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Basic Science

Effect of curcumin on the inflammatory reaction and functional recovery after spinal cord injury in a hyperglycemic rat model

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Abstract

BACKGROUND CONTEXT: Curcumin has anti-inflammatory and antioxidant activities.
OBJECTIVE: This study aimed to investigate the effects of curcumin on the histological changes and functional recovery following spinal cord injury (SCI).
STUDY DESIGN: One hundred twenty-eight Sprague-Dawley rats were distributed into a sham, SCI only, SCI-hyperglycemia, and SCI-hyperglycemia-curcumin (200 mg/kg/day, i.p.) groups.
METHODS: SCI was induced using a clip at T9-10 and hyperglycemia was induced by streptozotocin (60–70 mg/kg, i.v.). Plasma malondialdehyde levels and superoxide dismutase activity was measured to determine oxidative stress. The activity of macrophages in the spinal cord after SCI was stained by the anti-CD68 antibody (ED-1). The tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-8 levels were measured by enzyme-linked immunosorbent assay and Western blot was used to verify the levels of mitogen-activated protein kinases and STAT3. The glial fibrillary acidic protein expression was evaluated by immunofluorescence analysis. Functional recovery was assessed according to the Basso, Beattie, and Bresnahan scale and histologic outcome was evaluated by the lesion volume and spared tissue area.
RESULTS: Superoxide dismutase activity increased, the malondialdehyde level decreased, and ED-1 macrophage marker level decreased in the SCI-hyperglycemia-curcumin group than in the SCI-hyperglycemia group at 2 weeks after SCI ($p<.01$). The SCI-hyperglycemia-curcumin group showed a statistically significant reduction in IL-6, IL-8, and TNF-α levels compared with the SCI-hyperglycemia group after SCI. The phosphorylated-extracellular signal-regulated kinase, phosphorylated-JNK, and phospho-p38 levels were significantly lower in the SCI-hypoglycemia-curcumin group than in the SCI-hypoglycemia group. The SCI-hyperglycemia-curcumin group showed a decrease in glial fibrillary acidic protein expression after SCI compared with the SCI-hyperglycemia

FDA device/drug status: Not applicable.

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Disclaimer: None of the authors has any financial interest in the subject under discussion in this paper.

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group. The SCI-hyperglycemia-curcumin group showed a lower lesion volume, higher spared tissue, and better functional recovery than the SCI-hyperglycemia group.
CONCLUSIONS: Curcumin may have a potential neuroprotective effect in SCI with hyperglycemia.
CLINICAL SIGNIFICANCE: Curcumin decreased the inflammatory response and decreased astrogliosis and improved the functional recovery and histologic outcomes in SCI with hyperglycemia.

Keywords: Spinal cord injury; Hyperglycemia; Curcumin; Inflammation; Oxidative stress; Functional recovery; Neuroprotective effect

Introduction

Traumatic spinal cord injury (SCI) causes long-term or permanent neurologic deficits that affect both motor and sensory systems [1]. The pathophysiology of acute SCI is complex and can be divided into primary and secondary injuries. The primary injury immediately disrupts cell membranes, destroys myelin and axons, and damages microvessels, thereby triggering devastating secondary injury. The secondary injury involves active biologic processes and a subsequent series of deleterious processes that lead to apoptosis [2−7]. Reactive species and inflammation play important roles in the pathogenesis of secondary injury after SCI [7,8]. Primary injury is immediate and irreversible, but secondary injury worsens over time and provides an opportunity for therapeutic intervention [3,4,6].

Diabetes mellitus is a systemic disorder that affects multiple organs and systems, including the cardiovascular system, kidneys, peripheral nerves, and retina [9,10]. Previous studies conclude that hyperglycemia can influence the functional recovery after SCI in humans and rats [5,11,12]. Additionally, hyperglycemia was reported to cause changes in the characteristic and cellular response of the microglia, neurons, and astrocytes of the spinal cord [5,11]. Therefore, hyperglycemia can influence the pathophysiology of the spinal cord after SCI [11,13,14]. The importance of hyperglycemic treatment after injury is well-known in the treatment of trauma patients including those with SCI.

Curcumin (1,7-bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) is a nonsteroidal, naturally occurring compound that is an Indian spice and commonly utilized as a dietary pigment. Curcumin has a wide range of pharmacological activities, including anti-inflammatory, antioxidant, anti-infectious, hypocholesterolemic, and anticancerous effects [15−17]. Several studies have reported the neuroprotective effect of curcumin on SCI. Experimental cortical ischemic studies in rats have demonstrated that curcumin inhibits xanthine oxidase and glutathione peroxidases, upregulates superoxide dismutase (SOD) thus decreasing levels of superoxide anions and malondialdehyde (MDA), and preserves cerebral capacity while decreasing neuronal damage [4,18,19]. Curcumin inhibits apoptosis in SCI and quenches astrocyte activation. It also inhibits glial scar formation and inflammation [20,21].

Considering the results of previous studies, we hypothesized that curcumin would have a therapeutic effect on SCI with hyperglycemia. However, little is known about the effect of curcumin on hyperglycemia and SCI. Therefore, we analyzed whether curcumin can have positive effect on the inflammatory process and functional/histologic outcomes after SCI and hyperglycemia.

Materials and methods

SCI models

All animal experiments were performed in accordance with the National Institute of Health guidelines on animal care and approved by the Institutional Animal Care Committee of our institute. The experimental procedures were approved by the committee for Ethics in Animal Experimentation from Kyungpook National University Industry Foundation (28 September 2017). All efforts were made to minimize the number of animals used and animal suffering. Animals were housed in a temperature-controlled room on a 12-hour light/dark circadian cycle. The experimental design is summarized in Fig. 1.

The surgical technique used for SCI in rats has been previously described [22,23]. Rats were anesthetized using an intraperitoneal injection of a mixture of xylazine (10 mg/kg) and ketamine (60 mg/kg). After laminectomy at T9−10, the extradural plane between the dura and the adjacent vertebrae was carefully dissected. A modified aneurysm clip with a closing force of 30 g (Aesculap, Tuttlingen, Germany) was held in the open position using an applicator. The clip was rapidly released from the applicator and applied vertically onto the exposed spinal cord for 2 minutes of compression. For the sham controls, the same surgical procedure was followed, but clip compression was not applied. Postoperatively, the muscle, fascia, and skin were sutured using 4-0 silk sutures. The rectal temperature was maintained at 37.0˚C ± 0.5˚C by a thermostatically regulated heating pad during surgery, and animals were placed overnight in a temperature-controlled and humidity-controlled chamber during recovery. To reduce postsurgery isolation-induced stress, rats were housed in pairs at an ambient temperature of 22˚C−25˚C in an alternating 12-hour light/dark cycle. Bladders were manually emptied twice daily until spontaneous voiding occurred (usually within 7−10 days).

