Tauroursodeoxycholic Acid Alleviates Secondary Injury in Spinal Cord Injury Mice Through Reducing Oxidative Stress, Apoptosis, and Inflammatory Response

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Research

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Abstract

Background

Tauroursodeoxycholic acid (TUDCA) is a hydrophilic bile acid derivative, which has been demonstrated to have neuroprotective effects in different neurological disease models. However, the effect and underlying mechanism of TUDCA on spinal cord injury (SCI) have not been fully elucidated. This study is aim to investigate the protective effects of TUDCA in SCI mouse model and the related mechanism involved.

Methods

The primary cortical neurons were isolated from E16.5 C57BL/6 mouse embryos. To evaluate the effect of TUDCA on oxidative stress in vitro, the cortical neurons were treated with H_2O_2 with or without TUDCA added. Mice were randomly divided into sham, SCI and TUDCA groups. SCI model was induced using a pneumatic impact device at T9-T10 level of vertebra. TUDCA (200 mg/kg) or equal volume of saline was intragastrically administrated daily post injury for 14 days.

Results

We found that TUDCA reduced reactive oxygen species (ROS) generation, lactate dehydrogenase (LDH) release and restored superoxide dismutase (SOD) activity to protect primary cortical neurons from oxidative stress in vitro. In vivo, TUDCA treatment significantly reduced tissue injury, oxidative stress, inflammatory response, and apoptosis; promoted axon regeneration and remyelination in the lesion site of spinal cord of SCI mice. The functional recovery test revealed that TUDCA treatment significantly ameliorated recovery of limb function.

Conclusions

TUDCA treatment can alleviate secondary injury and promote functional recovery through reducing oxidative stress, inflammatory response and apoptosis induced by primary injury, and promote axon regeneration and remyelination, which could be used as a potential therapy for human SCI recovery.

Introduction

Spinal Cord Injury (SCI) refers to complete or incomplete spinal motor and sensory dysfunction caused by injury to spinal cord [1]. SCI is a serious disabling disease, which brings a heavy burden to patients' families and society. The pathophysiological process of SCI includes primary and secondary injuries [2-4]. The primary injury is caused by initial mechanical injury to the spinal cord which is an irreversible process [1, 5]. Subsequently, the hemorrhage, intravascular thrombosis, and vascular spasm will cause ischemia and edema leading to aggregated primary damage, which is called the secondary injury [1, 6]. The mechanisms of secondary injury involved oxidative stress, inflammatory response, excitatory toxicity, cell apoptosis and microcirculation disturbance[7, 8]. Therefore, inhibiting the progression of second

injury timely through attenuating oxidative stress and inflammation to reduce the neuronal cell death and promote axon regeneration is an effective strategy to alleviate neurological impairment [9, 10].

Tauroursodeoxycho.lic acid (TUDCA) is a hydrophilic bile acid which is the amino acid taurine conjugate of ursodeoxycholic acid [11, 12]. TUDCA is approved by the U.S. Food and Drug Administration (FDA) for the treatment of liver diseases such as cholestasis [13], cirrhosis [14], and hepatitis [15]. It can cross the blood-brain barrier, with no associated toxicity. There are growing researches to study the potential therapeutic effects of TUDCA on non-liver diseases, particularly on neurological disease models [16, 17]. TUDCA has been reported to show protective effects against apoptosis, inflammation, oxidative stress and/or mitochondrial dysfunction in different neurological diseases models, such as Parkinson's disease (PD)[18-20], Huntington's disease (HD) [11, 21], and [16, 22].

Increasing evidences have shown that TUDCA, as a chemical chaperone, had various neuroprotective activities, including anti-apoptosis, anti-inflammatory response, and anti-oxidative stress in different animal models of neurological diseases. For instance, Keene et al., reported that TUDCA treatment improved locomotor and sensorimotor recovery through reducing striatal atrophy, decreasing striatal apoptosis and the size of ubiquitinated neuronal intranuclear huntingtin inclusions in a transgenic animal model of Huntington's disease [19]. In a PD mouse model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), TUDCA has been shown to improve motor symptoms by preventing the decrease of dopaminergic fibers and ATP levels, as well as inhibiting mitochondrial dysfunction and neuroinflammation [23]. Wu et al., reported that TUDCA treatment improved cognitive impairment and neurotoxicity induced by LPS in mice through reducing LPS-induced apoptosis and ameliorated synaptic plasticity impairments [24]. In addition, TUDCA has also been shown to activate Nrf2 to limit reactive oxygen species (ROS)-mediated damage [20], and enhance mitochondrial biogenesis, and early neurogenesis [25].

Although studies have elaborated the protective properties of TUDCA in neurological diseases, few studies state the effects of TUDCA on SCI recovery and the involved mechanism. Thus, we aim to investigate the effects of TUDCA on oxidative stress, inflammation, and apoptosis to evaluate the protective property of TUDCA on axon regeneration and function recovery after SCI, and explore the underlying mechanism in this study.

Material And Methods

Animals

C57BL/6 mice were purchased from the Guangdong Medical Experimental Animal Center. The mice were housed in temperature-controlled conditions and supplied with free food and water. All animal experiments were approved by the Ethics Committee of Guangzhou University of Chinese Medicine and performed according to the guidelines of the Chinese National Institutes of Health (Guangzhou, China, Certificate No. 44005800012426).

TUDCA preparation

TUDCA used in this study (purity >98% of the total weight) was purchased from Shanghai yuanye Bio-Technology Company Limited (Shanghai, China). TUDCA was dissolved in 0.9% normal saline.

Primary culture of cortical neurons

Cortical neurons were extracted from embryos of pregnant C57BL/6 mice (E16.5) according to protocol [26]. Shortly, the cerebral cortex was separated and cut into approximately 1-mm pieces in precooled Dulbecco's Modified Eagle Medium: F-12 (DMEM/F12 medium, Gibco). Subsequently, the tissues were digested with 200 ug/ml papain (sigma, Beijing, China) for 25min at 37℃. After digestion with papain, the solution was filtered using a 100-um cell strainer (BD Falcon) and then centrifuged at 800rpm for 5min. The cell pellet was resuspended in complete Dulbecco's Modified Eagle Medium (DMEM; Gibco) containing 10% fetal bovine serum (FBS, Gibco). And then the cells were seeded into poly-D-lysine (sigma, Beijing, China) pre-coated 6-well plates, 24-well plates, or 96-well plates, and incubated in 5% CO_2 at 37℃. 4 hours later, the cells were refreshed and cultured in Neurobasal medium (Gibco) containing 0.5mM L-glutamine (Gibco) and 2% B27 (B-27™ Supplement, Gibco). Fresh neurobasal medium containing L-glutamine and B27 was used to replace half of the medium every two days until the seventh day. All cells used in the assay were at day 4.

Cell viability assay

Cell viability was assessed using the Cell Counting Kit-8 (CCK-8, KeyGEN, China) assay according to the protocol of the kit. Cortical neurons were seeded into 96-well plates (2×10^4 cells per well) precoated with poly-D-Lysine, and cortical neurons were treated with H_2O_2 with or without TUDCA at the indicated concentrations for 24 hours or 48 hours. Then 10 μL CCK-8 solution was added to the culture per well, and incubated at 37℃ for 2 hours. Finally, the absorbance of the culture medium at 450 nm read on microplate reader (Bio-Rad) was analyzed to determined cell viability.

