CU, Karavelioglu E, Tokyol C, Can Y (2014) Neuroprotective effect of alpha-lipoic acid and methylprednisolone on the spinal cord ischemia/reperfusion injury in rabbits. Br J Neurosurg: 1-6.

Chen HC, Fong TH, Lee AW, Chiu WT (2012) Autophagy is activated in injured neurons and inhibited by methylprednisolone after experimental spinal cord injury. Spine (Phila Pa 1976) 37:470-475.

Crowe MJ, Bresnahan JC, Shuman SL, Masters JN, Beattie MS (1997) Apoptosis and delayed degeneration after spinal cord injury in rats and monkeys. Nat Med 3:73-76.

Edinger AL, Thompson CB (2004) Death by design: apoptosis, necrosis and autophagy. Curr Opin Cell Biol 16:663-669.

Fehlings MG, Wilson JR, Cho N (2014) Methylprednisolone for the treatment of acute spinal cord injury: counterpoint. Neurosurgery 61 Suppl 1:36-42.

Fitzwalter BE, Thorburn A (2015) Recent insights into cell death and autophagy. FEBS J 282:4279-4288.

Gao K, Shen Z, Yuan Y, Han D, Song C, Guo Y, Mei X (2015a) Simvastatin inhibits neural cell apoptosis and promotes locomotor recovery via activation of Wnt/beta-catenin signaling pathway after spinal cord injury. J Neurochem doi: 10.1111/jnc.13382.

Gao K, Wang YS, Yuan YJ, Wan ZH, Yao TC, Li HH, Tang PF, Mei XF (2015b) Neuroprotective effect of rapamycin on spinal cord injury via activation of the Wnt/beta-catenin signaling pathway. Neural Regen Res 10:951-957.

Harrop JS (2014) Spinal cord injury: debating the efficacy of methylprednisolone. Neurosurgery 61 Suppl 1:30-31.

Hou H, Zhang L, Liu D, Mao Z, Du H, Tang P (2014) Acute spinal cord injury in rats induces autophagy activation. Turk Neurosurg 24:369-373.

Juurlink BH, Paterson PG (1998) Review of oxidative stress in brain and spinal cord injury: suggestions for pharmacological and nutritional management strategies. J Spinal Cord Med 21:309-334.

Kanno H, Ozawa H, Sekiguchi A, Itoi E (2009a) The role of autophagy in spinal cord injury. Autophagy 5:390-392.

Kanno H, Ozawa H, Sekiguchi A, Itoi E (2009b) Spinal cord injury induces upregulation of Beclin 1 and promotes autophagic cell death. Neurobiol Dis 33:143-148.

Kanno H, Ozawa H, Sekiguchi A, Yamaya S, Itoi E (2011) Induction of autophagy and autophagic cell death in damaged neural tissue after acute spinal cord injury in mice. Spine (Phila Pa 1976) 36:E1427-1434.

Lee JM, Yan P, Xiao Q, Chen S, Lee KY, Hsu CY, Xu J (2008) Methylprednisolone protects oligodendrocytes but not neurons after spinal cord injury. J Neurosci 28:3141-3149.

Liu JC, Patel A, Vaccaro AR, Lammertse DP, Chen D (2009) Methylprednisolone after traumatic spinal cord injury: yes or no? PM R 1:669-673.

Magraoui FE, Reidick C, Meyer HE, Platta HW (2015) Autophagy-related deubiquitinating enzymes involved in health and disease. Cells 4:596-621.

Marino G, Niso-Santano M, Baehrecke EH, Kroemer G (2014) Self-consumption: the interplay of autophagy and apoptosis. Nat Rev Mol Cell Biol 15:81-94.

Mazzocca AD, McCarthy MB, Intravia J, Beitzel K, Apostolakos J, Cote MP, Bradley J, Arciero RA (2013) An in vitro evaluation of the anti-inflammatory effects of platelet-rich plasma, ketorolac, and methylprednisolone. Arthroscopy 29:675-683.

Mukhopadhyay S, Panda PK, Sinha N, Das DN, Bhutia SK (2014) Autophagy and apoptosis: where do they meet? Apoptosis 19:555-566.

Silva NA, Sousa N, Reis RL, Salgado AJ (2014) From basics to clinical: a comprehensive review on spinal cord injury. Prog Neurobiol 114:25-57.

Springer JE, Azbill RD, Knapp PE (1999) Activation of the caspase-3 apoptotic cascade in traumatic spinal cord injury. Nat Med 5:943-946.

Walker CL, Walker MJ, Liu NK, Risberg EC, Gao X, Chen J, Xu XM (2012) Systemic bisperoxovanadium activates Akt/mTOR, reduces autophagy, and enhances recovery following cervical spinal cord injury. PLoS One 7:e30012.

Webb AA, Ngan S, Fowler JD (2010) Spinal cord injury I: a synopsis of the basic science. Can Vet J 51:485-492.

Xu J, Fan G, Chen S, Wu Y, Xu XM, Hsu CY (1998) Methylprednisolone inhibition of TNF-alpha expression and NF-kB activation after spinal cord injury in rats. Brain Res Mol Brain Res 59:135-142.

Yao W, Dai W, Jiang L, Lay EY, Zhong Z, Ritchie RO, Li X, Ke H, Lane NE (2015) Sclerostin-antibody treatment of glucocorticoid-induced osteoporosis maintained bone mass and strength. Osteoporos Int 27:283-294.

Copyedited by Slone-Murphy J, Robens J, Wang J, Qiu Y, Li CH, Song LP, Zhao M