

## Research Article

# Curcumin promotes functional recovery and inhibits neuronal apoptosis after spinal cord injury through the modulation of autophagy

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**Objective:** The study was aimed to investigate whether the neuroprotective role of curcumin is associated with regulation of autophagy.

**Methods:** Rat spinal cord injury (SCI) models were established according to Allen's weight-drop trauma method. Curcumin was administered 30 min after the contusion and continued weekly. At 3, 7, 14, 21, and 28 days after SCI, functional recovery was evaluated using the Basso, Beattie and Bresnahan (BBB) scoring and the oblique plate test, following which, spinal cord tissues were obtained. Histological changes and apoptosis were then measured with H&E staining and TUNEL assay. Glia activation, inflammatory infiltration, inflammatory factor release, and myelination were observed through immunohistochemical (IHC) staining and ELISA. Autophagy and Akt activation were detected by western blotting. After autophagy was inhibited by injection of chloroquine, TUNEL, inflammatory factor release, myelin basic protein (MBP) IHC staining and functional recovery evaluation were performed again.

**Results:** Curcumin treatment promoted functional recovery after SCI and reduced neuron apoptosis, improved spinal cord integrity, recovery, and re-myelination, and suppressed the inflammatory response. Autophagy was enhanced and Akt/mTOR pathway was inhibited by curcumin. Autophagy inhibition partially eliminated the protective effect of curcumin on SCI.

**Conclusion:** Curcumin may exert its therapeutic effect on SCI through the enhancement of autophagy, in which, inhibition of the Akt/mTOR signaling pathway may be also involved.

**Keywords:** Curcumin, Spinal cord injury, Autophagy, Akt, mTOR

## Introduction

Spinal cord injury (SCI) is a major cause of death and long-term disability worldwide.<sup>1,2</sup> Patients with SCI usually suffer enormous physical and emotional pain as injuries lead to long-term sensorimotor, cognitive, and psychiatric changes.<sup>2</sup> Over the years, a number of animal models have been established and developed to investigate the mechanisms and the functional consequences of SCI as well as for the evaluation of potential therapeutic interventions.<sup>3,4</sup> It is known that trauma

causes both direct tissue damage, which is considered to be the primary injury, and biochemical changes that lead to progressive cell loss, referred to as secondary injury.<sup>2,5</sup>

Basal levels of autophagy are essential for homeostasis in terminally differentiated cells like neurons. However, pathologically increased autophagy has been reported as a contributor to cell death.<sup>6</sup> When essential autophagy genes were knocked out, mice developed severe neurodegeneration, and the underlying mechanism of which was proposed to be cell injury and death caused by inhibited autophagy flux.<sup>6,7</sup> In experimental studies, combining an autophagy inhibitor with chemotherapy augments therapeutic benefits and helps overcome drug resistance in cancer.<sup>8,9</sup> Clinical trials

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using the late stage autophagy inhibitor chloroquine or its derivative hydroxychloroquine showed that patients have safely gone through the combinatory treatment of chloroquine-hydroxychloroquine with standard chemotherapies and exhibited prolonged survival in certain types of cancer.<sup>10</sup> Therefore, chloroquine has been used as an autophagy inhibitor in basic experiments in the laboratory. Impaired autophagy flux has been associated with many neurodegenerative disorders, such as Alzheimer's, Parkinson's, and Huntington's disease.<sup>11</sup> Emerging data suggest that autophagy flux may be either increased or decreased after SCI depending on the location and severity of the injury.<sup>12,13</sup> Thus, it is not yet known whether autophagy plays a beneficial or detrimental role after injury. However, it is clear that restoration and augmentation of autophagy flux improve functional recovery after injury by increasing cell survival, suggesting that the autophagy pathway is a potential therapeutic target for treatment of SCI.<sup>13</sup>

Curcumin, an active component of *Curcuma longa*, was initially reported having antibacterial function in 1949.<sup>14</sup> Studies have shown that issues with fast metabolism, low bioavailability, and low chemical stability have been overcome in the clinical application of curcumin and its analogs for the treatment of several inflammatory conditions in cancer,<sup>15</sup> cardiovascular disease,<sup>16</sup> and central nervous system disorder/trauma.<sup>17</sup> Additional reports have demonstrated the positive influence of curcumin for treatment of SCI and revealed that curcumin contributes to neurological recovery.<sup>18</sup> However, to the best of our knowledge, the therapeutic role of curcumin in SCI with respect to autophagy, autophagy flux, and related signaling pathways remains unreported. Thus, in the present study, we investigated the treatment of SCI using curcumin in a rat model and its influence on autophagy induction and related signaling pathways.