Streptozotocin-induced hyperglycemic rat model and curcumin treatment

Female Sprague-Dawley rats weighing 250−320 g were used. Hyperglycemia was induced by a single intravenous

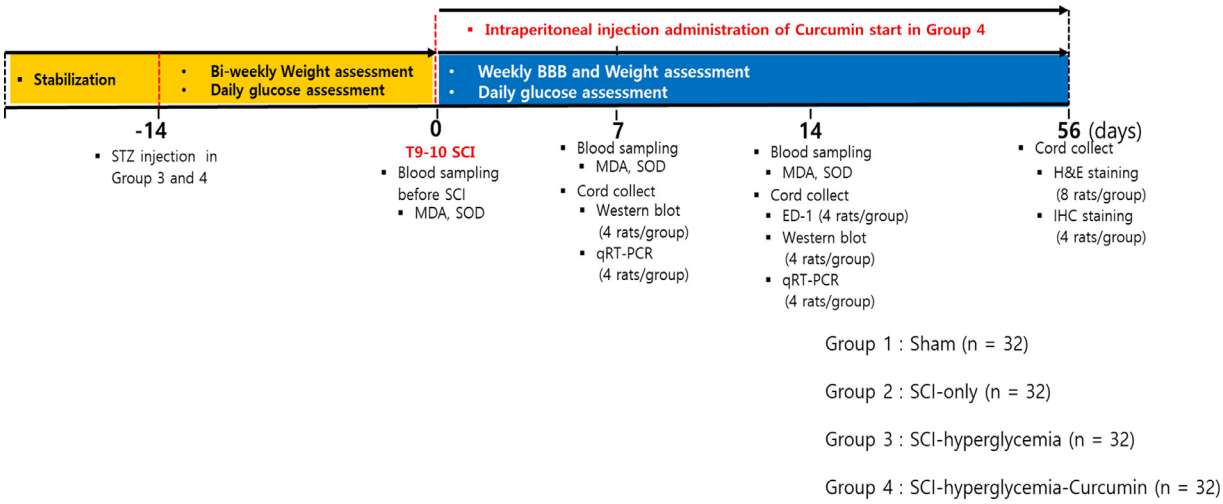

Fig. 1. Experimental animal design. The spinal cord injury (SCI)-hyperglycemia-curcumin group was treated after the SCI group started a curcumin injection after SCI. STZ, streptozotocin; MDA, malondialdehyde; SOD, superoxide dismutase; qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction; ED-1, anti-CD68 antibody; H&E, hematoxylin and eosin; IHC, immunohistochemistry.

injection of 60–70 mg/kg of streptozotocin (dissolved in a 0.1-mM citrate buffer [pH 4.5] immediately before use) under light ether anesthesia, and an equal volume of citrate buffer was administered to the sham rats at 2 weeks before the SCI model. The curcumin dose (200 mg/kg/day for 8 weeks) was similar to doses used in previous studies [4], and the drug was dissolved in 1.0-mL dimethyl sulfoxide before intraperitoneal injection. Changes in glucose concentration and weight in each group are summarized in Table 1. According to Fig. 1, blood sampling for MDA and SOD (0, 7, and 14 days), Western blot (7 and 14 days), immunofluorescence staining (14 days), real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR; 7 and 14 days), and hematoxylin and eosin staining (56 days) were performed.

Plasma biochemical markers for antioxidation

At 1 and 2 weeks after SCI, blood samples were collected by subclavian puncture. Plasma MDA levels and the sensitive biomarkers for lipid peroxidation (LP) were estimated using the NW LSS NW KMDA01 assay (Northwest Life Science Specialties, Vancouver, WA, USA), and activity is expressed as micromoles per gram of protein. SOD is a class of enzymes that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide, which results in a decreased MDA level. SOD activity was determined using a SOD assay kit (catalog number: 706002-Cayman Chemical, Ann Arbor, MI, USA). SOD activity is expressed as units per gram of protein.

Immunofluorescence staining and quantification of ED-1 and glial fibrillary acidic protein

After SCI, four rats from each of the four groups were euthanized and intracardially perfused with 4% paraformaldehyde in a 0.1-M sodium phosphate buffer (PB, pH = 7.4). The serial coronal sections through the rostral-caudal axis of the spinal cord were sectioned at a thickness of 5 μm on a cryostat from four rats per group after SCI. Every 30th cross-section from both the rostral and caudal sides (3 mm from the epicenter) was floated on the surface of the 0.1-M PB. To detect ED-1 (a marker for anti-CD68 antibody macrophages) and glial fibrillary acidic protein (GFAP) (a marker for gliosis), spinal cord sections were blocked with 4% normal serum in 0.5% Triton X-100 for 1 hour at room temperature, incubated overnight at 4°C with a 1:500 dilution of mouse monoclonal anti-rat ED-1 (Serotec, Oxford, UK) and a mouse monoclonal anti-mouse (1:200; Abcam, Cambridge, UK). The sections for detecting ED-1 were incubated in a 1:200 dilution of biotinylated anti-mouse immunoglobulin (Ig)-G (Sigma-Aldrich, St. Louis, MO, USA) and a 1:200 dilution of anti-rabbit IgG (Vector Laboratory, Burlingame, CA, USA) in a 0.1-M PB containing 4% normal serum and 0.5% Triton X-100 at 25°C for 2 hours. Then, the sections were incubated in a Vectastain ABC-HRP Kit (Peroxidase, Standard, Vector Laboratory) for 1.5 hours. Finally, staining was visualized through the reaction with 3,3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide in 0.25-M Tris for 1–3 minutes using a diaminobenzidine tetrahydrochloride reagent set (Kirkegaard & Perry, Gaithersburg, MD, USA). The labeled tissues were photographed using a Zeiss Axiopan microscope with high-powered differential interference contrast (DIC) optics (Carl Zeiss, Thornwood, NY, USA). For comparison, we randomly designed four rectangular areas (250 × 250-μm fields) to count the labeled cells in 128 sampled areas in both the gray and white matters, respectively. The Labworks, version 4.5, computer-assisted image analyzer (UVP, Upland, CA, USA) was used for the enumeration of immune-positive cells. The section for detecting GFAP was incubated for 2 hours with Alexa Fluor 594 goat anti-mouse IgG and Alexa Fluor 488 goat anti-rabbit IgG (both 1:300; Invitrogen, Grand Island, NY, USA).

Table 1
The changes of body weight, food intake, and serum glucose concentration in rats of the sham, SCI only, SCI-hyperglycemia and SCI-hyperglycemia-curcumin groups.

	Sham group	SCI only group	SCI-hyperglycemia group	SCI-hyperglycemia-curcumin group
Body weight (g)				
Initial	264.5 ± 8.3	267.3 ± 7.2	270.1 ± 8.3	268.3 ± 7.4
Final	320.5 ± 11.2	322.7 ± 10.3	254.5 ± 6.9 (**)	258.4 ± 7.3
Food intake (g/day)	25.4 ± 2.6	26.4 ± 3.2	24.7 ± 2.3	25.3 ± 2.1
Blood glucose level (mg/dL)				
Baseline (before STZ injection)	104.4 ± 4.9	106.3 ± 5.1	104.5 ±5.0	107.3 ± 4.6
After STZ injection	N/A	N/A	403.9 ± 12.5 (**)	411.3 ± 14.1 (**)

SCI, spinal cord injury, STZ, streptozocin, N/A, not available
**, p<.01 compared with the sham group.

The nuclei were stained with 4′,6-diamidino-2-phenylindole, and sections were washed with phosphate-buffered saline and mounted with a specific medium (Dako Cytomation, Milan, Italy). We randomly designated four rectangular areas (200 × 200 μm) for the quantification of the GFAP intensities in × 400 magnifications. The area of the captured images under × 400 magnification (200 × 200 μm) measured 1,849,600 (1360 × 1360) pixels. The stained immunofluorescence pixels per image were calculated and quantified using an intensity measurement method available in the ImageJ software package (National Institutes of Health, Bethesda, MD, USA) [24].

qRT-PCR

Segments of the spinal cord (10 mm) including the epicenter were collected at 1 and 2 weeks after SCI. Ribonucleic acid (RNA) quantity and purity were measured using a Nano Drop ND-1000 Spectrometer (Nano Drop Technologies, Wilmington, DE, USA) and an Eppendorf biophotometer (Eppendorf, Hamburg, Germany). Complementary DNA was made from 1 μg of RNA using a Prime Script RT reagent kit (Takara, Otsu, Shiga, Japan). qRT-PCR was performed using a SYBR Green Master Mix, and the detection of mRNA was analyzed using an ABI Step One Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The primers were purchased from Bioneer (Daejeon, Korea) (Supplementary Table S1). PCR amplifications were performed at 95°C for 30 seconds, followed by 45 cycles of thermal cycling at 95°C for 5 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. Melt curves were developed on completion of the cycles to remove nonspecific products. The relative expression levels of tumor necrosis alpha (TNF-α), interleukin (IL)-6, and IL-8 were normalized using the 2-ΔΔCT method [25]. These experiments were repeated three times.

