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Curcumin Provides Neuroprotection After Spinal Cord Injury

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Background. **Traumatic spinal cord injury (SCI) is a major cause of long-term disability. However, therapeutic agents targeting SCI are sorely lacking. The aim of this study was to investigate whether curcumin has neuroprotective effects after SCI in rats.**

Materials and methods. **Studies were performed in 39 male Sprague-Dawley rats after spinal cord hemisection. The animals were randomly divided into three groups: sham, vehicle, and curcumin. The Basso, Beattie, and Bresnahan (BBB) scale was used to evaluate functional outcome. Specimens were tested for histologic, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL), and immunohistochemical staining. Primary cultured astrocytes were used to test the inhibitory effect of curcumin on glial reactivation.**

Results. **The BBB scores for the affected hindlimb after hemisection were significantly improved in the curcumin-treated group compared with the vehicle group (on d 3 and 7; $P < 0.001$). Immunohistochemistry of NeuN revealed remarkable neuronal loss in the vehicle group after hemisection. In comparison, curcumin significantly protected neurons after SCI (curcumin compared with vehicle; $P < 0.001$). Furthermore, curcumin significantly attenuated apoptosis after SCI (curcumin compared with vehicle; $P < 0.001$). RT-PCR demonstrated that the expression of glial fibrillary acidic protein (GFAP) was significantly inhibited by curcumin.**

Conclusions. **Curcumin inhibited apoptosis and neuron loss, quenched astrocyte activation, and significantly improved neurologic deficit 7 d after spinal cord hemisection. By down-regulating GFAP expression, curcumin seems to attenuate astrocyte reactivation, which may be beneficial for neuronal survival. This is the first report demonstrating the successful treatment of SCI by curcumin.**

Key Words: **curcumin; spinal cord injury; hemisection; BBB scale; astrocyte primary culture; functional outcome; apoptosis; GFAP; astrocyte reactivation; neuroprotection.**

INTRODUCTION

Spinal cord injury (SCI) is a devastating condition for the individual patient and costly to society as a whole by requiring substantial long-term health care expenditures. A variety of molecular pathways are likely involved in the underlying pathologies of SCI, such as hypoxia, ischemia, lipid peroxidation, free radical production, neutral protease activation, prostaglandin production, and programmed cell death or apoptosis [1]. Primary injury to the spinal cord immediately disrupts cell membranes, destroys myelin and axons, and damages microvessels, thereby triggering devastating secondary injuries [2]. Secondary injury cascades are active biologic processes, including local inflammation, production of free radicals, and hyperoxidation [3–5]. The end result is that days or weeks after SCI, some of the neurons and glial cells die, even though they survived the initial injury [6, 7].

Spinal cord injury leads to an inflammatory response by activation of microglia, macrophages, and astrocytes [8, 9]. Microglia and astrocytes become activated, undergo proliferation, and increase in size after SCI. Increased glial fibrillary acidic protein (GFAP) expression is a hallmark of reactive astrocytes, and this

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cytoskeletal protein contributes to the barrier effect produced by the glial scar that mitigates axonal extension. Hence, astrocytes undergo reactive astrogliosis and produce a glial scar, which subsequently inhibits regeneration of neurons [10–12]. The observation that astrocytes enhance neuronal survival has been proposed to be due to the limiting of neuronal death from excitotoxins and oxidants, such as glutamate and reactive oxygen species [13, 14]. The disturbance of the interactions between astrocytes and neurons is related to numerous neurologic disorders, including cerebral ischemia, neurodegeneration, cerebral edema, and hepatic encephalopathy [15]. The role of astrocytes in protecting neurons has been an area of significant research interest and has emerged as a potential therapeutic target in the treatment of SCI.

Currently, methylprednisolone is the only recognized therapy for SCI, and its neuroprotective effect appears to be mediated via the inhibition of inflammatory reactions and lipid peroxidation [16]. However, the therapeutic effect of methylprednisolone is relatively minor [6], and the side effects associated with methylprednisolone are significant, including gastrointestinal bleeding, gastritis, and Cushing's syndrome [17]. Previously, we demonstrated that p35-p25-Cdk5 activation, τ hyperphosphorylation, and apoptosis could be the reasons for the neural damage after spinal cord hemisection [6, 9]. Accordingly, any novel treatment that has fewer complications and allows for significant recovery at both the functional and molecular levels would be an important therapeutic advancement.

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 is commonly utilized as a dietary pigment. Curcumin exhibits a variety of pharmacologic effects, including anti-inflammatory, anticarcinogenic, anti-infectious, antioxidant, and hypocholesterolemic activities [1, 18]. Recent studies have shown that curcumin has an antiapoptotic effect, both against dexamethane-induced apoptosis in rat thymocytes and chemotherapy-induced apoptosis in breast cancer cells [19, 20]. Curcumin can also prevent ultraviolet irradiation-induced apoptotic changes, including loss of mitochondrial membrane potential, mitochondrial release of cytochrome *c*, and increases in reactive oxygen species [21]. The neuroprotective effect of curcumin for SCI has not yet been reported in the literature. To delineate the nature and mechanisms underlying the potential therapeutic value of curcumin for SCI, we evaluated the effects of intraperitoneal curcumin injection in rats after spinal cord hemisection and examined the effect of curcumin on glial reactivation *in vitro* on primary cultured astrocytes. The specific aims were to answer the following four questions: (1) Do alterations in motor function occur after curcumin treatment? (2) What is the effect of curcumin on neuronal survival after cord hemisection? (3) Can curcumin inhibit reactive astrogliosis and consequently prevent the formation of glial scarring, which suppresses neuronal regeneration after cord hemisection? (4) Does inhibition of astrocyte activation and attenuation of GFAP expression occur *in vitro* in reactivated astrocytes mimicking SCI? The overall objective of this study was to determine the neuroprotective, antiapoptotic, and anti-inflammatory effects of curcumin treatment for SCI.