## Materials and methods

### Rat SCI model and treatments

A total of 160 male SD (Sprague Dawley) rats (Eight weeks old, weighing  $235 \pm 15$  g), provided by Kunming University of Science and Technology, were used in this study. This study was approved by the Ethics Committee of Kunming University of Science and Technology (ethics approval no.: 2015FB095). Rats were housed with free access to water and food in a room conditioned with 12 h light–dark cycles. The rats were initially divided into two types: sham operated rats ( $n = 30$ ), and SCI rats ( $n = 100$ ). In sham rats, laminectomy was performed without SCI, and they were treated with 98% normal saline (NS) solution ( $V_{NS}: V_{\text{water}} = 98/2$ ). SCI rats were established according to

a modified Allen's weight-drop trauma method.<sup>19</sup> Briefly, the rats were anesthetized with intraperitoneal injection of chloral hydrate (300 mg/kg) and operated under aseptic conditions. The skin was cut, the lamina was exposed, and a laminectomy was performed to expose the spinal cord at T9–T11. The exposed spinal cord segment was subjected to the subsequent SCI induced by dropping a 10-g iron body with a diameter of 10 mm from a height of 20 mm onto the spinal cord surface. Three days after the surgery, behavioral symptoms were evaluated. Successful SCI exhibits following characteristics in the rats: formation of tail sway reflex, spinal cord ischemia, flicking of legs, and the appearance of paralysis. These 100 SCI rats were subsequently subjected to injection with dimethyl sulfoxide (DMSO) ( $n = 50$ ) and curcumin ( $n = 50$ ). Curcumin (Sigma-Aldrich, Santa Clara, CA, USA) was dissolved in 2.0% DMSO with 98.0% normal saline solution. The group treated with the vehicle (2.0% DMSO with 98.0% normal saline solution; percutaneous, intramuscular injection near the site of injury within 30 min following the contusion) was designated as DMSO group, serving as a negative control.

In order to find out the role of autophagy in the protective effect of curcumin on SCI, we established 30 more SCI rat models using the same method mentioned above. The SCI rats received either: DMSO ( $n = 10$ ), curcumin (Cur,  $n = 10$ ), or curcumin combined with chloroquine (Cur + CQ,  $n = 10$ ). These treatments were administered every week via percutaneous, intramuscular injection near the site of injury within 30 min following the contusion and continued weekly for 3 weeks (DMSO: 1 ml/kg BW (body weight); curcumin: 60 mg/ml/kg BW in DMSO;<sup>20,21</sup> Cur + CQ: 60 mg/ml/kg curcumin in DMSO + 50 mg/kg chloroquine).<sup>22</sup> Rats in the sham group received DMSO injection within 10 min after laminectomy.

### Evaluation of functional recovery

Locomotor activity was assessed using Basso, Beattie and Bresnahan (BBB) scoring in accordance with a previous report<sup>23</sup> and functional tests were conducted three days and weekly after the treatment (time points of 3, 7, 14, 21, 28 days). Hind-limb movements were observed by two independent, blinded examiners as the rats were forced towards the edges of an open field.<sup>23</sup> Rats that exhibited any functional recovery within 72 h of injury were excluded from the study.

In addition, Rivlin's oblique plate test was performed to evaluate each rat's ability to grip and maintain posture as previously described.<sup>24</sup> The action of each rat was measured three times by placing it in the middle of an oblique plate. In each trial, the plate was

periodically lifted by 5°, and the maximum angle was recorded as the angle at which the rat could maintain its position for five seconds without sliding down.

### *Hematoxylin–eosin (H&E) staining and immunohistochemistry*

The rats were euthanized using an overdose of 2% pentobarbital sodium (120 mg/kg; intraperitoneal injection) when the corresponding treatment was finished. Tissue segments containing the lesion were paraffin embedded. Tissues were cut into 5- $\mu$ m-thick sections, which were taken 2 mm rostral to the epicenter of the injury, and mounted. Parts of them were fixed in 10% formalin for 2 days and used for histopathological analysis, and other parts were stored in liquid nitrogen, which were intended for western blotting.

For histopathological observation, the sections were subjected to routine H&E staining. Simply, the paraffin sections spinal cord were dewaxed with xylene and an alcohol series. Slides were placed in hematoxylin for 5 min, followed by a dip in 2% acid alcohol and then in lithium carbonate. The slides were dipped in eosin for 2 min and then dehydrated with ethanol before xylene. The area of preserved tissue and the cavity in spinal cord were measured with ImageJ.

Immunohistochemical staining was performed after deparaffinization. Intrinsic peroxidase activity was blocked by incubation in 5% H<sub>2</sub>O<sub>2</sub> in PBS solution for 20 min. Sections were incubated overnight at 4°C with anti-rat primary antibody against myelin basic protein (MBP, 1:500; Cell Signaling, Boston, USA), Iba-1 (1:1000; Wako Pure Chemical Industries, Osaka, Japan), and GFAP (1:200; Abcam, Cambridge, UK). Sections were then incubated with conjugated secondary antibody (Cell Signaling, Boston, USA) for 1 h at 37°C. Sections were rinsed with PBS and mounted. Immunostaining was examined with an Olympus BX60 microscope, and the results were semi-quantified by Image Pro-Plus.