Western blot analysis

Segments of the spinal cord (10 mm) including the epicenter at the middle were collected at 1 and 2 weeks after SCI. The thoracic spinal cord was rapidly dissected and then immediately frozen in liquid nitrogen. Frozen tissue was mixed with a RIPA buffer (25-mM Tris-Cl, pH 7.6; 150-mM NaCl; 1% NP-40, 1% sodium deoxycholate; 0.1% sodium dodecyl sulfate) containing a protease inhibitor cocktail (Roche, Mannheim, Germany) immediately after homogenization. The homogenate was centrifuged at 13,000 rpm for 30 minutes at 4°C, and the supernatants were determined using the bicinchoninic acid protein assay (Sigma-Aldrich). Proteins were separated by a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel and transferred to a nitrocellulose membrane. The membrane was immediately placed in a blocking solution (5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween-20) for 1 hour at room temperature. The membrane was incubated with a 1:1,000 dilution of mouse monoclonal antiphospho-ERK (Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit polyclonal anti-p-p38 (Cell Signaling, Danvers, MA, USA), mouse monoclonal antiphospho-JNK (Santa Cruz Biotechnology), rabbit monoclonal antiphospho-stat3 (Abcam), and mouse monoclonal anti-β-actin (Sigma-Aldrich) overnight at 4°C, and then with a horseradish peroxidase-conjugated secondary antibody for 1 hour at room temperature. The proteins were detected with a chemiluminescence reagent. Immune-positive bands used Image J, version 1.46r, a computer-assisted image analyzer (National Institutes of Health). These experiments were repeated three times.

Functional outcome measurement

The rats were tested for functional deficits at pre-SCI, 1 day, 3 days, and each week for 8 weeks after SCI using the open field locomotor rating scale developed by Basso, Beattie, and Bresnahan (BBB score, 0∼21 points) [26]. Two evaluators were unaware of the group allocations, and they observed functional scores of each hind limb for 1 minute. Functional scores were recorded and averaged.

Tissue preparation and quantification of the lesion volume and spared tissue area

At 8 weeks after SCI, eight rats from each of the four groups were euthanized. Following decapitation, a 1.5-cm

segment of the spinal cord centered at the injury site was immediately harvested from the vertebral canal and postfixed in 10% formalin overnight. The serial coronal sections through the rostral-caudal axis of the spinal cord (5 μm) were collected at lengths of 100 μm each, and the epicenter and both rostral and caudal 30th (3 mm) and 60th (6 mm) sections from the epicenter were stained with a hematoxylin and eosin solution (BBC Biochemical, Mount Vernon, USA). The slides were examined and photographed using a Zeiss Axioplan microscope (Carl Zeiss) [22,23] with high-powered DIC optics. The area of cavitation and total spared tissue area of each section were calculated and quantified using ImageJ software (National Institutes of Health). The total cavity volume was calculated by summation of the measured cavity area of each section multiplied by the intersection distance.

Statistical analysis

For multiple comparisons, enzyme-linked immunoassay, densitometry, and qRT-PCR were performed. To analyze differences in the BBB scores between the groups over time, a repeated-measures analysis of variance with post-hoc Tukey's test was performed. The values for groups are presented as the average ± standard error of mean, and significance was accepted for p values <.05. All statistical comparisons were computed using SPSS 19.0 (SPSS, Chicago, IL, USA).

Results

Curcumin increased SOD activity and decreased the MDA level after SCI

SOD activities were significantly lower in the SCI-hyperglycemia group than in the sham and SCI only groups at 1 and 2 weeks after SCI (p<.01). However, the SCI-hyperglycemia-curcumin group showed a statistically significant increase in SOD activity compared with the SCI-hyperglycemia group at 2 weeks (p<.01). Significant differences in MDA levels appeared after SCI. The MDA levels were significantly elevated in the SCI-hyperglycemia group than in the SCI only group at 2 weeks. However, the SCI-hyperglycemia-curcumin group showed a statistically significant decrease in MDA levels compared with the SCI-hyperglycemia group at 2 weeks (p<.01; Fig. 2).

Curcumin decreased the anti-CD68 antibody macrophage marker (ED-1) in the spinal cord at 1 and 2 weeks after SCI

To investigate the activity of macrophages in the spinal cord after SCI, CD68, a protein highly expressed by cells in the monocyte lineage or macrophages, was stained by the anti-CD68 antibody (ED-1). The SCI-hyperglycemia group showed higher densities of ED-1 macrophages than the SCI only group. However, the SCI-hyperglycemia-curcumin group had a significantly lower density of ED-1 macrophages

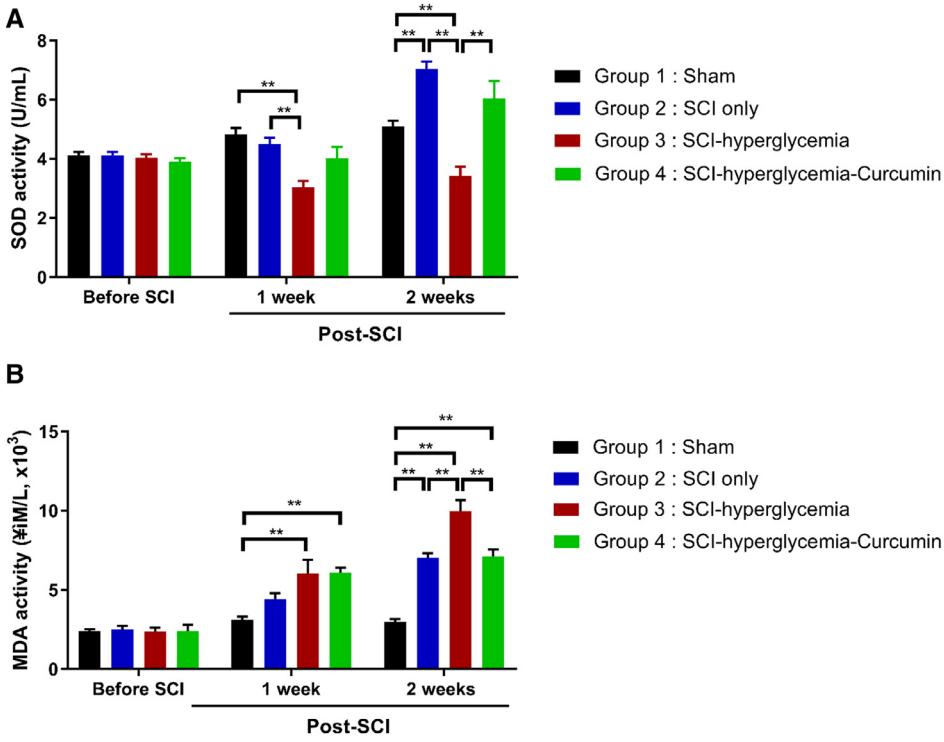

Fig. 2. The superoxide dismutase (SOD) activity (A) and malondialdehyde (MDA) level (B) after spinal cord injury (SCI). * p<.05, ** p<.01.

in white and gray matters than the SCI-hyperglycemia group at 1 and 2 weeks after SCI (p<.01; Fig. 3).