ROS generation

Intracellular ROS generation were measured using DCFH-DA fluorescent probe (KeyGEN, China). After treatment, cortical neurons were washed with PBS, then 20 μM DCFH-DA fluorescent probe were added into the cultures with serum free medium. Cells were further cultured at 37°C for 1 h according to instructions of the probe. ROS production in cells was determined by DCF fluorescence observed under fluorescence microscopy (Olympus IX73).

LDH, SOD and GSH measurement

The biomedical kits (Jiancheng Institute of Biology, Nanjing, China) were used to measure and normalize the level of LDH, SOD and reduced GSH according to the protocol of the kit.

SCI model and treatment

The model of SCI was induced under sterile conditions and performed according to Allen's method as previously described [27]. Before surgery, the mice were anesthetized using 1% pentobarbital sodium (50 mg/kg) by intraperitoneal injection (i.p.), and the spinal cord were exposed at the T9-T10 levels by laminectomy. The SCI model was induced using a pneumatic impact device. The force was set at 0.5 m/s, and the duration time was 80 ms. After surgery, bladders were manually voided twice a day until the mice could urinate normally. The mice were randomly divided into sham, SCI and TUDCA groups. The mice in the sham group were only subjected to surgical procedure without SCI. TUDCA at 200 mg/kg dosage was given to the mice by oral route once daily post injury. Meanwhile, equal volume of saline was given orally once daily in the sham group and SCI group.

Functional behavior evaluation

Hindlimb motor function was evaluated using the Basso-Beattie-Bresnahan (BBB) locomotion scale and the footprint test at different timepoints post-injury. The BBB scale ranges from 0 to 21 (0 = complete paralysis to 21 = normal gait) based on hindlimb joints movement and coordination.

Footprint test was performed by dipping the posterior limb of the mice with black dye. Then the mice were encouraged to walk straight to across a narrow path to record the footprints. The footprints were scanned, and the digitized images were used to analyze their gaits.

Tissue preparation

The mice were sacrificed under anesthetized and transcranial perfused with 0.9% normal saline at specific time points. For western blot, the spinal cord tissues around the lesion epicenter (± 0.5cm) were dissected out, then homogenized in RIPA buffer (Beyotime, Jiangsu, China) with 10 μl/ml protease inhibitor cocktail. The tissue lysates were centrifuged at 12,000 rpm for 15 min at 4℃, and then the supernatants were collected. Protein assay kit (BCA, Beyotime, Jiangsu, China) was used to determine the protein concentration.

For immunofluorescence staining and histological assessment, the mice were perfused 4% paraformaldehyde (PFA in 0.1 M PBS, pH 7.4) transcardially under anesthetic after normal saline perfusion. Spinal cord tissues were dissected out and post-fixed with 4% paraformaldehyde overnight. Tissues were dehydrated sequentially with 70%, 80%, 95% and 100% ethanol. Then the processed tisues were embedded in an appropriate orientation. The paraffin-embedded spinal cord was sectioned at 5mm using a microtome, and sections were mounted onto histological glass slides and dried overnight for immunostaining or stored at room temperature until use.

Immunofluorescence Staining on cells and sections

The primary cortical neurons cultured on the coverslips were fixed at designated time points with 4% PFA for at least 2 hours at 4℃. The sections were dewaxed in xylene three times each for 5 minutes and hydrated through a series of alcohol of decreasing concentrations (100% to 95% to 80% to 70% and then tap water twice, each step for 5 minutes). The prepared tissue sections and cells were blocked in PBS

with 10% normal horse serum at room temperature for 1 hour, and then incubated at 4℃ overnight in PBS with the following primary antibodies from different species diluted in 10% normal horse serum: microtubule-associated protein 2 (MAP2, 1:200, Boster Biological Engineering Co.), β-tubulin III (Tuj1, 1:200, Millipore), GFAP (1:500, Boster Biological Engineering Co.), GAP43 (1:200, NOVUS), MBP (1:200, NOVUS), and CD68 (1:300, Boster Biological Engineering Co.). Secondary antibodies tagged with different Alexa fluor® fluorochrome (1: 300) were used. The immunostained sections or cells were examined under a fluorescence microscope (Olympus IX73).

TUNEL assay

Apoptotic cells in the sections were identified by TUNEL staining using the TUNEL cell apoptosis detection kit (Yeasen Biotech, Shanghai, China) following the manufacturer's protocol. The immunofluorescent images were captured under a fluorescence microscope (Olympus IX73). After TUNEL labeling, the numbers of apoptotic cells (TUNEL positive cells) and the total number of cells (DAPI positive cells) on each section were counted.

Statistical analysis

All values are presented as the means ± SD. All data were conducted in GraphPad Prism 8 software (GraphPad Software Inc.). Statistical analysis was performed using one-way ANOVA for more than two groups, or using unpaired Student's t test to compare two groups. In all the analyses, $p<0.05$ was considered statistically significant (expressed as *$p<0.05$ or **$p<0.01$).

Results

TUDCA protected cortical neurons from H_2O_2-induced injury

The mouse cortical neurons were treated with different concentrations of TUDCA in a range of 50 μM to 800 μM for 48 hours. As shown in Figure 1A, cells treated with TUDCA showed similar cell viabilities as control, indicating TUDCA is nontoxic to mouse cortical neuron. Refer to previous reports, TUDCA at 200 μM was used in further experiments. To determine the effect of TUDCA on H_2O_2-induced injury, cells were treated with H_2O_2 (300 or 400 μM) or H_2O_2 plus TUDCA (200 μM) for 24 hours. H_2O_2 treatment caused a significant decrease in cell viability compared to control, while co-treatment with TUDCA significantly increased the cell viability compared to cells exposed to H_2O_2 alone. To further clarify the role of TUDCA in oxidative stress, we measured the intracellular ROS generation, levels of LDH and SOD activity. As shown in Figure 1, compared to control, H_2O_2 treatment induced ROS generation, LDH release and reduced SOD activity. While TUDCA treatment significantly reduced ROS generation and LDH release caused by H_2O_2 exposure and restored SOD activity (Figure 1C-F).

TUDCA attenuated H_2O_2-induced axon degeneration in cortical neurons

Oxidative stress has been demonstrated to contribute to axon degeneration in numerous neurological disorders. As our results showed that TUDCA could decrease oxidative stress, thus we want to know whether TUDCA could attenuate axon degeneration induced by oxidative stress. Labeled Tuj1 (class III beta-tubulin, a marker of neurons) was implemented to evaluate the effect of TUDCA in primary cortical neurons. As shown in Figure 2, TUDCA significantly attenuated H_2O_2-induced axon degeneration in primary cortical neurons as compared with control.