MATERIALS AND METHODS

Animal Care

Male Sprague-Dawley (SD) rats (Academia Sinica, Taipei, Taiwan), weighing 280 to 330 g, were kept two per cage for at least 5 d after their arrival at our laboratory. The rats had access to food and water *ad libitum* and were housed within a room with a 12:12 h dark-light cycle. This study was performed in accordance with the guidelines provided by the Experimental Animal Laboratory and approved by the Animal Care and Use Committee at Wan Fang Medical Center.

Spinal Cord Hemisection Injury in Rats

For the hemisection procedure, rats received isoflurane anesthesia and were placed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) to position the spinal cord. By means of an adjustable wire knife, the rats had their spinal cord lesioned on the left side (34 rats) or were submitted to a sham operation (five rats). Laminectomy was performed at the eleventh thoracic vertebrae accessed with a fine diamond drill. The guide of the wire knife was placed in a vertical plane close to the lateral surface of the lower thoracic level of the spinal cord. This level was chosen so that the unlesioned cranial and caudal segments of the spinal cord could be analyzed. The knife, which was previously turned medially, was then extended 1.5 mm and the guide was lifted 4.0 mm to hemitransect the spinal cord. Iridectomy scissors were used to ensure the completeness of the hemisection. The fascia and skin were closed with sutures for each layer, and animals were allowed to recover on a 36.5 °C heating pad. Postoperative treatments included saline (1.0 mL subcutaneously) for rehydration. Following surgery, animals were maintained under the same preoperative conditions, and were eating and drinking within 3 h after surgery. Weight loss was minimal, occurring acutely over the first 2 postoperative days, and was not greater than 5% of the total body weight. The extent of the hemisection lesion, assessed histologically, was confined unilaterally and included the dorsal column, Lissauer's tract, the lateral and ventral column systems, and gray matter. Locomotor function was observed and recorded using the Basso, Beattie, and Bresnahan (BBB) locomotor rating scale [22] to ensure that a motor deficit of the ipsilateral limb occurred and did not impair the somatosensory behavioral tests. Animals that demonstrated a loss of locomotion in both hindlimbs, indicating bilateral corticospinal tract transection, were excluded from the study. The sham operation consisted of only a laminectomy.

Treatment of SCI

Rats were randomly divided into two groups (each group $n = 17$). The curcumin group received intraperitoneal administration of curcumin 40 mg/kg once daily beginning 1 d before the hemisection surgery and continuing for 6 d. The vehicle group received intraperitoneal administration of dimethyl sulfoxide (DMSO) 40 mg/kg once daily

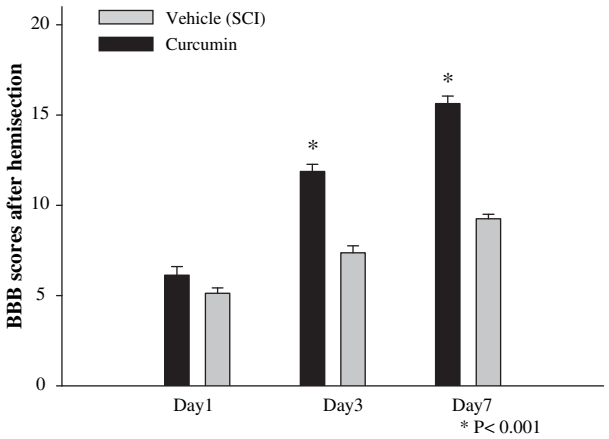

FIG. 1. Curcumin improved neurologic deficits after spinal cord hemisection. Treatment with curcumin resulted in a significant improvement of the BBB scores for the affected hindlimb compared with vehicle after SCI (curcumin compared with vehicle $*P < 0.001$ on d 3 and 7, $n = 17$ in each group).

beginning 1 d before the hemisection surgery and continuing for 6 d. No medication was administrated in the sham group. Upon recovery from anesthesia, hemitransected animals were initially evaluated neurologically with the affected hindlimb paralyzed. BBB locomotor rating scales [22] were recorded 1, 3, and 7 d after injury. Curcumin was purchased from Sigma-Aldrich (St. Louis, MO) and dissolved in DMSO. DMSO was also obtained from Sigma-Aldrich.