### *NeuN & TUNEL (TdT mediated dUTP nick end labeling) double staining*

Neuronal death was assessed by double staining of NeuN and TUNEL, using a commercially available kit (Merck, Darmstadt, Germany). The sections were dewaxed, dehydrated, and rinsed for the TUNEL technique. The sections were permeabilized and subjected to antigen retrieval using proteinase K. Endogenous peroxidase was blocked using a methanol solution and hydrogen peroxide (3% H<sub>2</sub>O<sub>2</sub>). Prior to TUNEL staining, sections were washed once with PBS and subsequently incubated overnight at 4°C with monoclonal mouse

anti-rat NeuN (1:200; Millipore, MA, USA) diluted in carrier solution I (1% goat serum, 1% BSA, and 0.3% Triton X-100 in PBS). The percentage of dead neuron cells = TUNEL<sup>+</sup>/NeuN<sup>+</sup> cells/DAPI<sup>+</sup> cells.

### *Wet/dry weight ratio assay*

Spinal cord water content was determined as previously described<sup>25</sup> using the wet/dry weight method. Spinal cord samples were taken 48 h after SCI. Spinal cords were removed, and 1.5 cm-size spinal tissues were obtained at the edge of the injury site. The tissues were weighed, heated at 110°C for 24 h, and re-weighed to obtain the dry weight. The percentage of water content was calculated using the following equation: (wet weight – dry weight)/wet weight  $\times$  100.

### *ELISA analysis*

Tissue samples were homogenized twice after adding stainless steel beads and Triton X-100 (2 mL, 0.1%) using a Bullet Blender (Next Advance, NY, USA). Homogenates were incubated at room temperature for 1 h and centrifuged for 15 min at 150  $\times$  g and 0°C. Equal amounts of each supernatant (200  $\mu$ L) were transferred to a microwell plate prior to addition of 800  $\mu$ L PBS. A commercially available ELISA kit (Immunoanalysis Corporation, Pomona, CA, USA) was used to evaluate the release of inflammatory cytokines, TNF- $\alpha$ , IL-6, and IL-1 $\beta$  in accordance with the manufacturer's instructions.

### *Western blot analysis*

Rats were decapitated 4 h, 3 and 7 days after the treatment and sham group. The spinal cord from the rats in each group was removed and stored at –80°C. Western blotting was conducted by researchers blinded to the groups. Spinal cord tissues were homogenized and total protein was extracted using RIPA lysis buffer. After assessment of protein concentration, 40  $\mu$ g of total protein was subjected to SDS-PAGE separation and transferred to PVDF membrane (Millipore, MA, USA). The membrane was then blocked with 5% non-fat milk at room temperature. The primary antibody (Abcam, Cambridge, UK) against caspase 3 (1: 500), LC3B (1:1,000), p62 (1:1,500), p-Akt (1:1,000), Akt (1:500), p-mTOR (1:2,000), or mTOR (1:2,000) was diluted with TBS at 1:2000 and membranes were incubated with the primary antibody at 4°C overnight. The secondary antibody was added and incubated for 45 min at room temperature and visualized with the enhanced chemiluminescence (ECL) technique. Imaging data were analyzed using the ImageJ (NIH, MD, USA) software using the expression level of GAPDH as an internal reference.

**Statistical analysis**

The data are presented as means ± standard deviations (SD) and were analyzed using SPSS version 16.0 (IBM, Chicago, IL, USA). Intergroup data were compared using analysis of variance (ANOVA). A *P*-value of less than 0.05 was regarded as statistically significant.

**Results**

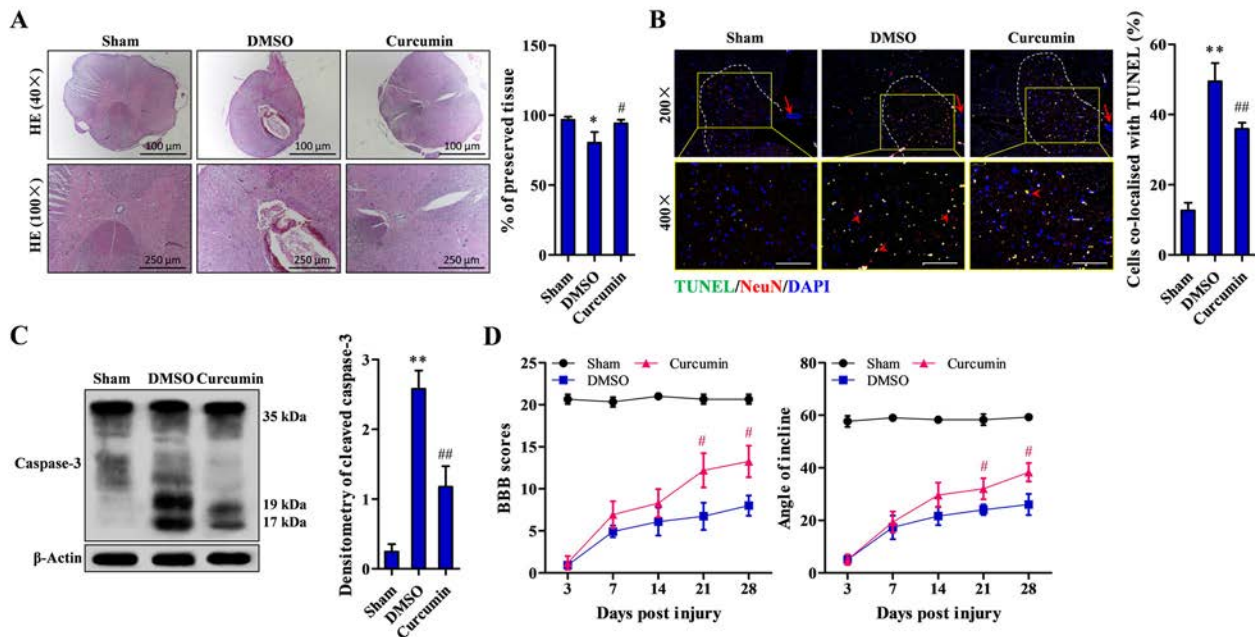
*Curcumin reduces damage to tissue structure, decreases neuronal apoptosis, and improves functional recovery after SCI*