Curcumin decreased the messenger RNA gene expression of IL-6, IL-8, and TNF-α at 1 and 2 weeks after SCI

To investigate the effect of hyperglycemia and curcumin on the inflammatory response in messenger RNA (mRNA) after SCI, qRT-PCR was performed. In the SCI-hyperglycemia and SCI-only groups, the expressions of IL-6, IL-8, and TNF-α were increased at 1 and 2 weeks after SCI. In contrast, the SCI-hyperglycemia-curcumin group showed a statistically significant reduction in expressions of IL-6 and IL-8, compared with the SCI-hyperglycemia group after SCI (p<.05; Fig. 4).

Curcumin decreased the extracellular signal-regulated kinase, JNK, and phospho-p38 in mitogen-activated protein kinases on hyperglycemia in SCI

We examined the inflammatory signal pathways such as mitogen-activated protein kinases including extracellular signal-regulated kinase (ERK), JNK, phospho-p38 (p-p38), and STAT3 using Western blot assays at 1 and 2 weeks after SCI with hyperglycemia (Fig. 5). The levels of p-ERK were 9.02 ± 0.58 and 10.73 ± 0.48 at 1 and 2 weeks, respectively, in the SCI-hyperglycemia group, and they were significantly higher than those in the SCI only groups (1.90 ± 0.11 at 1 week and 3.04 ± 0.21 at 2 weeks) (p<.01). However, the levels of p-EPK were 7.11 ± 0.32 and 6.09 ± 0.21 at 1 and 2 weeks, respectively, in the SCI-hypoglycemia-curcumin group, and they were significantly

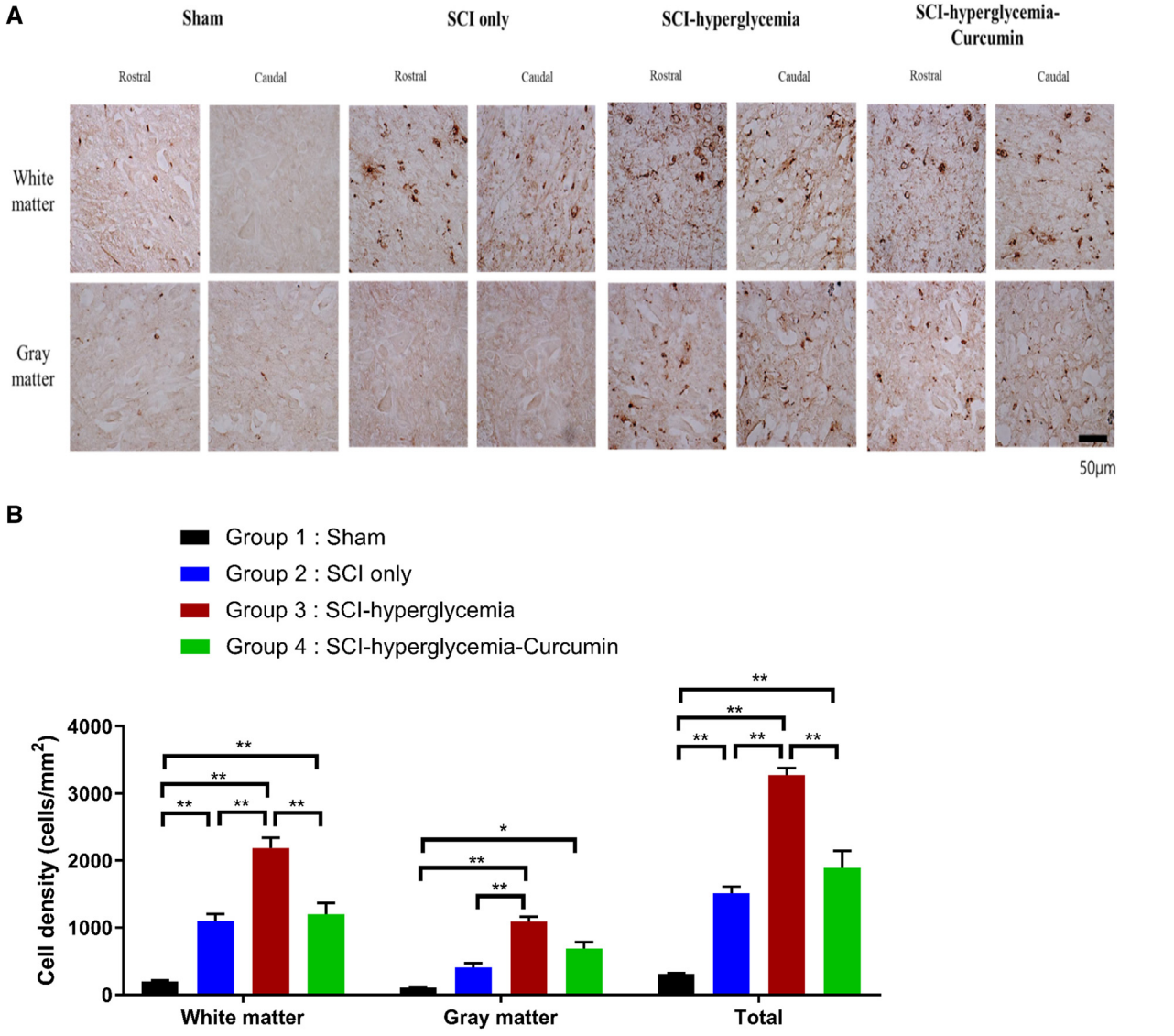

Fig. 3. Densities of the anti-CD68 antibody (ED-1) in immunoreactive cells of the spinal cord (A), Bar graphs show the cell density of ED-1 at 2 weeks after SCI. SCI, spinal cord injury. * p<.05, ** p<.01.