Histology, Immunohistochemistry, and Cell Count

On the seventh day after hemisection spinal cord injury, animals were deeply anesthetized by isoflurane and perfused through the left ventricle with phosphate-buffered saline (PBS), followed by cold 4% paraformaldehyde in 0.15 M sodium phosphate buffer, pH 7.4. The spinal cord was removed immediately, postfixed for 8 h in the same fixative at 4 °C, and cryoprotected for 2 to 3 d in 15% and 30% sucrose. The spinal cord was frozen in powdered dry ice and stored at −80 °C until analyzed. Five-micrometer sections were cut on a frozen stage with a sliding microtome at the center of spinal cord hemisection. The sections were prepared for either immunostaining or apoptosis staining. For immunohistochemistry, sections were washed in PBS and incubated in 3% normal goat serum with 0.3% Triton X-100 in PBS for 1 h. The free-floating sections were incubated at 4 °C with anti-NeuN (neuron-nuclear specific protein) (Chemicon, Temecula, CA) or anti-GFAP (DakoCytomation, Glostrup, Denmark) antibodies. Immunoreactivity was visualized using the Vectastain Elite ABC Peroxidase method (Vector Laboratories, Burlingame, CA) with diaminobenzidine (DAB) as the chromogen. Apoptosis within the hemisection was detected by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) using an apoptosis detection kit (Oncogene Research Products, Cambridge, MA). TUNEL staining was performed according to the manufacturer's instructions. The negative control for the TUNEL staining was generated by omission of the Klenow enzyme, while the negative control sections for the immunohistochemical studies were incubated as above but without primary antibodies. Cell counting was performed on every sixth section at the center of spinal cord hemisection stained with the above antibodies at a magnification of ×400. Only cells with clearly visible stain were counted. All data are presented as mean ± SEM of five consecutive cell quantifications.

Animals for Primary Astrocyte Cultures

Neonatal 0- to 2-d-old SD rats were obtained from the National Institute of Experimental Animal Research (Taipei, Taiwan). Rats were euthanized by an overdose of sevoflurane (Abbott, Osaka, Japan) to minimize pain or discomfort in accordance with the National Institutes of Health guidelines (Bethesda, MD) regarding the care and use of animals for experimental procedures. The procedures for euthanizing postnatal rats in this study were reviewed and approved by the Experimental Animal Review Committee at Taipei Medical University.

Primary Astrocyte Cultures

Astrocyte cultures were prepared as described previously [23, 24] with some modification. In brief, cerebral cortices were harvested from 1- to 2-d-old SD rats, homogenized by mechanical dissociation, and the cell suspension was diluted in Dulbecco's modified Eagle's medium (DMEM) with F12 media (Invitrogen, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (FBS). Cells were seeded onto 75-cm^2 flasks at an initial density of 2×10^6 cells/flask. Six to 8 d after seeding, microglia and oligodendrocytes were removed by orbital shaking of the culture flasks at 160 rpm for 24 h at 37 °C. The suspended cells were decanted to obtain a pure astrocyte layer adhered at the bottom. The purified astrocytes were subcultured onto 35-mm culture dishes with DMEM/F12 medium containing 10% FBS in a humidified tissue culture incubator with 5% CO_2 maintained at 37 °C. The characteristic morphology indicated that the astrocyte culture was a minimum of 85% pure. Experiments were performed on astrocytes grown for 7 d subsequent to astrocyte reactivation.

Curcumin Treatment of Reactivated Astrocytes

To study the effect of curcumin on reactivated astrocytes, primary astrocyte cultures (10^6 in a 35 mm dish) at 7 d *in vitro* (DIV) were incubated with 0 and 1 μM curcumin for 24 h. The mRNA level of GFAP was analyzed by RT-PCR to determine astroglial activation *in vitro*. Each experiment was performed at least in duplicate and was carried out using at least three different astrocyte cultures.

Reversed Transcription-Polymerase Chain Reaction Analysis of GFAP mRNA

Total RNA was prepared by directly lysing the cultured astrocytes in extraction buffer (Trizol/phenol/chloroform), and reverse transcribing the mRNA into cDNA using oligo-dT and SuperScript II reverse transcriptase (Invitrogen). The cDNAs were subjected to PCR to measure the expression of GFAP and the housekeeping gene GAPDH as an internal control.

The PCR protocol included 25 cycles of denaturation for 1 minute at 94 °C, annealing for 1 min at 55 to 60 °C, and extension for 1 min at 72 °C. Reaction products were separated by electrophoresis on a 1.2% agarose gel. Bands were visualized and quantified using an electrophoresis image analysis system (Eastman Kodak Co., Rochester, NY). Net intensity of each band was normalized to the band intensity of GAPDH and divided by the normalized intensity of the control group to obtain the ratio-to-control value.

Real-Time PCR

Primer-probe sets were designed for the TaqMan Gene Expression Assays. By means of FAM labeled MGB probes, the level of GFAP mRNA was quantified by an ABI PRISM 7300 HT real-time PCR system (Applied Biosystems, Foster City, CA). The relative mRNA level was normalized to a housekeeping gene (β-actin), which was used as an internal control. Each measurement was performed at least in duplicate, and the threshold cycle (Ct) (the fractional cycle number at which the amount of amplified target reached a fixed threshold) was determined.