TUDCA reduced tissue damage and improved motor function after SCI

To determine the effect of TUDCA treatment in functional recovery of SCI mice, we performed Basso-Beattie-Bresnahan (BBB) rating scale and footprint test (Figure 3A). The BBB scores decreased significantly in the Saline and TUDCA groups at 1, 3, 7 and 14 days, and the BBB scores of mice that treated with TUDCA were higher than Saline group at day 14 after SCI (Figure 3B). The footprint test revealed that mice in Saline group showed inconsistent behavior with extensive dragging, while mice in TUDCA group showed a relatively consistent posterior limb footprint at day 14 after SCI (Figure 3A). H&E staining were performed to elaborate the histological morphology of the injury spinal cord at day 14 after SCI (Figure 3C). Compared with sham group, obvious malformation and cavity were observed in the injured site of spinal cord at day 14 after SCI. In the TUDCA group, the lesion area decreased significantly with less damaged tissue. Moreover, normal neurons were observed in sham group by Nissl staining. But in SCI group, there were few neurons with normal Nissl at the injured site of the spinal cord. However, the number of neurons in the lesion area was significantly increased after TUDCA treatment (Figure 3D and E). Consistent with our assumption, TUDCA treatment could decrease the damage of tissue, protect neurons in the lesion area, and improve functional recovery of SCI mice.

TUDCA attenuated oxidative stress through Nrf2 signaling pathway after SCI

Oxidative stress has been reported to participate in secondary injury progressing after SCI. As reactive ROS are difficult to measure directly in vivo, so we determined the level of reduced GSH and SOD activity to evaluate the effect of TUDCA on oxidative stress in SCI mice. The level of reduced GSH and SOD activity decreased significantly at day 3 after SCI, while TUDCA treatment restored the level of GSH and SOD activity (Figure 4A&B). Nuclear factor erythroid 2-related factor 2 (Nrf2), the "master regulator" of cellular resistance to oxidants was evaluated by western blot. The result showed that Nrf2 and NADPH quinine oxidoreductase-1 (NQO-1) expression were increased by TUDCA at day3, which activated the antioxidant response (Figure 4C-E). In addition, TUNEL staining was performed to evaluate the cell apoptosis rate after SCI. As shown is Figure 4F, few TUNEL positive cells were observed at the spinal cord in sham group, while the amount of apoptosis cells at the lesion cites of spinal cord increased significantly at day 14 after SCI. However, TUDCA treatment remarkably decreased the cell apoptosis (Figure 4G).

TUDCA reduced glial scar and promoted axonal generation after SCI

Glial scar and axonal generation in the injured spinal cord is critical for motor function recovery after SCI, so we performed immunofluorescence and western blot to elaborate the glial scar and axonal regeneration in SCI mice after TUDCA treatment. MAP2 is a neuron-specific cytoskeletal protein that is enriched in dendrites, and is used to label the mature neurons in nervous system. Spinal cord injury leads to the mature neurons lost in or around the lesion site. Immunostaining of MAP2 was used to measure the distance from lesion center to nearest neuron. Compared to SCI group, the distance of injured spinal cord in TUDCA group significantly decreased (Figure 5A&B). Following the SCI, astrocytes were activated and migrated to the lesion cite to create a barrier surrounding the lesion site completely by around 14 days post SCI. As shown in Figure 5A, an obvious glial scar with activated astrocytes surrounding was observed in SCI groups, and MAP2 positive axons were not observed in the lesion site surrounded by GFAP positive astrocytes. The glial scar area was measured by Image J (Figure 5C). Compared with SCI group, the glial scar area decreased significantly in TUDCA group; and the border of glial scar surrounded by activated astrocytes was not as obvious as SCI group (Figure 5A). Moreover, the western blot analysis showed the GFAP expression level was decreased in TUDCA groups. This indicated that TUDCA treatment inhibited reactive astrogliosis to prevent astrocytes form an overlapping wall of densely packed and adhered cells in the lesion penumbra.

Double immunofluorescence staining of GFAP and GAP43 (Growth Associated Protein 43) was performed to observe the axon regeneration in the lesion cite (Figure 5D). GAP43 is closely related to nerve regeneration and plays a key role in guiding axon growth and regulating axon formation of new connections. In the SCI group, few GAP43 positive axons were observed in the lesion area, whereas TUDCA group showed GAP43 positive axons which revealed that TUDCA treatment elicited the axon outgrowth. The western blot analysis results for GAP43 also revealed that GAP43 expression restored after TUDCA treatment.

TUDCA promoted remyelination

After SCI, direct damage caused necrosis and apoptosis of oligodendrocytes which lead to axonal demyelination. Myelin plays a key role in maintaining the integrity of axons. Therefore, remyelination is important for functional recovery of axons after spinal cord injury. Myelin basic protein (MBP) is an indispensable and structural hydrophilic protein of myelinated axons, which has long been used to identify myelinated axons and active remyelination. Therefore, MBP and GFAP double immunofluorescence staining was performed to evaluate the axon remyelination in the lesion site at day 14 after SCI. In SCI group, few MBP positive axons were observed in the fibrotic scar surrounding by activated astrocytes at the lesion site. But in the TUDCA group, MBP expression increased significantly in the lesion site of the spinal cord (Figure 6A). The western blot results also showed that TUDCA treatment promoted axon remyelination after SCI (Figure 6B&C).

TUDCA reduced inflammatory reaction

Inflammation induces apoptosis of the cells around the lesion after SCI. Inhibiting excessive inflammation is essential to prevent secondary injury processing and promote functional recovery. M1

macrophages have been reported to dominate lesion site at the early injury and initiate secondary damage. Immunofluorescence staining of CD68 was performed to evaluate the distribution of M1 macrophages. As shown in Figure 7, few numbers of M1 macrophages were observed in the sham group, while the number of CD68 positive macrophages remarkably increased and occupied the lesion site after SCI. Whereas TUDCA treatment evidently reduced the number and distribution of M1 macrophages in the injured spinal cord.

Discussion

The pathophysiology of SCI is a complicated process which can be divided into primary and second injuries. Timely and effective intervention to interrupt secondary injury can effectively reduce the further neurological damage and improve functional recovery [5, 28]. In the present study, we found that TUDCA could protect primary cortical neurons and promote functional recovery through inhibiting oxidative stress, inflammatory response and apoptosis induced by primary injury, which might be used to promote SCI recovery.

TUDCA, a chemical chaperone, has been reported to possess neuroprotective properties, including anti-apoptosis, anti-inflammation, and anti-oxidative stress in different animal model of neurological diseases [19, 29-31]. It could activate Nrf2 signaling to prevent reactive oxygen species production, decrease oxidative stress and its mediated damage in PD [20]. As a chemical chaperone, TUDCA abolishes unfolded protein response (UPR) activation to improve axonal degeneration in X-linked adrenoleukodystrophy [32]. In a neuroinflammation mouse model, TUDCA showed an anti-inflammation effect through reducing glial cell activation and increasing intracellular cAMP levels in microglia [31, 33]. It is a powerful neuroprotective agent with multiple actions, but its role in spinal cord injury has not been elaborated clearly. Therefore, we aim to state the protective effect of TUDCA on functional recovery and the underlying mechanism in SCI mice.