Histological observation of the injured spinal cords treated with DMSO exhibited a central cavity with severe focal necrosis, widespread edema, diffuse hemorrhage and severe focal necrosis in both gray-matter and white-matter and tissue loss (*P* < 0.05, Fig. 1A). Curcumin treatment resulted in a notable amelioration of necrosis and considerable tissue preservation after 28 days of treatment (*P* < 0.05, Fig. 1A). TUNEL staining revealed significantly more neuronal death in injured spinal cords treated with DMSO compared to the sham control group (*P* < 0.01). A significant reduction in the percentage of apoptotic neurons was seen in the curcumin treatment group compared to the DMSO group (*P* < 0.01) (Fig. 1B). Cleaved caspase-3 could be barely found in the DMSO group, compared to the

which, sham control group showed significantly increased caspase-3 cleavage (*P* < 0.05). But the cleavage in the curcumin treatment group was significantly lower compared to the DMSO group (*P* < 0.01) (Fig. 1C). Hind limb locomotion function was assessed with BBB scoring. As shown in Fig. 1D, the BBB score was significantly higher in the curcumin group compared to the DMSO group 21 days after SCI and was even higher after 28 days (*P* < 0.05).

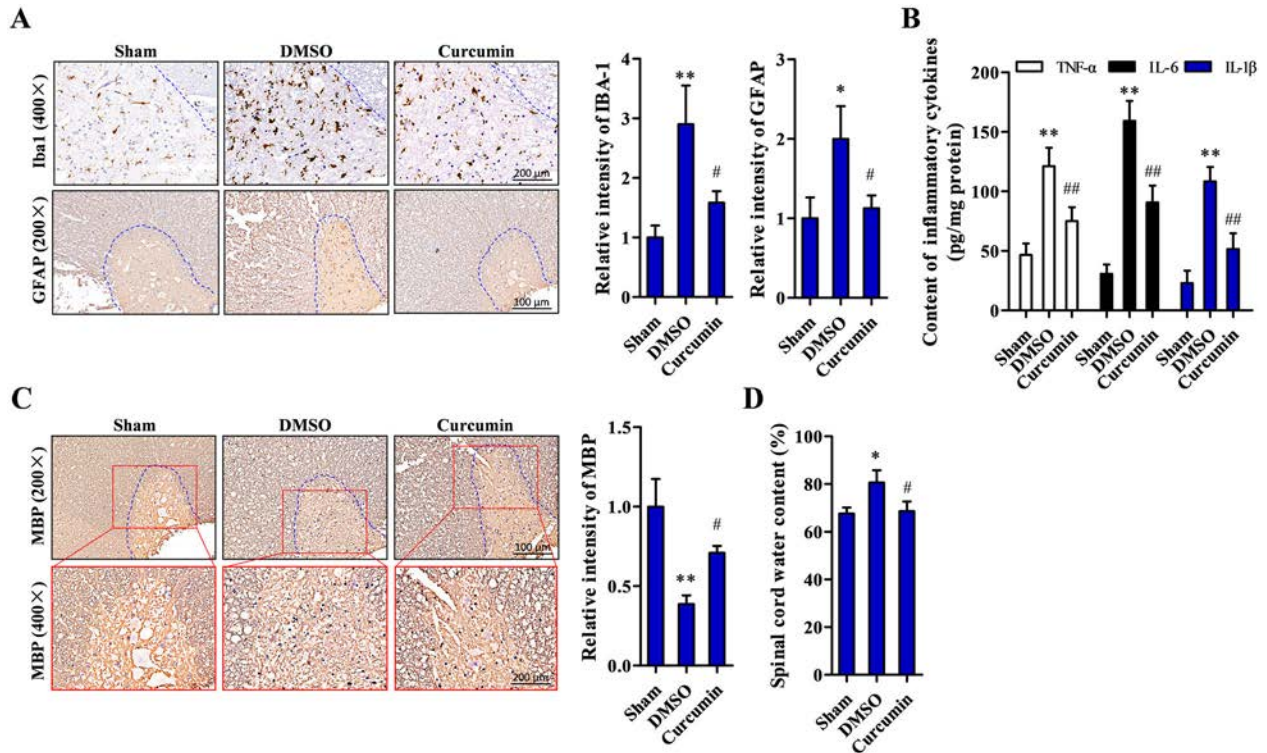
*Curcumin decreases gliosis, inflammation response, and improves myelination after SCI*