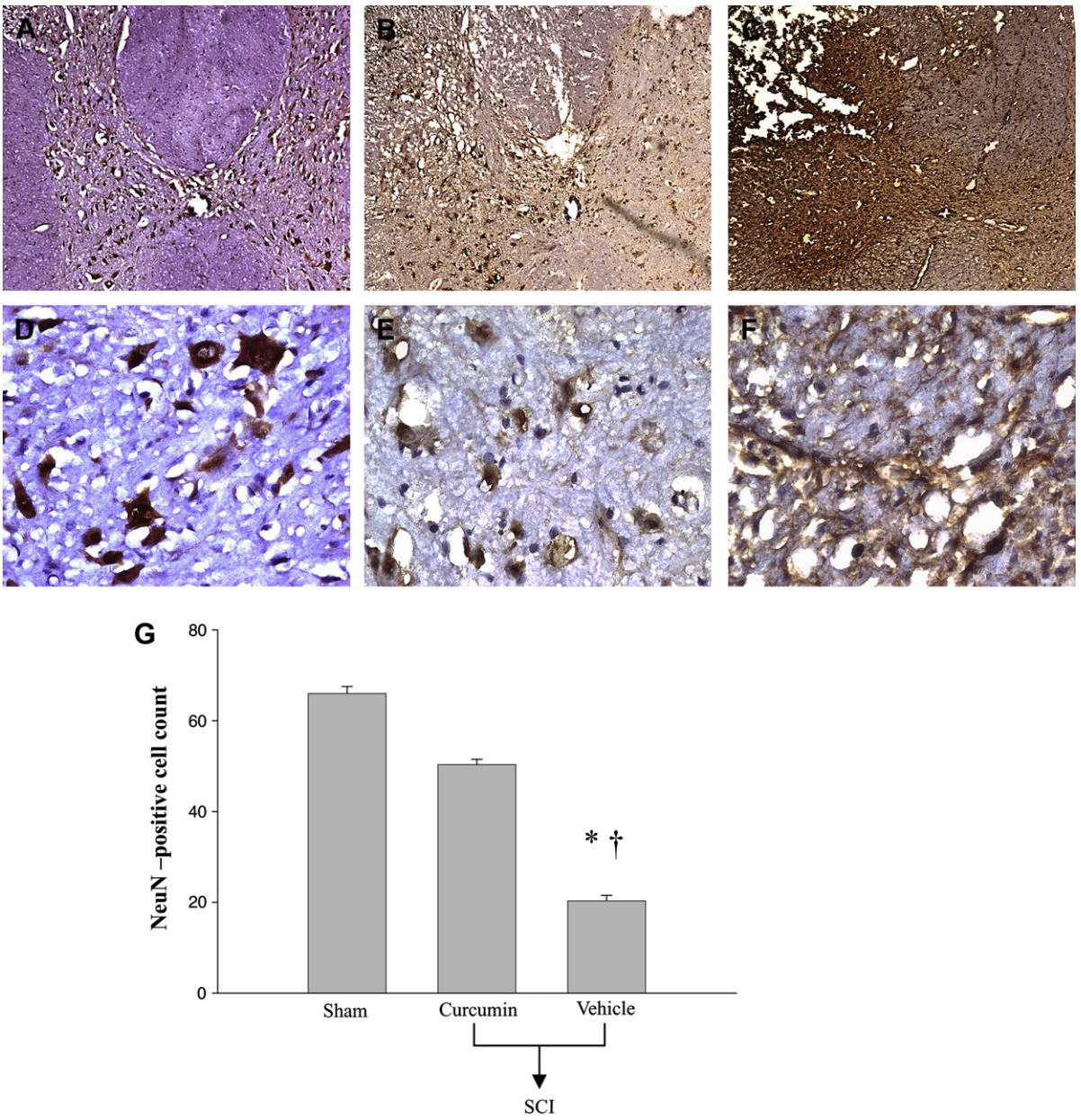

FIG. 2. Representative photographs of NeuN-stained sections from the spinal cord of sham animals (A), (D) *versus* hemisectioned animals with curcumin treatment (B), (E) and hemisectioned animals with vehicle treatment (C), (F). Note that there are numerous normal neurons in the sham group (D), and that NeuN staining is relatively preserved in the curcumin group (E) compared with the severe neuronal loss in the vehicle group (F) after SCI (original magnification (A)–(C): 50×; (D)–(F): ×400). (G) Vertical bars indicate the mean (± SEM) number of neurons per tissue section for each of the groups (sham *versus* vehicle: $^{*}P < 0.001$; curcumin *versus* vehicle $^{\dagger}P < 0.001$, $n = 5$ in each group). (Color version of figure is available online.)

Immunofluorescence Staining

To study the effect of curcumin on glial reactivation, cultured astrocytes at 7 DIV were incubated with 0 and 5 μM curcumin for 24 h. GFAP immunofluorescence staining was performed to biochemically determine astroglial activation *in vitro*. After 24 h, astrocytes were rinsed three times in PBS and fixed in paraformaldehyde for 20 min at room temperature (RT). After rinsing with PBS, cells were treated with blocking solution for 30 min. Blocking was performed with 2% goat serum, 3% BSA, and 0.5% Triton X-100. The primary antibody used was a mouse anti-GFAP monoclonal IgG at a concentration of 1:1000 (Chemicon). Texas red-conjugated AffiniPure Goat anti-mouse IgG (H + L) (Jackson ImmunoResearch Laboratories, West Grove, PA) was used at a concentration of 1:200. Glass cover slips were mounted with fluorescent mounting medium (Vector Laboratories, Burlingame, CA). Nuclei were visualized with 4',6-diamidino-2-phenyindole (DAPI) stain at a concentration of 1:5000. The cells were observed under a fluorescence microscope.