Oxidative stress has been reported to participate in and accelerate secondary injury processing in spinal cord injury after the physical trauma to the spinal cord [34, 35]. Therefore, suppression of oxidative stress has been considered as an effective therapeutic strategy to improve SCI recovery. First, we checked the protective effect of TUDCA on H_2O_2-induced oxidative stress in primary cortical neurons. The results showed that TUDCA increased cell viability by inhibiting ROS production, LDH release, and restoring SOD activity in vitro. And the immunofluorescence results showed that TUDCA treatment alleviated axon degeneration caused by oxidative stress.

Then we further investigate whether TUDCA could promote functional recovery in SCI mice. The functional measurement revealed that TUDCA treatment improved motor functional recovery after SCI. TUDCA treatment significantly decreased the lesion area, while increased the number of neurons with normal morphology and Nissl bodies. The level of reduced GSH and SOD activity after SCI was increased by TUDCA, implying the extent of oxidative stress was inhibited [35]. Nrf2 is a transcription factor which regulates antioxidant enzymes expression including NQO-1, and glutathione S-transferases (GST) [36]. In

this study, we found TUDCA reduced oxidative stress by Nrf2/NQO-1 signaling pathway after SCI. TUNEL staining showed that TUDCA treatment reduced cell apoptosis in the lesion site caused by SCI. Therefore, TUDCA exerts neuroprotective effects by reducing oxidative stress, as well as alleviating oxidative stress mediated cell apoptosis and tissue damage through Nrf2/NQO-1 signaling pathway.

At the site of injury, extensive neuronal apoptosis, axon degeneration and demyelination, and initiation of immune responses would trigger widespread secondary injury leading to dysfunction after SCI [37]. The glial scar formation process helps resolve inflammation to an extent, but a prominent and permanent glial scar forms a dense barrier to inhibit axon regeneration at the lesion site [38]. So, an intervention which not only blocks proinflammatory factors but also reduces the glial scar area is beneficial for axon regeneration and remyelination, and improving functional recovery. Therefore, we evaluate the effect of TUDCA on inflammation and glial scar formation in SCI mice. GFAP and GAP43 double labeling was used to evaluate the fibrotic component area of glial scar and the axon regeneration at the lesion site of the spinal cord. Our data showed that at day 14 after SCI, GFAP expression increased significantly, and a glial scar with GFAP positive astrocytes in the penumbra formed in the injured spinal cord. We also observed that reactive astrogliosis at the inner margin of the lesion penumbra created palisading-like patterns with thick hypertrophied processes that densely overlap and pack around the lesion in SCI mice. This is consistent with the previous reports [3, 39]. While the GFAP expression was remarkably decreased with TUDCA treatment, the border formed by active astrocytes in the lesion penumbra was not obvious as SCI group, and the fibrotic component area of glial scar reduced. The result demonstrated that TUDCA treatment reduced the glial scar, and provided a micro-environment to benefit axon regeneration. Double immunofluorescence of GFAP and GAP43, and western blot analysis showed that TUDCA treatment promoted axon regeneration at the lesion site.

Following SCI, activated microglia/macrophages not only release proinflammatory cytokines that cause cytotoxicity and demyelination but also produce neuroprotective molecules that preserve myelination and stimulate axon regeneration and sprouting [40]. According to the role of microglia/macrophages in SCI, they are divided into two subpopulations, M1 cells release proinflammatory cytokines and M2 cells promote remyelination [41, 42]. Therefore, shift M1/M2 polarization will lead to a shift between inflammation and remyelination. This is important for functional recovery after SCI [43]. M1 macrophages have been reported to be the dominant macrophage type found at the lesion site [44]. In this study, we found that CD68 positive M1 macrophage mainly distributed in the fibrotic component of glial scar, while there were a few cells were MBP positive. TUDCA treatment evidently reduced the number and distribution of M1 macrophage, and increased the cell remyelination at the lesion site, indicating that TUDCA treatment can mitigate inflammatory response and enhance remyelination process after SCI in mice.

Conclusion

In conclusion, the present study provided evidence showing that TUDCA treatment significantly attenuates secondary injury and improves motor function recovery in SCI mice. TUDCA alleviates

oxidative stress through Nrf2/NQO-1 signaling pathway, and reduces M1 macrophages to protect motor neuron and promote axon regeneration and remyelination. The exact mechanisms involved have not been elaborated clearly, hence, future studies should further explore and elaborate how TUDCA affects the microenvironment or the proliferation and differentiation of endogenous neural stem cells, reactive astrocytes and microglial to promote axon regeneration and remyelination. TUDCA may be considered as a potential effective intervention for SCI treatment.

Abbreviations

AD: Alzheimer's diseases

BBB: Basso-Beattie-Bresnahan

CCK-8: Cell Counting Kit-8

FDA: Food and Drug Administration

GAP43: Growth Associated Protein 43

GFAP: Glial fibrillary acidic protein

GSH: Glutathione

HD: Huntington's disease

LDH: Lactate dehydrogenase

MAP2: Microtubule Associated Protein 2

MBP: Myelin basic protein

MPTP: 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine

NQO-1: NADPH quinine oxidoreductase-1

Nrf2: Nuclear factor erythroid 2-related factor 2

PD: Parkinson's disease

PFA: Paraformaldehyde

ROS: Reactive oxygen species

SCI: Spinal cord injury

SOD: Superoxide dismutase

TUDCA: Tauroursodeoxycholic acid

Tuj1: β-tubulin III

UPR: unfolded protein response

Declarations

Ethics approval and consent to participate

All animal experiments were approved by the Ethics Committee of Guangzhou University of Chinese Medicine and performed according to the guidelines of the Chinese National Institutes of Health.

Consent for publication

Not applicable

Availability of data and materials

All data used in the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Y.H.H. and J.Y.L. performed and analyzed the experiments. T.C.D, T.D.H., X.L., Z.F.X., J.H.Z., D.L., and Y.H. performed experiments. L.L.X.and D.K.L. were responsible for the overall study design and for writing the manuscript.