Our histopathological examination revealed that Iba-1 signal intensity in the DMSO group was approximately 3-fold higher than in the sham control group (*P* < 0.05) and more than 1-fold lower in the curcumin treatment group compared to the DMSO group (*P* < 0.05). Likewise, the percentage of GFAP-positive cells was significantly increased in the DMSO group and significantly decreased in the curcumin treatment group (both *P* < 0.05) (Fig. 2A). To determine the effect of curcumin on the inflammatory response, ELISAs were performed to determine inflammatory cytokine concentrations. TNF-α, IL-6, and IL-1β were significantly higher in the DMSO group (*P* < 0.01) compared to the sham group, and their levels were remarkably



**Figure 1. Decreased damage to tissue structure and improved functional recovery due to 28-day curcumin treatment after SCI. (A)** Representative images of H&E staining of the injury site after SCI; 100× magnified pictures were taken from 40× photos. **(B)** Neuron apoptosis in left ventral horn (within the dotted lines) detected by TUNEL staining; apoptotic neurons were co-localized with TUNEL (arrow head); scale bar: 200 μm; arrow: central canal. **(C)** Caspase-3 activity examined by western blotting. **(D)** Functional recovery assessed by BBB scoring and the oblique plate test. Sham (*n* = 10), DMSO (*n* = 10), curcumin (*n* = 10) treatment group. Each experiment was conducted in triplicate. \**P* < 0.05, \*\**P* < 0.01 vs. sham controls; #*P* < 0.05, ##*P* < 0.01 vs. DMSO.





**Figure 2.** Decreased gliosis and inflammation, and improved myelination by curcumin treatment after SCI. (A) Examination of astrocytes and gliosis level by their representative markers Iba-1 and GFAP in left ventral horn (within the dotted lines) using immunohistochemistry. (B) Detection of inflammatory cytokine TNF- $\alpha$ , IL-6, and IL-1 $\beta$  by ELISA kit. (C) Myelination status observed by immunohistochemical staining of MBP at the site of injured tissue. (D) Spinal edema determined by wet/dry weight ratio assay. Sham ( $n = 15$ ), DMSO ( $n = 20$ ), curcumin ( $n = 20$ ) treatment group. Each experiment was conducted in triplicate. \* $P < 0.05$ , \*\* $P < 0.01$  vs. sham controls; # $P < 0.05$ , ## $P < 0.01$  vs DMSO.

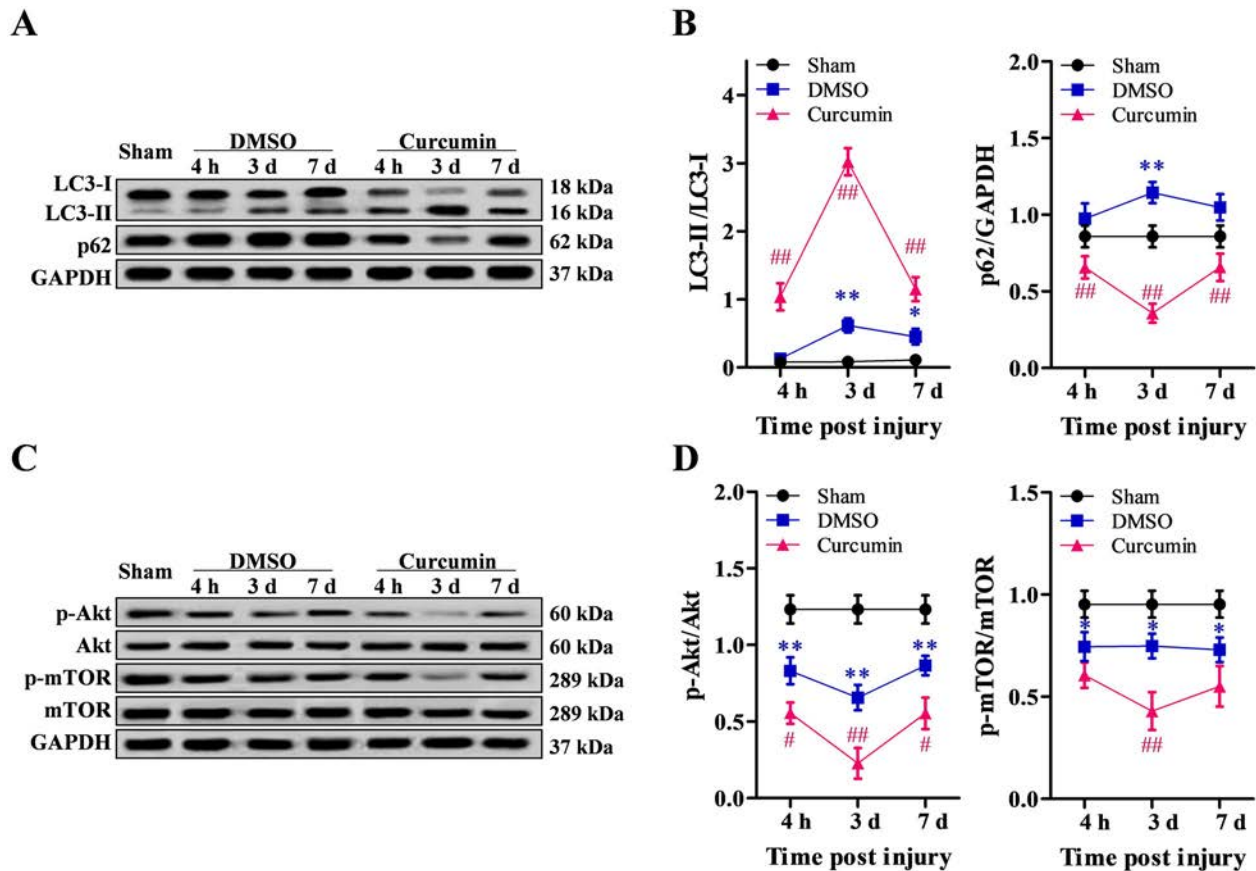
reduced ( $P < 0.01$ ) with curcumin treatment compared to DMSO alone (Fig. 2B). In addition, myelination of nerves was determined by examining the expression level of myelin basic protein (MBP) by immunohistochemical staining analysis. MBP was lower ( $P < 0.01$ ) in the DMSO group than the sham controls, but significantly higher ( $P < 0.05$ ) in the curcumin treatment group compared to the DMSO group (Fig. 2C). The effect of curcumin on spinal cord edema was assessed with the wet/dry ratio method. The results showed that the spinal cord water content was higher in the DMSO group compared to the sham controls ( $P < 0.05$ ) but was significantly lower in the curcumin treatment group compared to the DMSO group ( $P < 0.05$ ) (Fig. 2D).