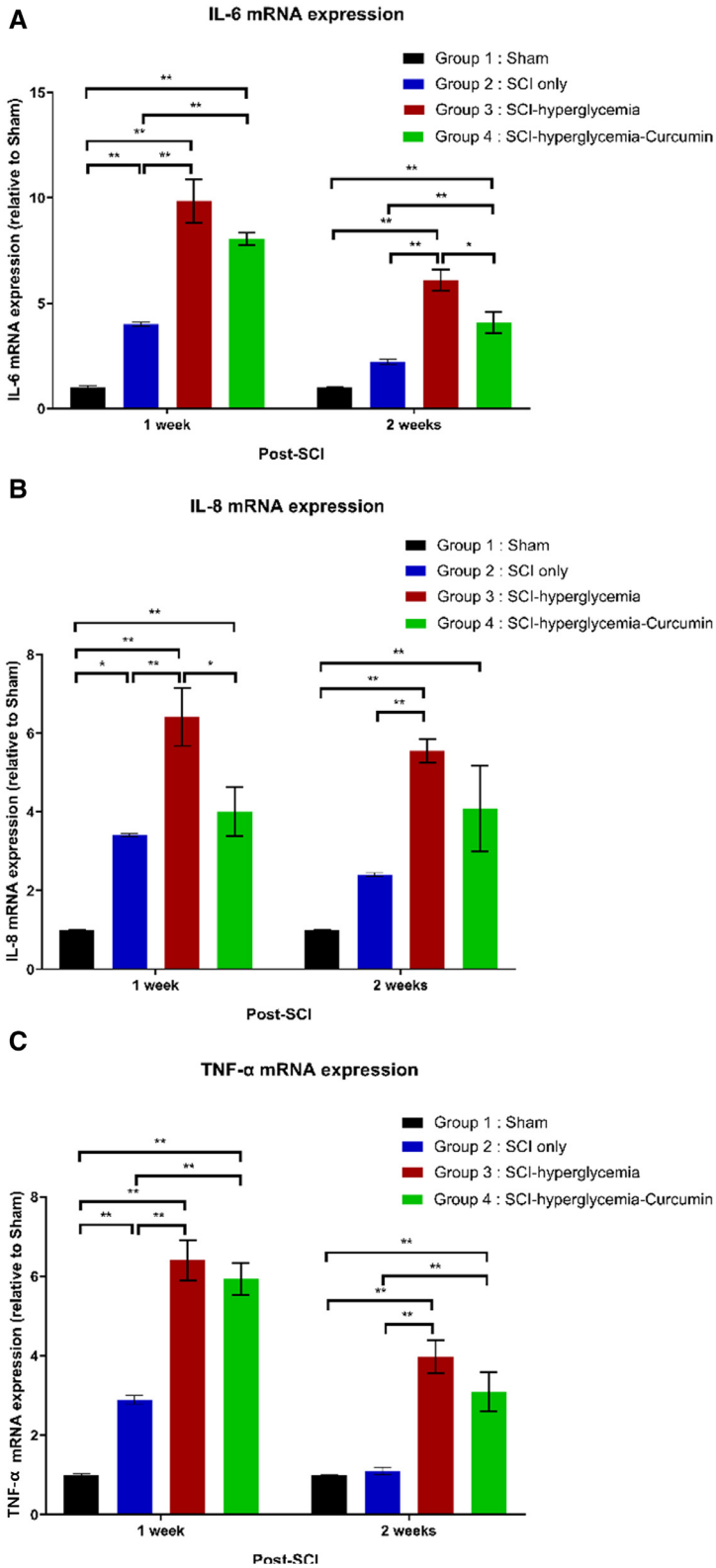

Fig. 4. The messenger RNA (mRNA) expression levels of interleukin (IL)-6 (A), IL-8 (B), and tumor necrosis factor (TNF)-α (C) at 1 and 2 weeks after spinal cord injury (SCI). * p<.05, ** p<.01.

decreased in the SCI-hypoglycemia group (p<.01). The levels of p-JNK were 7.84 ± 0.15 and 6.04 ± 0.21 at 1 and 2 weeks, respectively, in the SCI-hyperglycemia group, and they were significantly higher than those in the SCI only group (4.40 ± 0.20 at 1 week and 2.39 ± 0.13 at 2 weeks) (p<.01). However, the levels of p-JNK in the SCI-hypoglycemia-curcumin group were 0.51 ± 0.20 and 2.19 ± 0.21 at 1 and 2 weeks, respectively, and they were significant decreased in the SCI-hypoglycemia group (p<.01). The levels of p-p38 were 9.11 ± 0.28 and 12.64 ± 0.50 at 1 and 2 weeks, respectively, in SCI-hyperglycemia, and they were significantly higher than those in the SCI only group (7.09 ± 0.39 at 1 week and 4.19 ± 0.33 at 2 weeks; p<.01). However, the levels of p-p38 in the SCI-hyperglycemia-curcumin group were 8.11 ± 0.76 and 6.44 ± 1.03 at 1 and 2 weeks, respectively, and they significantly decreased in the SCI-hyperglycemia group (p<.01).

Curcumin decreased the glial fibrillary acidic protein expression via the STAT3 pathway in SCI with hyperglycemia

To determine whether the effect of curcumin on hyperglycemia in SCI increases the *GFAP* expression, we performed western blot assays of p-STAT, which is well known to have a key role in the signaling pathway for astrogliosis [27], at 1 and 2 weeks, and immunohistochemistry staining for *GFAP* at 8 weeks after SCI. The levels of p-STAT were 7.43 ± 0.11 and 13.28 ± 0.40 at 1 and 2 weeks, respectively, in the in SCI-hyperglycemia group,

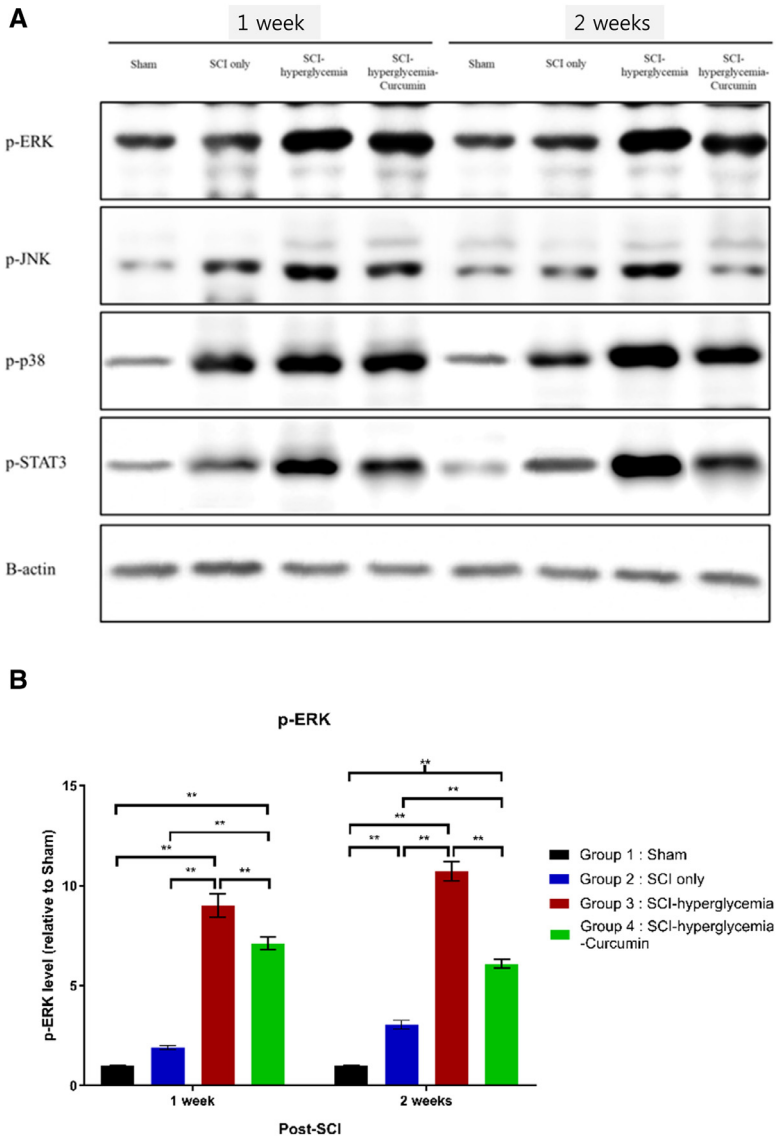

Fig. 5. The effect of hyperglycemia and curcumin on the phosphorylation of MAP kinases and STAT3. The bar graphs shows activation of the extracellular signal-regulated kinase (ERK) (B), JNK (C), p-p38 mitogen activated protein (MAP) kinases (D) and STAT3 (E) at 1 and 2 weeks after spinal cord injury (SCI). * p<.05, ** p<.01.