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Figures

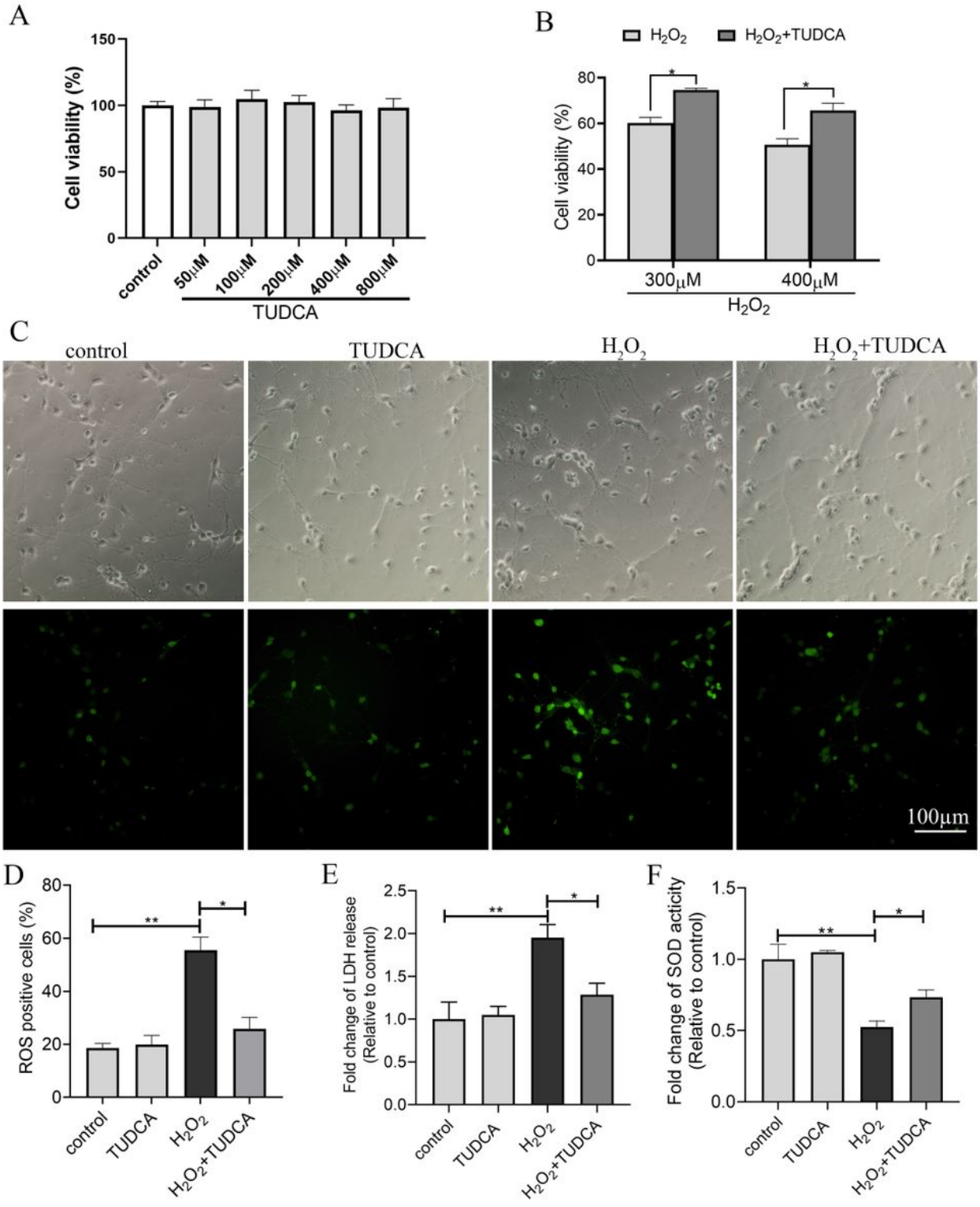

Figure 1

TUDCA protected cortical neurons from H2O2 exposure. (A) Cells were treated with different concentrations of TUDCA for 48h. (B) Cortical neurons were exposed to H2O2 and treated with TUDCA for 24h. Cell viability was measured by CCK-8 assay. (C&D) Detection of intracellular ROS generation using DCFH-DA. (F) Cortical neurons were treated with 300μM H2O2 and 200μM TUDCA for 24h, then SOD

activity was measured. All experiments were performed in triplicated and data were presented as means ± S.D.

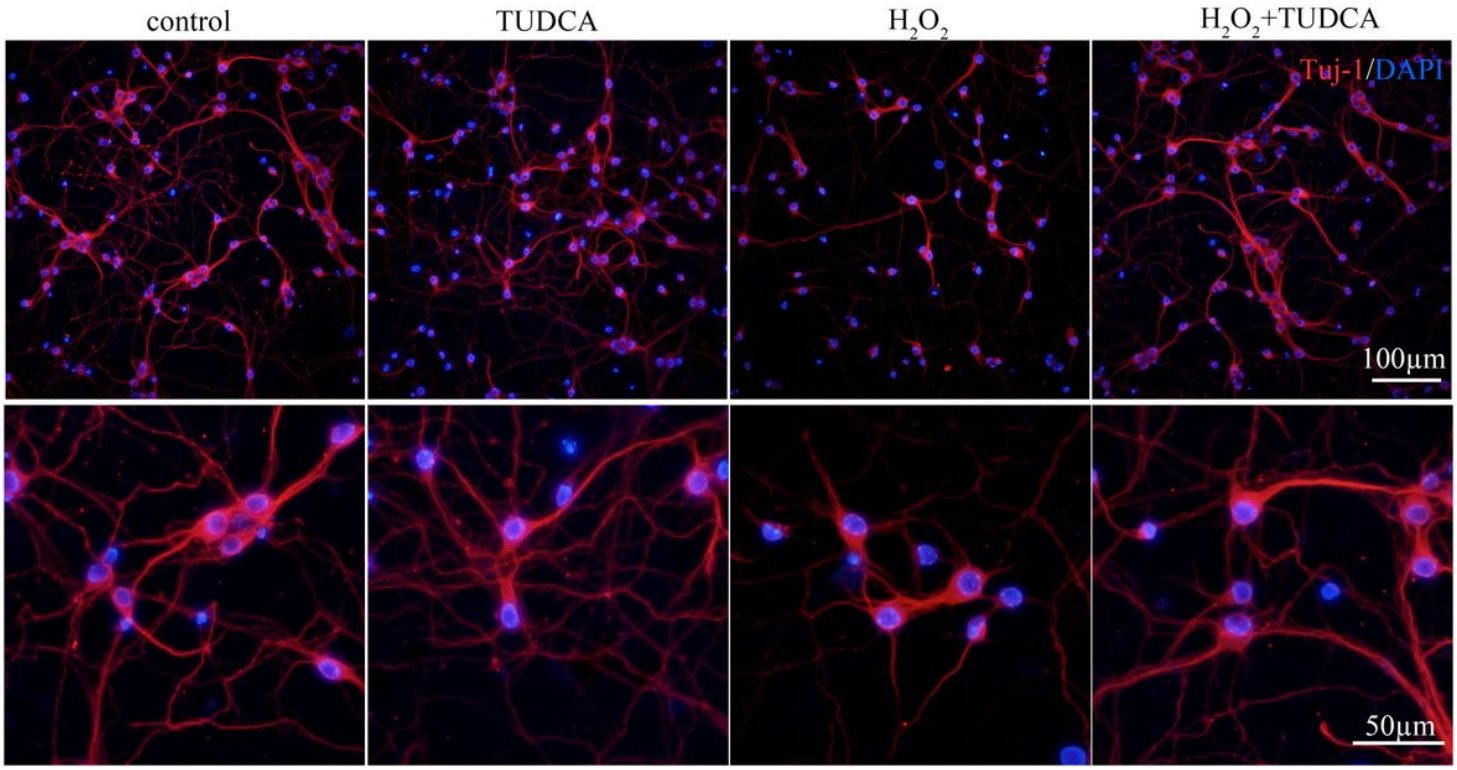

Figure 2

TUDCA alleviated axon degeneration caused by H2O2 exposure. Immunofluorescence images showing the axon labeled with Tuj1 in primary cortical neurons.