#### *Curcumin promotes autophagy flux and downregulates Akt activation after SCI*

To examine the induction of autophagy after SCI, we assessed the levels of autophagy protein LC3 (MAP1LC3B) in the injured spinal cord by western blotting. Conversion of LC3-I to LC3-II is an essential marker of autophagosome formation and accumulation.

With curcumin treatment, LC3-II/LC3-I ratio was significantly increased 4 h after injury compared to DMSO alone ( $P < 0.01$ ) and reached peak expression on day 3, followed by a reduction on day 7 after injury. On the other hand, the expression of p62 (normalized to GAPDH) was lower in the curcumin treatment group compared to the DMSO group ( $P < 0.01$ ), and was higher in the DMSO group compared to the sham controls ( $P < 0.05$ ) (Fig. 3A, B). Because autophagy flux is often associated with Akt/mTOR pathway,<sup>26,27</sup> we examined the expression of Akt and mTOR molecules by western blot analysis. The results showed that both Akt and mTOR expression were significantly lower in the DMSO group compared to the sham controls ( $P < 0.05$ ), and were further decreased in the curcumin treatment group compared to the DMSO group ( $P < 0.05$ ) (Fig. 3C, D).

*Inhibition of autophagy partly abolishes the protective effect of curcumin on spinal cord injury*  
We examined the consequences of inhibiting autophagy on the protective effect of curcumin after spinal cord injury by combined treatment with curcumin and



**Figure 3.** Autophagy flux promoted by curcumin treatment after SCI through the Akt/mTOR signaling pathway. (A, B) Autophagy flux detected 4 h, 3, and 7 days after mechanical injury by western blot analysis. (C, D) Activation of the Akt/mTOR signaling pathway examined 4 h, 3, and 7 days after treatment by western blot analysis. Sham ( $n = 5$ ), DMSO ( $n = 30$ ), curcumin ( $n = 30$ ) treatment group. Each experiment was conducted in triplicate. \* $P < 0.05$ , \*\* $P < 0.01$ , vs. sham control; # $P < 0.05$ , ## $P < 0.01$ , vs. DMSO.