Statistical Analysis

All data are presented as mean ± SEM (at least three separate experiments). Statistical analysis was performed using a one-way ANOVA. To determine the difference in BBB scores over time between and within groups, statistical analysis was performed using repeated

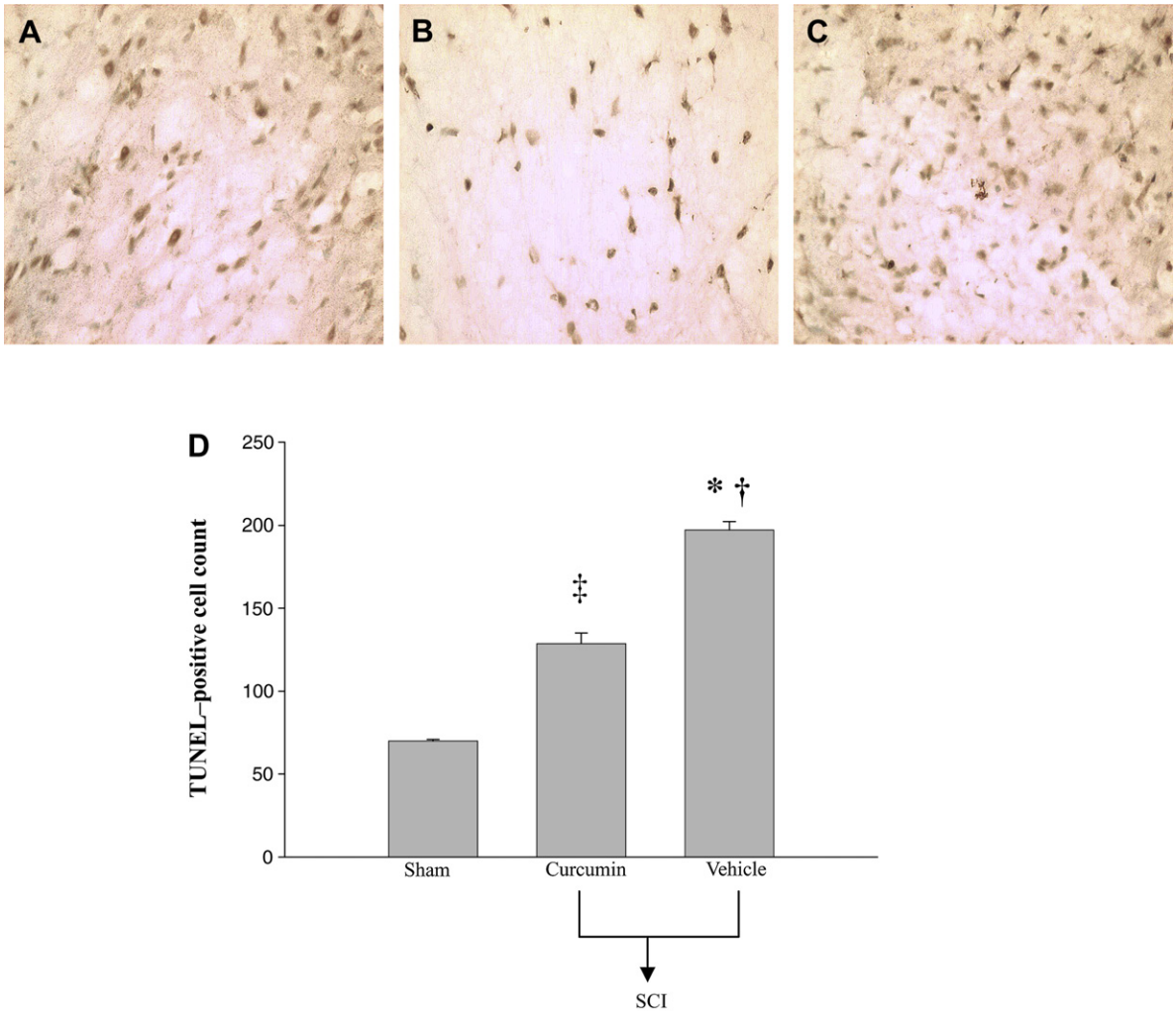

FIG. 3. Representative photograph of triphosphate nick-end labeling (TUNEL)-stained sections from the spinal cord of sham controls (A) compared with hemisectioned animals with curcumin treatment (B) and hemisectioned animals with vehicle treatment (C) and (D). Vertical bars indicate the mean (± SEM) number of TUNEL-stained cells per tissue section for each group (sham *versus* vehicle $^{*}P < 0.001$; curcumin *versus* vehicle $^{\dagger}P < 0.001$; curcumin *versus* sham $^{\ddagger}P < 0.001$, $n = 5$ in each group). (Color version of figure is available online.)

measure ANOVA. $P < 0.05$ was considered significant. For BBB scale analysis, a Mann-Whitney U test was used.

RESULTS AND DISCUSSION

Curcumin Improved Neurological Function, Reduced Apoptosis, and Limited Neuronal Damage after Spinal Cord Hemisection

To evaluate the extent of neurological impairment after SCI, we used the BBB locomotor scales. The BBB scores for the affected hindlimb after hemisection were significantly improved in the curcumin-treated group compared with the vehicle group over time ($P < 0.001$). Meanwhile, BBB scores over time were significantly different in each group (both $P < 0.001$). In addition, there were significant differences in BBB scores between the curcumin-treated and vehicle groups at d 3 and 7 after SCI (both $P < 0.001$) (Fig. 1). Immunohistochemistry of NeuN, a neuron-specific marker, revealed remarkable neuronal loss in the vehicle group after hemisection (sham compared with vehicle; $P < 0.001$) (Fig. 2C and F). In comparison, curcumin significantly protected neurons after SCI (curcumin compared with vehicle; $P < 0.001$) (Fig. 2B and E). Furthermore, 7 d after SCI, apoptosis was detected by TUNEL staining in the vehicle group (Fig. 3C), while apoptosis was reduced in the curcumin-treated group (Fig. 3B). Curcumin significantly attenuated apoptosis after SCI (curcumin compared with vehicle; $P < 0.001$) (Fig. 3D). Thus, the neuroprotection observed with curcumin treatment after spinal cord hemisection could be due to antiapoptotic effects and the prevention of neuronal damage.