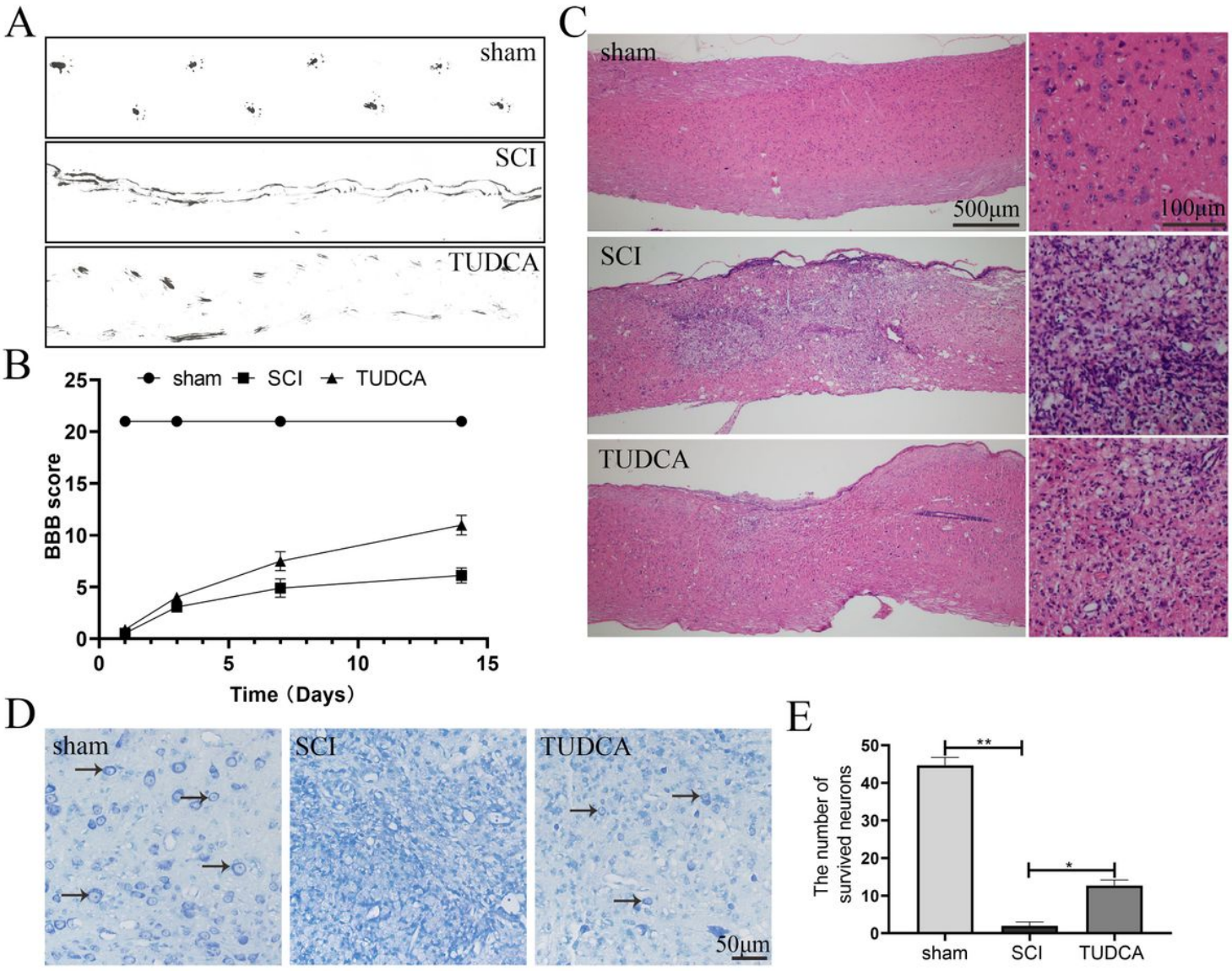

Figure 3

TUDCA improved pathology and motor function after SCI. (A) Footprint analyses of the different groups at 14 days post-injury. (B) The Basso-Beattie-Bresnahan (BBB) locomotion scores of the different groups. (C) Representative images from H&E staining in longitudinal section at 14 days post-injury. (D&E) The survived neurons were stained by Nissl Staining in different groups. All experiments were performed in triplicated and data were presented as means ± S.D, n=4 per group.