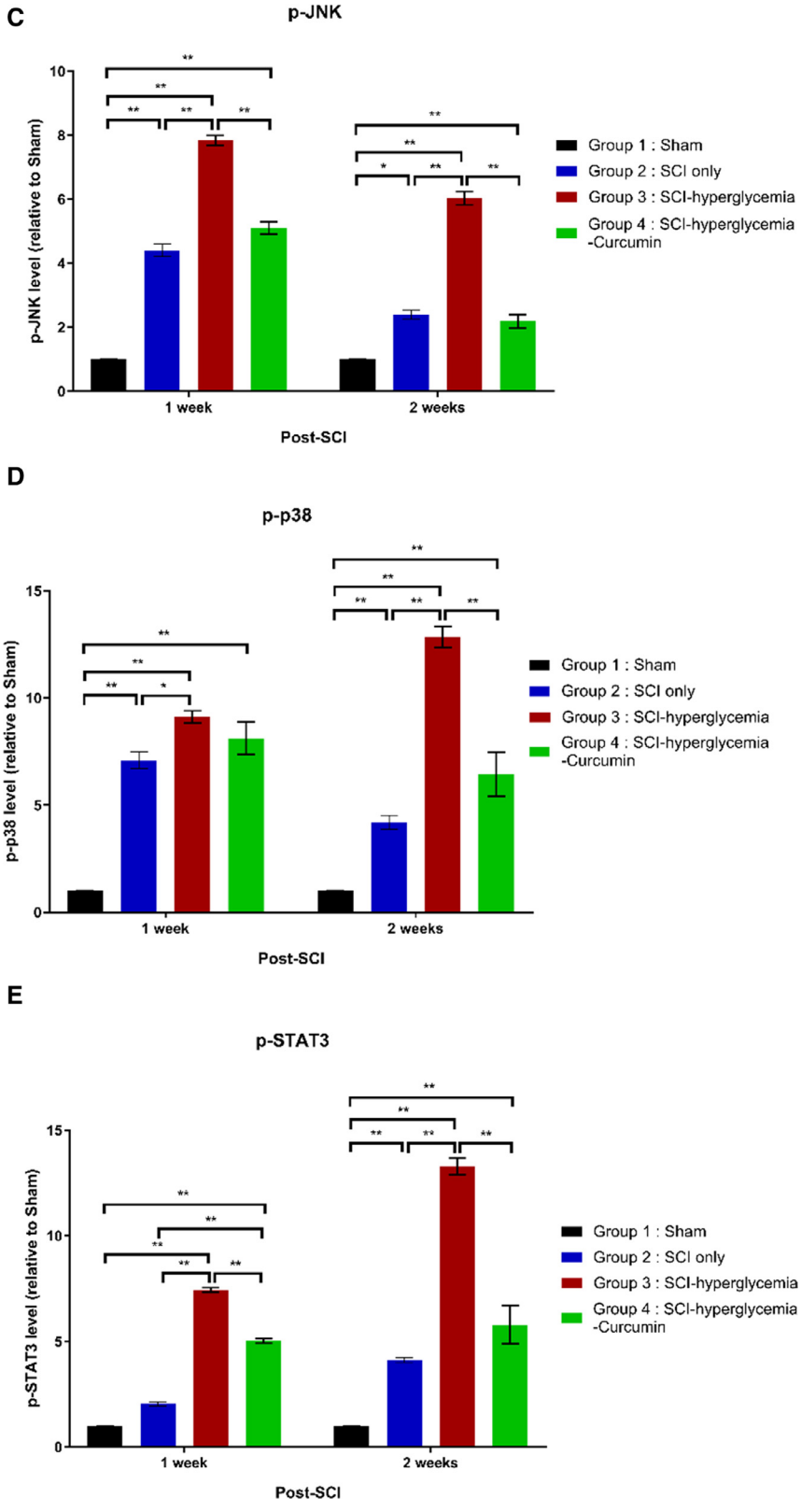

Fig. 5. Continued

and they were significantly higher than those in the SCI only groups (2.04 ± 0.09 at 1 week and 4.11 ± 0.11 at 2 weeks; (p<.01). However, the levels of p-STAT were 5.02 ± 0.11 and 5.78 ± 0.89 at 1 and 2 weeks, respectively, in the SCI-hyperglycemia-curcumin group, and they were significantly decreased in the SCI-hyperglycemia group (p<.01; Fig. 6). Additionally, the *GFAP* expression was significantly higher in the SCI-hyperglycemia group (938,574 ± 40,031) than in the SCI only group (459,834 ± 20,934) and SCI-hyperglycemia-curcumin group (70,3945 ± 49,024) (p<.01, Fig. 5). These results indicate that curcumin decreases astrogliosis after SCI via the STAT3 signaling pathway.

Curcumin influenced functional recovery in SCI with hyperglycemia

According to the BBB scores, the SCI-hyperglycemia group had lower functional recovery than the SCI only group. However, the SCI-hyperglycemia-curcumin group showed statistically significant functional recovery from 6 weeks after SCI compared with the SCI-hyperglycemia group (Fig. 7).

Curcumin influenced the histologic outcomes after SCI

To investigate the effect of hyperglycemia and curcumin on the spared tissue area and lesion volume in the spinal cord after SCI, the rats were sacrificed at 8 weeks after SCI. The SCI-hyperglycemia group showed a higher lesion volume and lower spared tissue area than the sham and SCI only groups. Additionally, the SCI-hyperglycemia-curcumin group showed a lower lesion volume and higher spared tissue area than the SCI-hypoglycemia group (Fig. 8). These results were consistent with the functional outcomes.

Discussion

Traumatic SCI typically results in permanent neurological deficits in the motor and sensory systems [1]. Many of the pathological changes after SCI are secondary to the initial damage, and they involve active biological processes, including local inflammation, generation of free radicals, and high-oxidative stress [2–4,6]. Many researchers have suggested that the degree of impairment following SCI may be reduced by early neuroprotective intervention immediately after SCI. Thus, this has been the subject of much research recently.

In the SCI rat model, our results indicate this was caused by poor functional recovery by increasing inflammation and astrogliosis. However, in hyperglycemia, curcumin promoted functional recovery significantly. This neuroprotective effect was caused by inhibiting inflammation and alleviating astrogliosis.

Curcumin, which has been used for centuries as a dietary spice and as a traditional Indian medicine that inhibits active nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases/extracellular signal regulated kinase, resulting in an anti-inflammatory effect [24]. Curcumin reduce astrogliosis in SCI and significantly decreased the expression of IL-1β and nitric oxide, as well as the number of Iba1$^+$ inflammatory cells at the lesion site [20]. Additionally, curcumin inhibited neuronal loss and apoptosis, and its neuroprotective effect was present in SCI [21]. However, there are few studies regarding the therapeutic potential of curcumin in hyperglycemia after SCI.

Major secondary deleterious mechanisms after SCI include damage of the cell membrane by molecular oxygen, which is involved in the process of lipid peroxidation. In this process, free radicals take electrons from the lipids, generally in the cell membranes, and this causes cell damage [2,4,8]. LP forms reactive aldehydes such as MDA, therefore, MDA is widely used as an indicator of LP. SOD is an enzyme that catalyzes the dismutation of superoxide radicals into ordinary molecular oxygen or hydrogen peroxide. Lin et al. reported that curcumin reduced MDA level and decreased SOD activity in the spinal ischemic/perfusion model, thereby improving neurologic function as cell apoptosis is reduced [18]. In another study, curcumin enhanced glutathione and SOD activity in the brain cortex and hippocampus [25]. According to our results, the MDA level was significantly increased after SCI, while SOD activity was decreased. However, the MDA level was significantly decreased, and SOD activity increased in the SCI-hyperglycemia-curcumin group. These findings suggest that curcumin increases oxidation and decreases the antioxidant defense mechanism, which results in the persistence of antioxidant activities for a prolonged period in the hyperglycemia state.

Inflammatory reaction is one of the main secondary deleterious mechanisms [2,3,22,26]. Uncontrolled hyperglycemia showed a 3.7-6-fold increased microglial activation, 54%–95% reduction in the number and activation of astrocytes, more than ninefold increase in neuronal and glial apoptosis, and a 1.5-2-fold increase in damaged neurons in the hippocampus and frontal cortex. Hyperglycemia may play a main causal role in activation of microglia [27]. Additionally, hyperglycemia increases microglia activity and expands the response of inflammatory cytokines such as TNF-α, IL-6, and IL-8 [11]. Curcumin dose-dependently inhibited M1 macrophage polarization and the production of TNF-α, IL-6, and IL-12B. Furthermore, curcumin significantly inhibited the phosphorylation of ERK, JNK, p-p38, and NF-κB [28]. In our study, curcumin reduced the inflammatory reaction though various pathways in the SCI-hyperglycemia state.