Curcumin Can Attenuate GFAP Expression after SCI

Astrocytes are the most abundant type of glial cells that provide support and supply nutrition for neurons

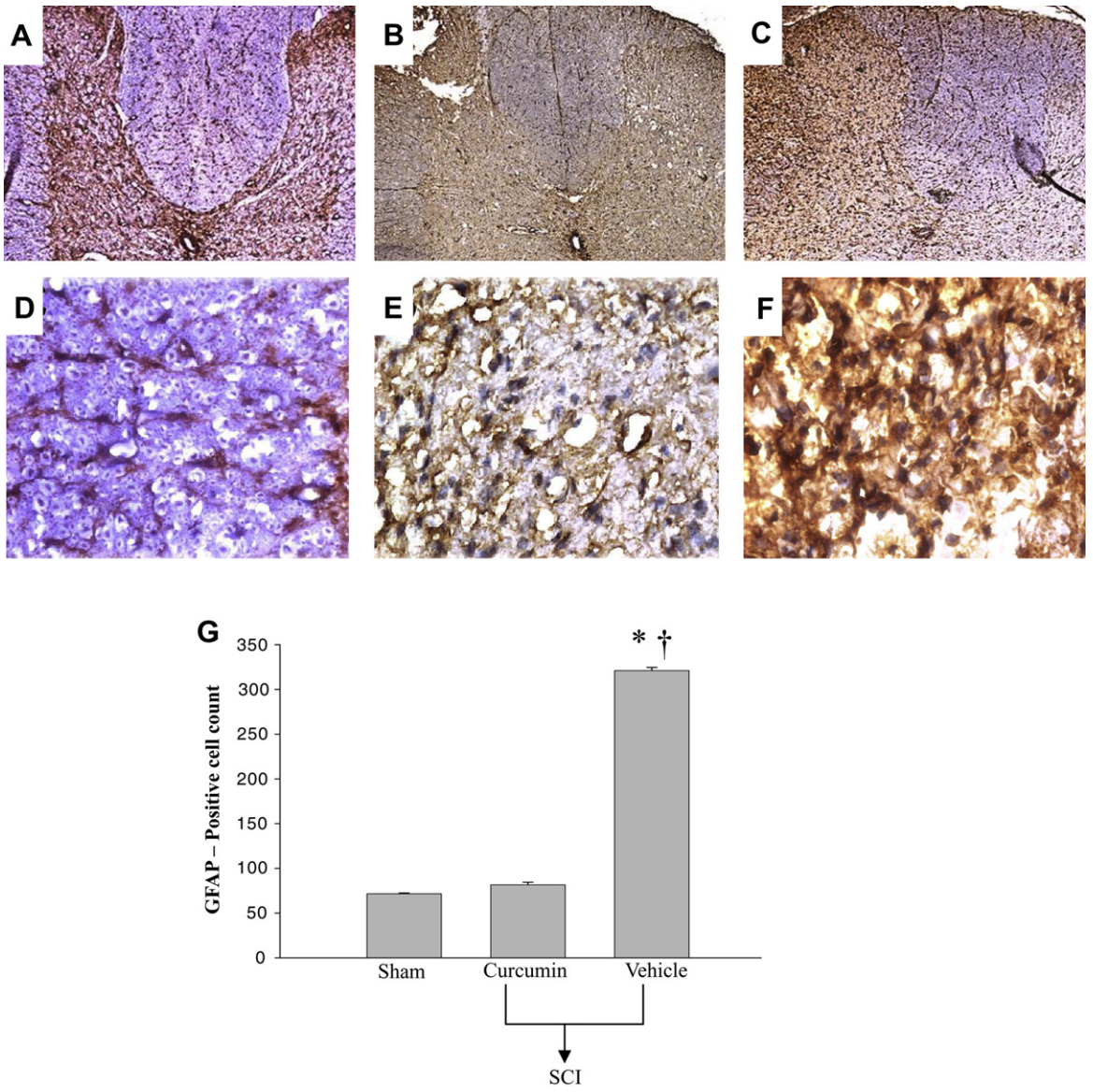

FIG. 4. Representative photographs of GFAP-stained sections from the spinal cord of sham control animals (A) and (D), as well as the curcumin-treated group (B) and (E), and the vehicle-treated group (C) and (F) 7 d after SCI. Strong staining for GFAP positive cells was observed in the group with the spinal cord lesion (C) and (F), while curcumin administration markedly attenuated this upregulation (B) and (E) (original magnification (A)–(C): ×50; (D)–(F): ×400). (G) Vertical bars indicate the mean (±SEM) number of GFAP-stained cells per tissue section for each group (sham *versus* vehicle: $^{*}P < 0.001$; curcumin *versus* vehicle $^{\dagger}P < 0.001$, $n = 5$ in each group). (Color version of figure is available online.)

in the central nervous system. Astrogliosis is a characteristic inflammatory reactive response of astrocytes after SCI. Reactive astrogliosis may impair axonal regeneration and functional neuronal recovery [6, 9, 16]. The expression of GFAP indicates hypertrophy and proliferation of astrocytes after neurological insults. Therefore, the expression of GFAP was investigated after spinal cord hemisection. Strong immunostaining for GFAP was demonstrated in the spinal cord lesion (Fig. 4C and F). However, this activation was greatly attenuated in the curcumin group (Fig. 4B and E). These results demonstrated that curcumin can abate the activation of astrocytes after spinal cord injury.