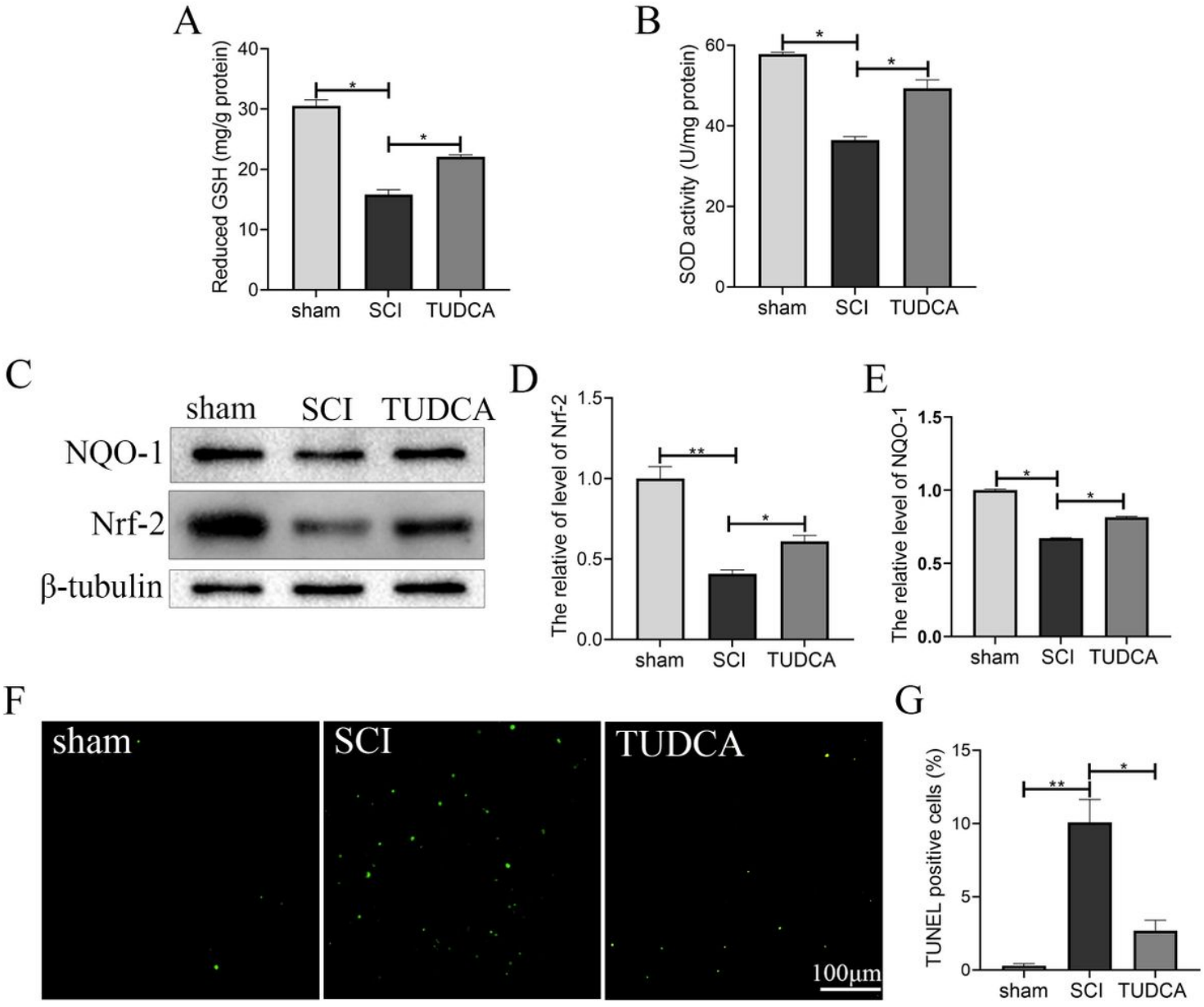

Figure 4

TUDCA exerted neuroprotective effects after SCI through Nrf2/NQO-1 signaling pathway. (A&B) The levels of reduced GSH and SOD activity were measured at day 7 after SCI. (C-E) Western blot analysis and quantification of Nrf2, NQO-1 expression at day 7 after SCI. (F&G) TUNEL was performed to analyze cell apoptosis rate at day 14 after SCI. All experiments were performed in triplicated and data were presented as means ± S.D, n=4 per group.

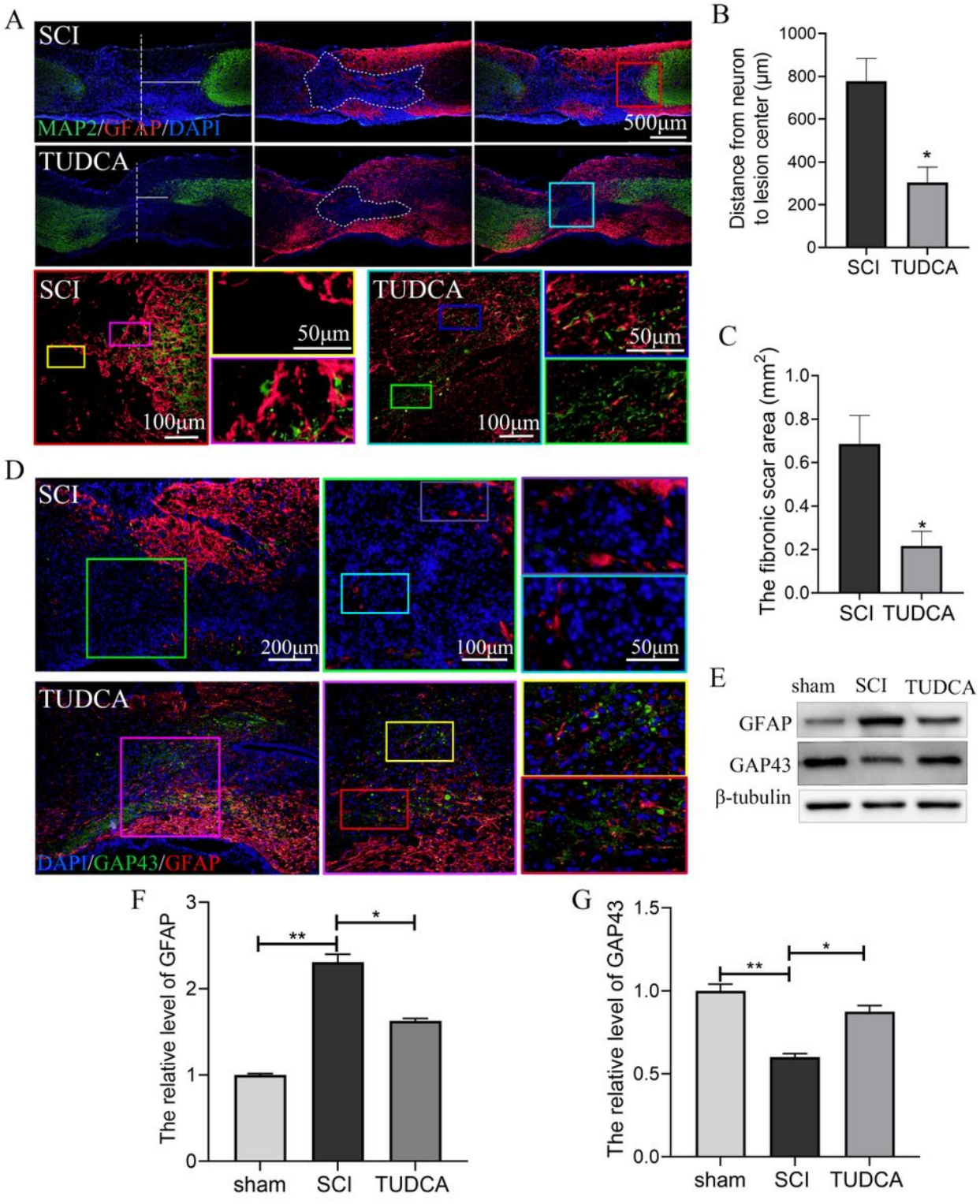

Figure 5

TUDCA promoted axonal regeneration after SCI. (A) Co-immunofluorescence images showed GFAP (red) and MAP2 (green) at day 14 after SCI. (B) Quantification of the distance from neurons to the lesion center from MAP2 immunofluorescence. (C) Quantification of the fibrotic scar surrounding by reactive astrocytes of spinal cord from GFAP immunofluorescence. (D) Co-immunofluorescence images showed the axonal regeneration (GAP43, green) within the GFAP (red). (E-G). Western blot analysis and

quantification of GFAP, GAP43 expression. All experiments were performed in triplicated and data were presented as means ± S.D, n=4 per group.