Astrogliosis, a cellular response characterized by massive activation of astrocytes is a pathological hallmark in several neurodegenerative diseases, and recognized by the upregulation of GFAP [29]. A dominant feature of astrogliosis is cellular hypertrophy with an attendant accumulation of GFAP-enriched intermediate filaments. Inhibition of STAT3 activation prevents the development of neuroinflammation by blocking lymphocyte adhesion and

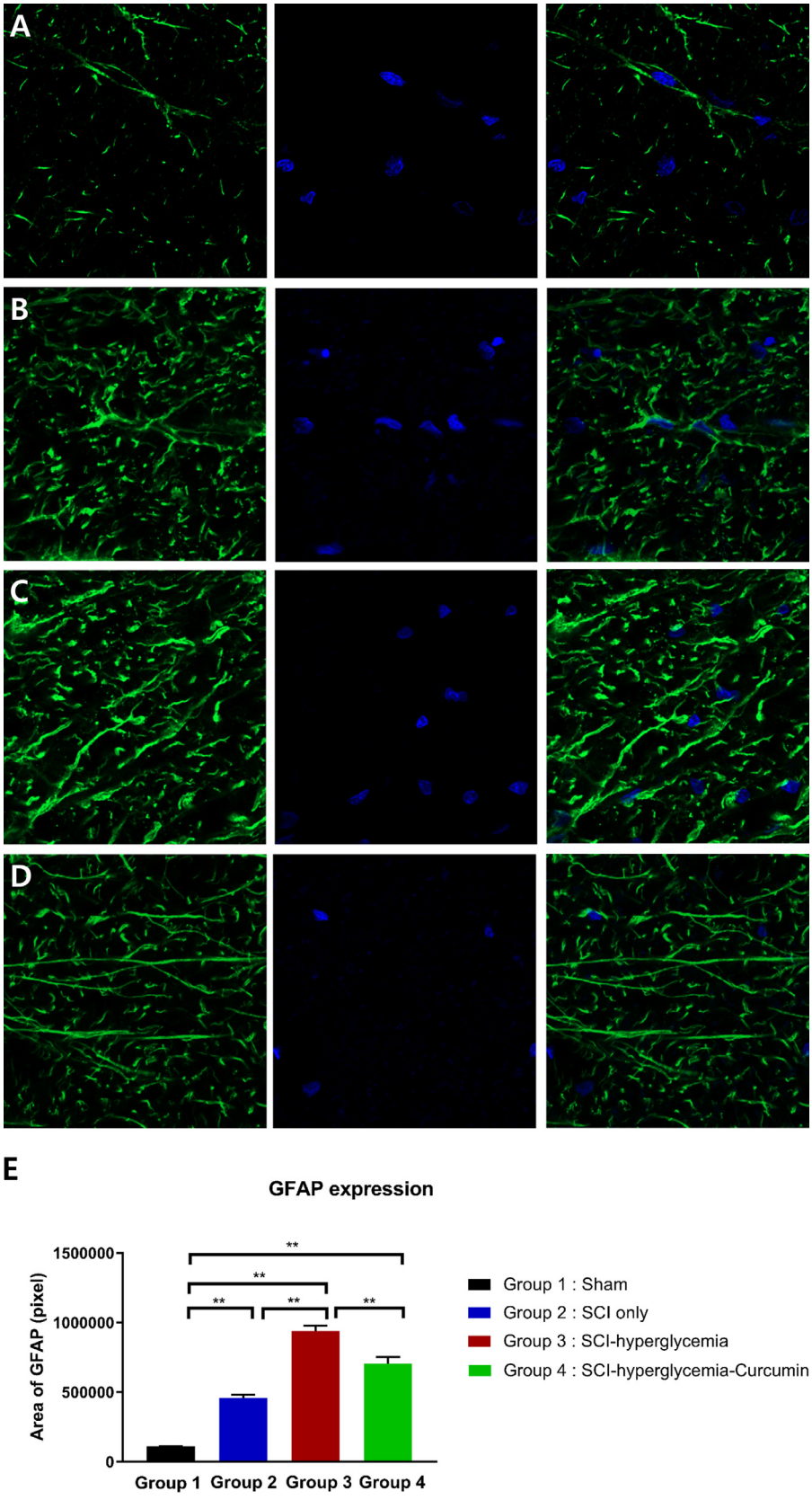

Fig. 6. The expression of glial fibrillary acidic protein (GFAP) after SCI. Sham group (A), spinal cord injury (SCI) only group (B), SCI-hyperglycemia group (C), and SCI-hyperglycemia-curcumin group (D). Bar graphs show the expression of GFAP at 1 and 2 weeks after SCI. SCI, spinal cord injury. * p<.05, ** p<.01.

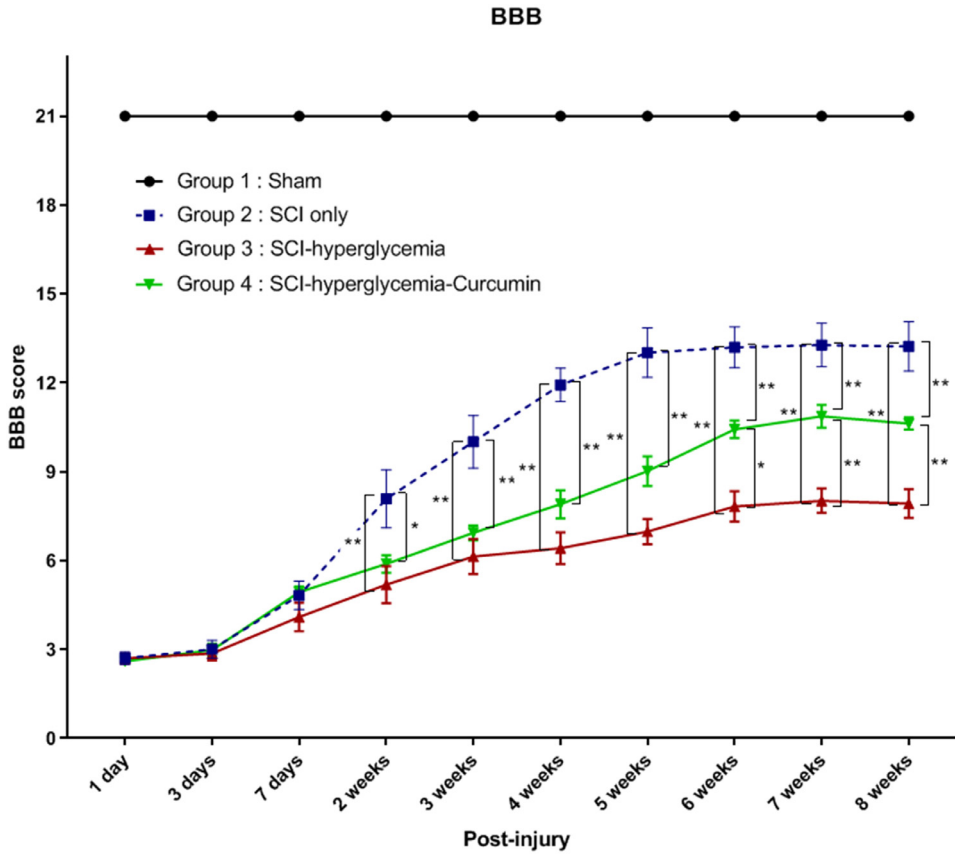

Fig. 7. The changes of functional recovery (Basso, Beattie, and Bresnahan [BBB] score) after spinal cord injury (SCI). * p<.05, ** p<.01.

preventing T-cell infiltration [30]. Enhancement of STAT3 directly inhibited neurogenesis and induced astrogliogenesis in neural stem cells [31]. Our study showed similar results. Curcumin in SCI-hyperglycemia can decrease the accumulation of GFAP and astrogliosis in the spinal cord after SCI during the chronic phase via the activated STAT3 pathway, thus decreasing the inflammatory reaction after SCI, limiting excessive astrogliosis, and alleviating the severity of SCI and thereby, improving functional recovery.