In addition, the beneficial effects of curcumin treatment were not solely restricted to neurons, even though the literature has stressed the antiapoptotic and protective effects of curcumin on neurons [1, 25–30]. However, little is known regarding the inhibitory effects of curcumin on astrocyte proliferation [31–34]. Spinal cord injury results in an inflammatory response characterized by the activation of astrocytes, which leads to reactive astrogliosis. Reactive astrocytes promote glial scar formation, which inhibits axonal regeneration and

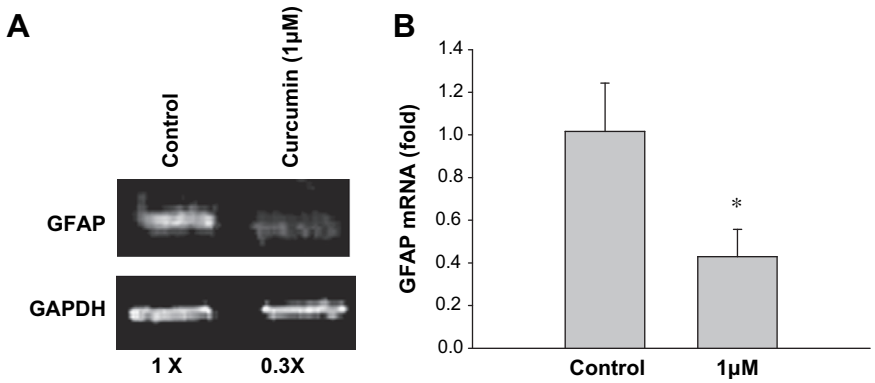

FIG. 5. Treatment of primary cultured astrocytes with curcumin. (A) mRNA levels of GFAP were determined by RT-PCR in reactivated astrocytes at 7 DIV after treatment with 1 μM of curcumin. Curcumin treatment significantly attenuated the upregulation of GFAP compared with control cells that received no treatment. The results shown are from one experiment that was repeated at least three times. (B) GFAP expression levels were determined using TaqMan real-time PCR with the ABI Prism 7900 Sequence Detection System. *Curcumin (1 μM) treated-cells were significantly different ($P < 0.001$) compared with control cells ($n = 3$).

neuronal tissue repair [6, 9, 16, 35]. In our study, we demonstrated marked activation of astrocytes after SCI, which was revealed by immunostaining for GFAP, and curcumin effectively abolished reactive astrogliosis. Curcumin treatment appeared to control these inflammatory responses and may have improved neuronal tissue repair and functional recovery after trauma. These beneficial effects were evident from the improvement of BBB scores after curcumin treatment in our rat cord injury model. Thus, the targeting of reactivated astrocytes provides a window of opportunity for the treatment of SCI. We further examined the effect of curcumin on glial reactivation *in vitro* in primary cultured astrocytes.

Curcumin Inhibits Astrocyte Reactivation *In Vitro*

Primary cultured astrocytes after 7 d *in vitro* that remained reactivated were used for the experiments in this study. This culture model was developed to mimic the conditions of glial reactivation following CNS injury *in vivo*. Based on the observation that curcumin can reduce the expression of GFAP in a rat hemisection model, the effects of curcumin on reactivated astrocytes were investigated *in vitro*. The expression of GFAP was assessed by RT-PCR. At a concentration of 1 μM, curcumin significantly down-regulated the expression of GFAP in reactivated astrocytes (Fig. 5A). The difference in GFAP mRNA isolated from reactivated astrocytes either with or without curcumin treatment was confirmed using quantitative real-time PCR ($P < 0.001$) (Fig. 5B). The results from our *in vitro* study suggested that curcumin reduced the expression of GFAP and inhibited astrocyte reactivation, which was consistent with the result of our animal study. By down-regulating GFAP expression, curcumin appeared to attenuate astrocyte reactivation, which may benefit neuronal survival. In this study, we tested the effects of curcumin using a low dose of 1 μM instead of a high dose. For the high dose, it is likely that the concentration would have become neurotoxic and stimulatory to the astrocytes, similar to adding an excitotoxin. It is always a concern when using a high concentration of a test compound for a study because it can be toxic with nonspecific modes of action, and not exhibit a specific pharmacologic mechanism. Many phytochemicals, like curcumin, have a biphasic dose-response relationship, which has beneficial effects at a low dose and toxic effects at a high dose [36, 37]. To prevent the toxic effects of high-dose curcumin, including carcinogenic, neurotoxic, and cardiotoxic insults [36, 37], smaller doses ($\leq$1 μM) were utilized in this study. Importantly, lower doses can be as effective as higher doses if used for a longer period [31]. In this study, our data clearly demonstrated that a low dose of curcumin (1 μM) could attenuate reactive astrogliosis in both semiquantitative and real-time PCR assays. Thus, glial inactivity could be one of the mechanisms underlying neuroprotection in curcumin therapy.