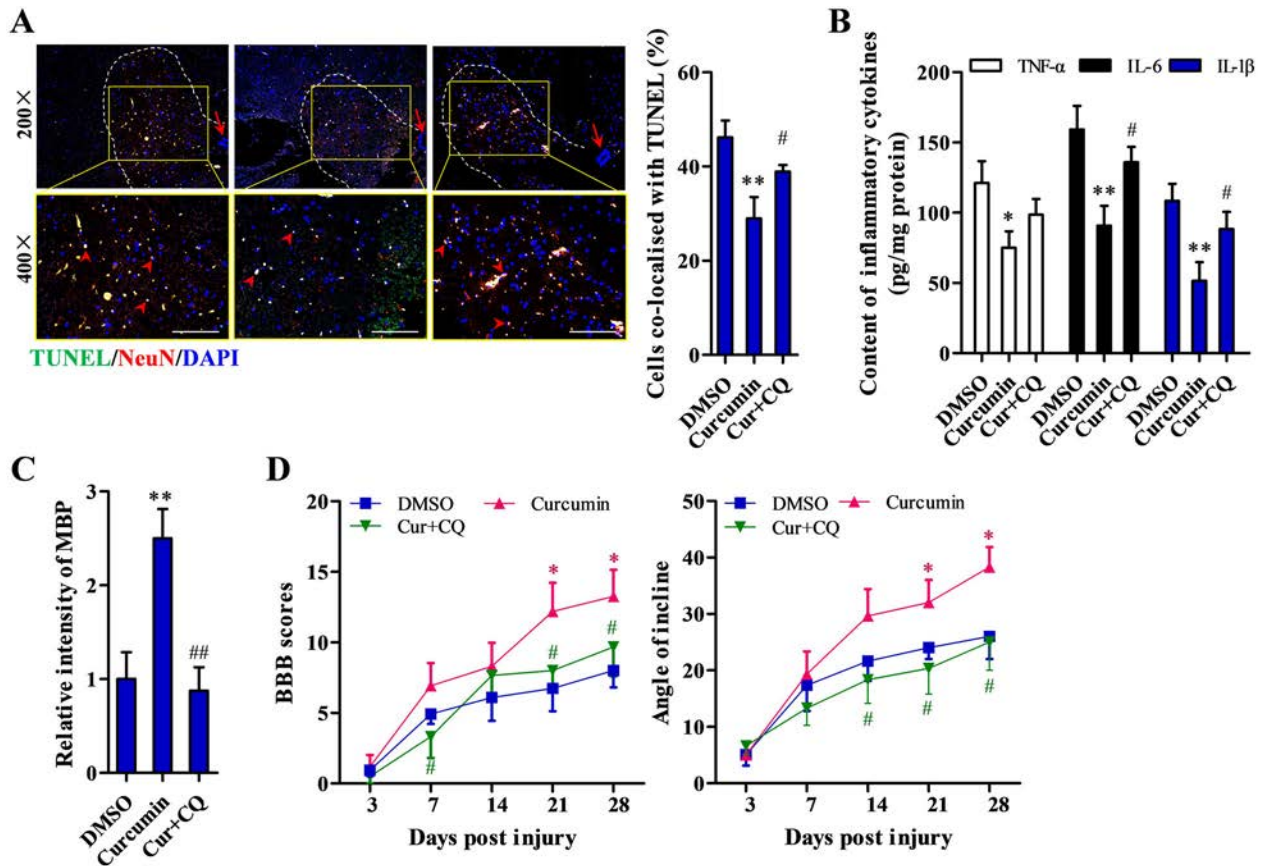
chloroquine (CQ, autophagy pathway inhibitor). The percentage of apoptotic cells was significantly increased with combined treatment (Cur + CQ) compared to curcumin treatment alone ( $P < 0.05$ ) (Fig. 4A). In addition, the concentrations of inflammatory cytokines, TNF- $\alpha$ , IL-6, and IL-1 $\beta$ , were increased with combined treatment compared to curcumin treatment only ( $P < 0.05$ ) (Fig. 4B). Immunohistochemical staining revealed an elevated number of MBP positive cells with curcumin treatment that was reduced by addition of chloroquine ( $P < 0.05$ ) (Fig. 4C). In functional recovery analysis, the significantly increased BBB score and angle of incline after curcumin treatment were markedly blocked by CQ (Curcumin + CQ v.s. Curcumin,  $P < 0.05$ ) 21 and 28 days after SCI (Fig. 4D).

## Discussion

This study demonstrates that the active compound curcumin enhances functional recovery after SCI as evidenced by higher locomotor ratings, amelioration of tissue damage, and reduced neuronal death.

Histopathological investigation revealed that glial scar formation and inflammatory responses were reduced and that spinal myelination was improved after curcumin treatment. In addition, curcumin promoted the induction of autophagy flux after SCI and inhibited the activation of the Akt/mTOR signaling pathway. To further explore the effect of curcumin on induction of autophagy flux, we inhibited the autophagy pathway. The results showed that autophagy inhibition partially abrogates the protective effect of curcumin after SCI as evidenced by increased tissue damage, neuronal death rate, and inflammatory cytokines, and reduced BBB score.

SCI causes the cell death of neurons, astrocytes, and oligodendrocytes and leads to massive loss of sensory and motor skills beneath the site of injury.<sup>28,29</sup> In addition, SCI provokes an inflammatory response<sup>30</sup> which contributes to activation of resident microglia cells<sup>31</sup> and demyelination.<sup>32</sup> In our work, overproduction of microglia and astrocytes was detected by examining the expression levels of Iba-1



**Figure 4.** Abrogation of curcumin's protective effect by inhibition of autophagy. (A) Nerve cell apoptosis detected by TUNEL staining and apoptotic neurons in left ventral horn (within the dotted lines) were co-localized with TUNEL (arrow head); scale bar: 200  $\mu$ m. (B) Detection of inflammatory cytokine TNF- $\alpha$ , IL-6, and IL-1 $\beta$  by ELISA detection kit. (C) Myelination status measured by immunohistochemical staining of MBP at the site of injured tissue. (D) Functional recovery assessed by BBB scoring and angle of incline analysis. DMSO ( $n = 10$ ), curcumin ( $n = 10$ ), and curcumin + CQ treatment group ( $n = 10$ ). Each experiment was conducted in triplicate. \* $P < 0.05$ , \*\* $P < 0.01$  vs. DMSO; # $P < 0.05$ , ## $P < 0.01$  vs. curcumin.