Curcumin helps in functional recovery by exerting antioxidant, anti-inflammatory, and antiastrogliosis effects on the SCI of hyperglycemic rats. However, the SCI-hyperglycemia-curcumin group showed worse functional recovery and histologic outcomes than the SCI only group. This does not mean that the neuroprotective effect of curcumin on SCI is weak. We did not include a SCI-curcumin group in our study. Previous studies have demonstrated the neuroprotective effect of curcumin injection after SCI [4]. As a result, curcumin enhances early functional recovery after SCI by diminishing cavitation volume, anti-inflammatory reactions, and antioxidant activity [4].

In previous studies, hyperglycemia reported impairing functional recovery after SCI. Clinically, blood glucose control after SCI is important [11]. In addition, if curcumin is used for treatment, synergistic effects can be expected.

This study has some limitations. The first limitation of the study is the bioavailability of curcumin. Although curcumin is safe for humans even at high doses (12 g/day), it shows poor bioavailability because of of its limited absorption and rapid metabolism and systemic elimination [32]. Yang et al. reported that the maximum serum curcumin level in rats was 0.36 ± 0.05 μg/mL after administering 10 mg/kg curcumin intravenously. However, the maximum serum curcumin level was only 0.06 ± 0.01 μg/mL when curcumin was administered orally at a 50-times higher dose (500 mg/kg) [33]. When curcumin was given orally in another study, 90% of the administered dose had left the stomach and small intestine after 30 minutes, but only 1% was present after 24 hours [34]. When inverted sacs of rat intestines were incubated at different concentrations of curcumin in a medium, 30%–80% of curcumin disappeared from the mucosal side, and none was found in the serosal fluid. Furthermore, at the highest concentration, only 3% of curcumin could be found in tissues [35]. According to Pan et al., after the administration of 0.1 g/kg curcumin intraperitoneally in mice, the concentrations measured in the intestine, spleen, liver, and kidney were 117, 26.1, 26.9, and 7.5 μg/g, respectively, but it was only 0.4 μg/g in the brain [36]. In our study, we did not measure the diffusion of curcumin into the spinal cord. Based on findings in the literature, we assume that SCI affected the selective permeability

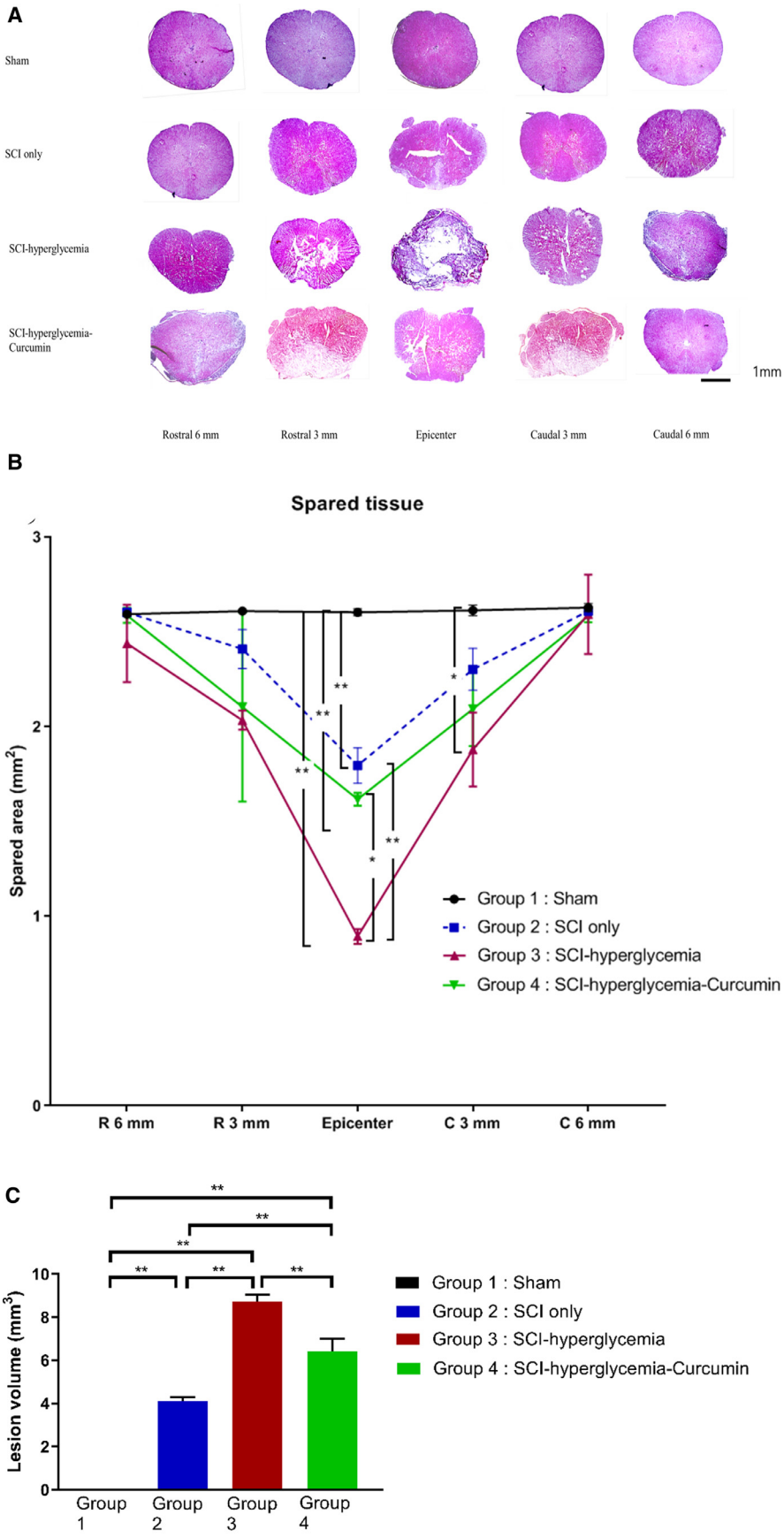

Fig. 8. The histologic outcomes (A) after spinal cord injury (SCI). The graphs shows the spared tissue area (B) and lesion volume (C) in the four group after SCI. * p<.05, ** p<.01.

of the blood-brain barrier, which might have resulted in higher diffusion rates than those under normal conditions [37]. In a 6-month randomized, placebo-controlled, double-blind clinical trial of the effects of curcumin in patients with Alzheimer's disease, curcumin increased vitamin E levels, although there was no improvement in cognitive function [38]. Another limitation is the lack of data on the effect of various metabolites of curcumin. Curcumin is converted to various forms of metabolites, such as glucuronides of tetrahydrocurcumin, hexahydrocurcumin, and dihydrocurcumin in the liver and blood [36,39]. It is unclear at this point whether or not curcumin metabolites have similar effects and activity as curcumin [40–42].

Conclusions

In our study, hyperglycemia after SCI increased the inflammatory response and oxygen-free radicals to the spinal cord and blood and showed poor functional recovery in a rat model. In contrast, curcumin decreased the inflammatory response and oxygen-free radicals in SCI with hyperglycemia. Additionally, curcumin decreased astrogliosis and improved the functional recovery and histologic outcomes. These results suggest that curcumin has a potential neuroprotective effect on SCI with hyperglycemia.

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Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.spinee.2019.07.013.

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