Morphological Alteration of GFAP Expression after Curcumin Administration

To investigate whether curcumin has inhibitory effects on GFAP expression at the protein level, immunofluorescent staining of GFAP on primary cultured astrocytes was examined *in vitro*. The purity of the primary cultured astrocytes reached 99%, as detected by DAPI (Fig. 6A and B) and anti-GFAP immunofluorescence (Fig. 6 G and H). The inhibitory effect of curcumin treatment on astrocytes was observed as a morphologic change (Fig. 6C, D, E, and F) and as a reduction in GFAP immunofluorescent staining. At 7 DIV, the control cells exhibited a large and circular morphology

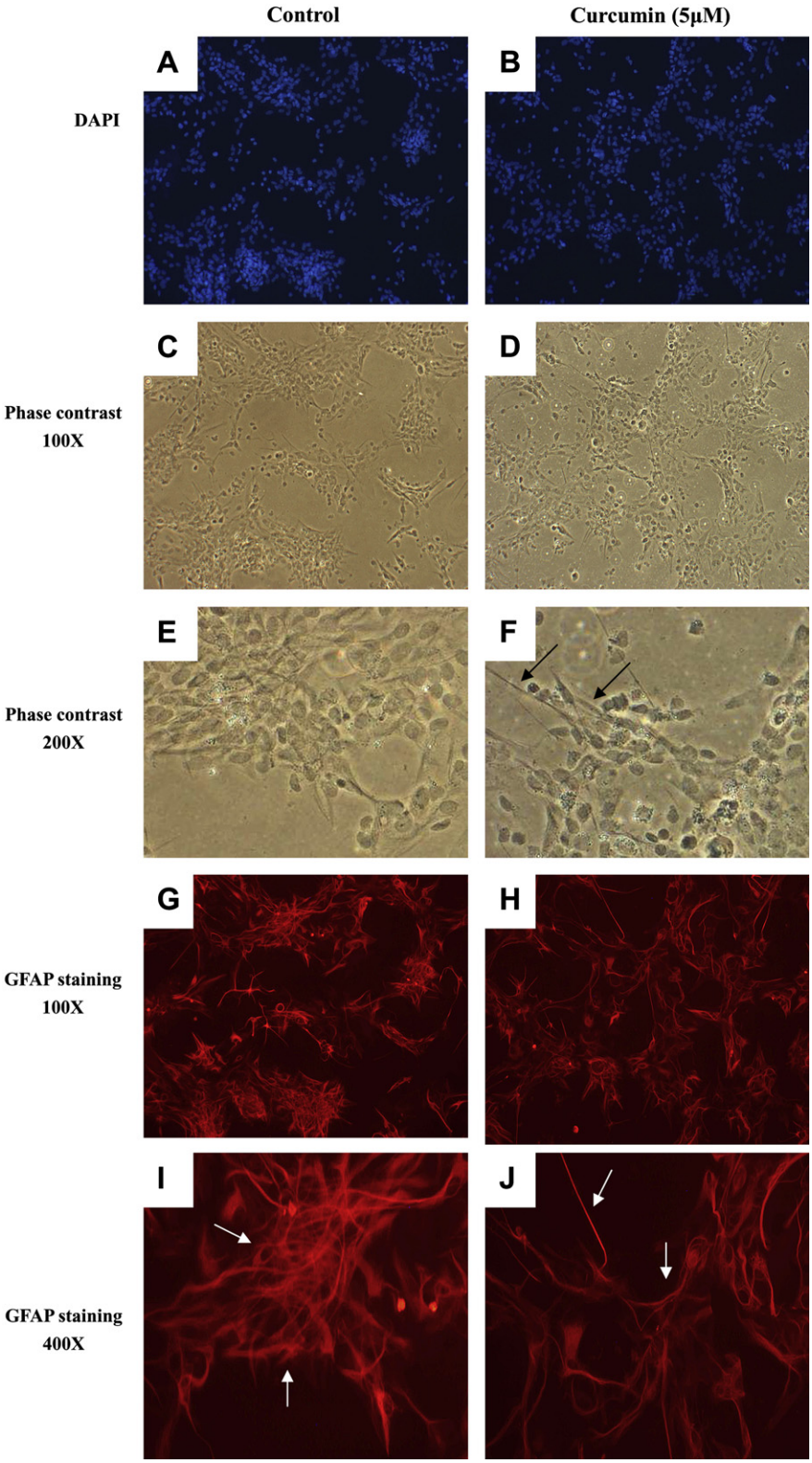

FIG. 6. Inhibitory effects of curcumin treatment on astrocyte morphology and GFAP immunofluorescent staining. At 7 DIV, the control cells (C) and (E) were large and circular in morphology, while the 5 μM curcumin-treated astrocytes (D) and (F) were smaller and elongated [arrowed in (F)]. The GFAP immunofluorescence exhibited by control cells (G) and (I) remained abundant and densely packed, indicating astrocytes remained reactivated [arrowed in (I)]. The GFAP expression of 5 μM curcumin-treated cells (H) and (J) was decreased and less densely packed [arrowed in (J)] ($n = 3$). (Color version of figure is available online.)

(Fig. 6C and E). In contrast, the 5 μM-treated cultured cells were much smaller and elongated (Fig. 6D and F). GFAP immunofluorescence observed in control cells remained abundant and densely packed, indicating that the astrocytes had remained reactivated (Fig. 6 G and I). In comparison, curcumin-treated cells showed GFAP expression that was much reduced and these cells were less densely packed (Fig. 6H and J). Moreover, these morphologic findings were compatible with the inhibitory effects of GFAP upregulation in our SCI experiments (Fig. 4 and Fig. 5).

In conclusion, we demonstrated that curcumin treatment provides neuroprotection against spinal cord injury-induced disability in rats by attenuation of neuron loss, prevention of neuronal apoptosis, and by decreasing astrocyte activation. By down-regulating GFAP expression, curcumin can attenuate astrocyte reactivation *in vitro*, which may benefit neuronal survival.

ACKNOWLEDGMENT

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