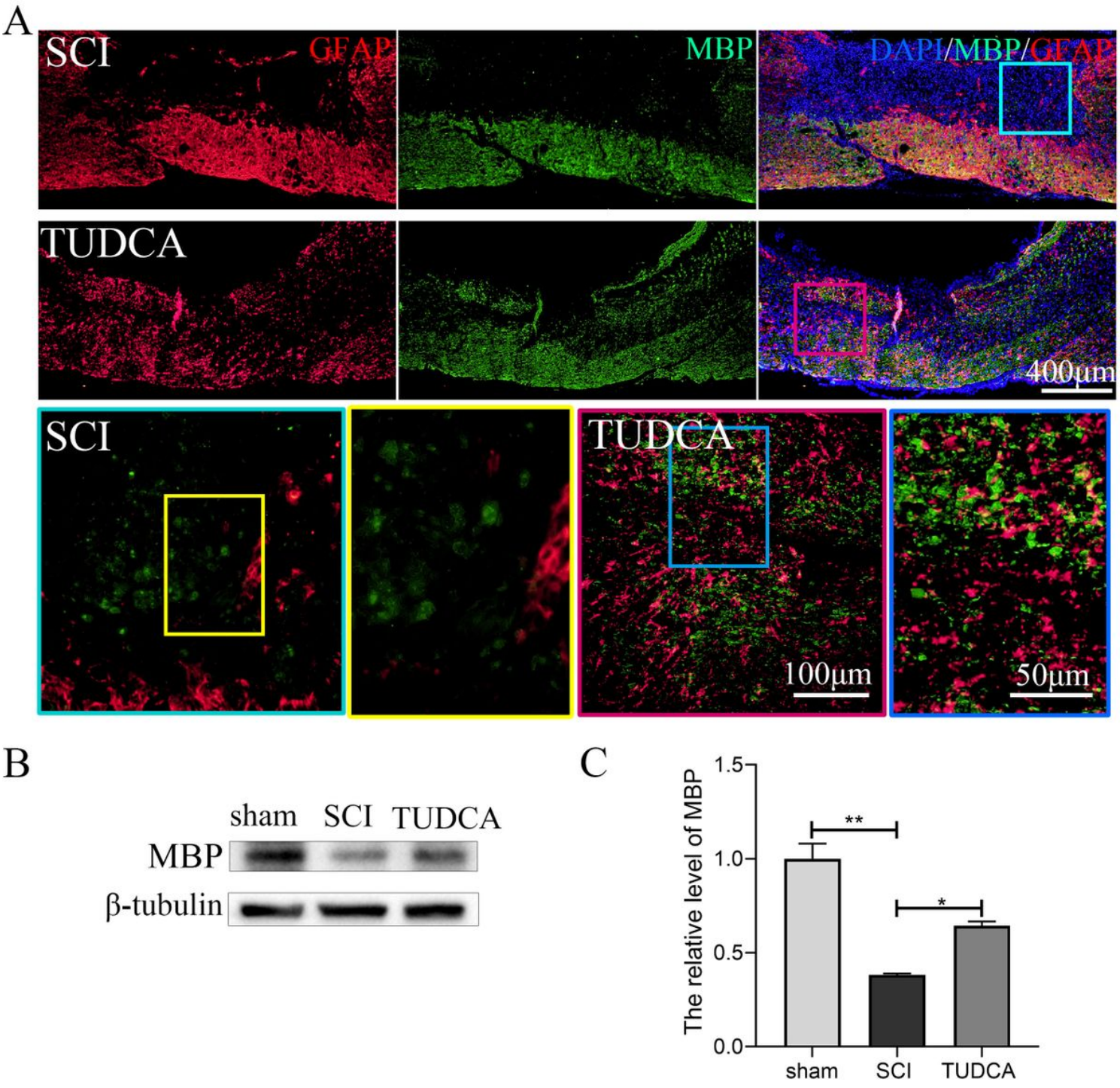

Figure 6

TUDCA treatment promoted remyelination. (A). immunofluorescence images of spinal cord at day 14 after SCI shows the distribution of MBP (green) and GFAP in the lesion site. (B, C) Western blot analysis and quantification data of MBP expression in each group. All experiments were performed in triplicated and data were presented as means ± S.D, n=4 per group.

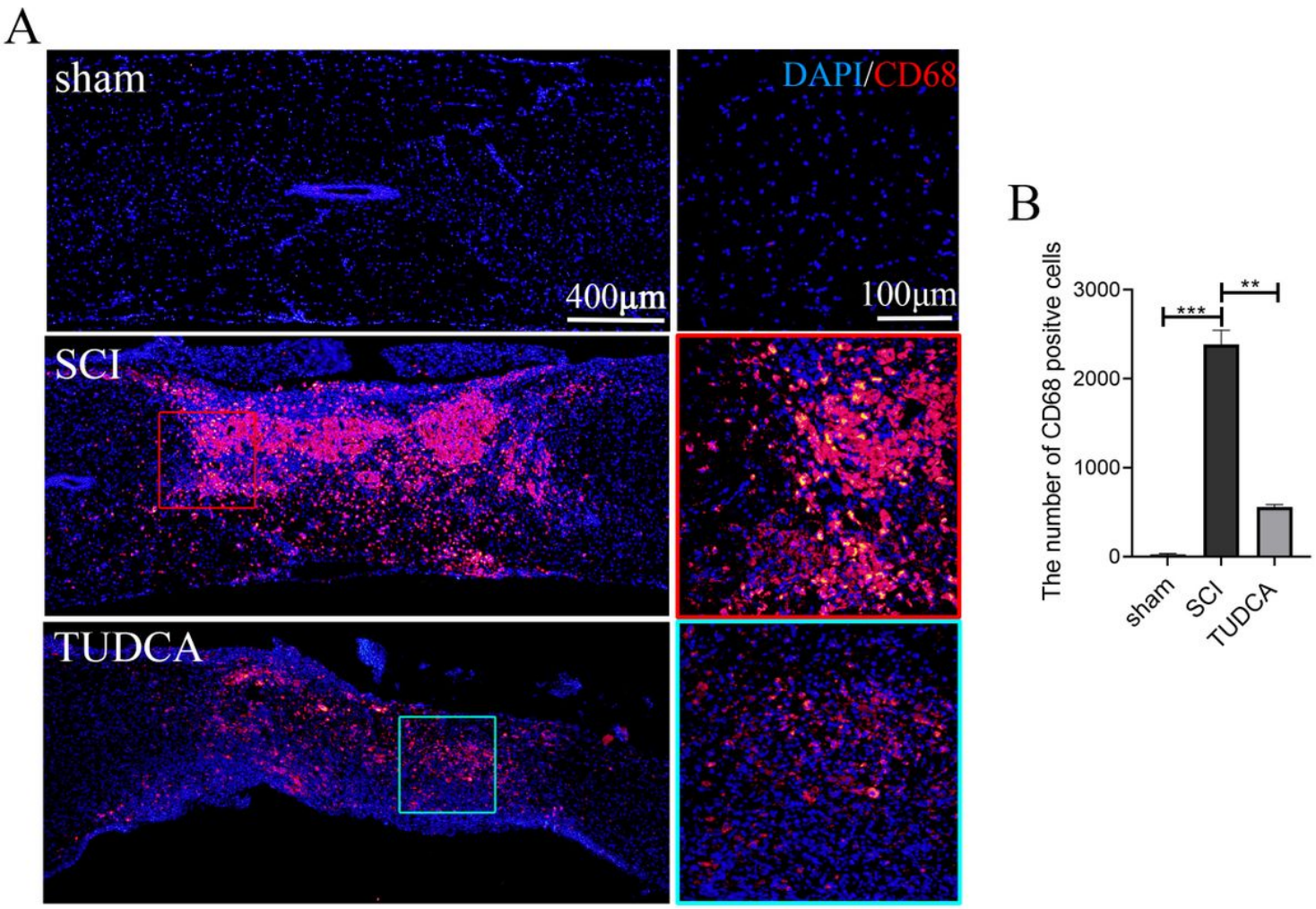

Figure 7

TUDCA treatment reduced the inflammatory reaction. (A) The immunofluorescence images of the spinal cord at day14 after SCI showed the M1 macrophages (CD68 positive). (B) Quantification the number of CD68 positive cells in spinal cord. All experiments were performed in triplicated and data were presented as means ± S.D, n=4 per group.