and GFAP in SCI rats and this elevation was significantly reduced with curcumin treatment. Furthermore, inflammatory cytokine concentrations, TNF- $\alpha$ , IL-6, and IL-1 $\beta$ , were significantly higher in the DMSO group compared to the sham group, and their levels were remarkably reduced with curcumin treatment. Considering the pathophysiology of SCI that includes neuronal death, reactive gliosis, axon demyelination, and syringomyelia around the lesion, alleviating secondary damage is a crucial element in the treatment of SCI.<sup>33</sup> Curcumin improves motor function recovery and reduces spinal cord edema by inhibiting the JAK/STAT signaling pathway.<sup>34</sup> In addition, it promotes spinal cord repair through inhibition of glial scar formation and inflammation.<sup>35</sup> Our results demonstrated that myelination of nerves (biomarker: myelin basic protein) was lower in the DMSO group than the sham controls, but significantly higher in the curcumin treatment group compared to the DMSO group. Along with

the therapeutic function to promote nerve myelination, our result also exhibited that curcumin could reduce spinal cord edema. These results may suggest that curcumin could be a potential candidate for treating loss of sensory and motor skills caused by SCI.

Since induction of autophagy flux could be beneficial for functional recovery after SCI, in this study we further investigated whether curcumin regulates the autophagy pathway after SCI in rats. In previous literature, nicotinate-curcumin, one of the curcumin derivatives, was reported to decrease lipid accumulation by restoring autophagy flux, suggesting therapeutic potential to reverse atherosclerosis.<sup>36</sup> In a study of transthyretin (TTR)-related amyloidosis disease, which is characterized by extracellular deposition of aggregates and associated with autophagy impairment, curcumin was suggested as an alternative therapeutic agent due to its potential role in modulating autophagy.<sup>37</sup> Interestingly, in malignant mesothelioma cell lines,



curcumin was reported to block autophagy and activate apoptosis.<sup>38</sup> It has also been reported that curcumin induces autophagy through inhibition of the Akt/mTOR pathway in Alzheimer's disease,<sup>39</sup> malignant glioma,<sup>40</sup> and human leukemia.<sup>41</sup> In agreement, our results demonstrated that the therapeutic role of curcumin in spinal cord injury was associated with elicitation of autophagy through inhibition of the Akt/mTOR pathway.

Increasing literatures reported that blocking of autophagic flux in neurons results from lysosome defects or the failure in fusion between autophagosomes and lysosomes, and leads to the development of some CNS diseases.<sup>42</sup> Upstream of mTOR, the survival PI3K/AKT pathway modulates mTOR activity that is also altered in neurodegenerative diseases of Alzheimer and Parkinson. Suppression of the PI3K/AKT/mTOR pathway promotes autophagy and activation of PI3K/AKT/mTOR inhibits autophagy.<sup>27</sup> Rapamycin specifically inhibits mTOR pathway and increases the formation of autophagosomes, and therefore stimulates the process of autophagy.<sup>43</sup> Hence, this agent was commonly used as an autophagic agonist. On the other hand, the behavior improvement and mitigation of histological destruction strongly supports the benefit of autophagy in SCI.<sup>12</sup> However, rapamycin tend to overstimulate autophagy and may lead to detrimental outcomes. As a natural component curcumin may play a safer role in suppressed mTOR induced autophagy. Our results suggest that, when autophagy was inhibited, therapeutic role of curcumin was diminished as evidenced by increased level of inflammatory cytokines, reduced neuron myelination, decreased BBB score. Discovery indicates that curcumin is a direct inhibitor of mTOR, which may remove the association of Raptor with mTOR at low concentration and suppress the binding of Rictor with mTOR at a high concentration,<sup>44</sup> but it is not supported by molecular insight into the regulation. Therefore, a much more detailed mechanism about the inhibition of curcumin in mTOR signaling pathway should be further studied.

In addition, we noticed that the autophagy flux reached a peak point followed by a decrease 7 days post injury/treatment. Given that the time-dependent accumulation of cellular damage is the general cause of aging, it is easy to infer that a gradual, age-related decline in autophagic activity may contribute to this result. However, this speculation needs further experiment for confirmation. Along with the decline in autophagy flux 7 days post treatment, Akt/mTOR expression was also decreased at the same time point. As to how

this result occurs, we could not have any clue yet. Our next experiment has already been launched to find an explanation for such phenomenon.

In summary, the present study demonstrates that curcumin promotes motor function recovery and reduces neuronal apoptosis. In addition, curcumin may exert its neuroprotective effect on SCI through the modulation of autophagy and inhibiting Akt/mTOR signaling.

## Disclaimer statements

**Conflicts of interest** The authors declare that they have no conflict of interest.

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