Shengmai-san–Mediated Enhancement of Regenerative Responses of Spinal Cord Axons After Injury in Rats

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Abstract. Shengmai-san (SMS) is a traditional Chinese medicine used to treat diverse symptoms including cardiovascular and neurological disorders. Here we investigated the effects of SMS on regenerative responses of spinal cord axons in rats that were given contusion injury at the lower thoracic level. The injury cavity was confined to a restricted area by SMS treatment, and the signals of glial scar protein chondroitin sulphate proteoglycan (CSPG) and inflammatory cell marker protein CD11β were heavily observed within the injury cavity in SMS-treated animals. Anterograde tracing of DiI-labeled corticospinal tract (CST) axons revealed increases in collateral arborization around and within the injury cavity and caudal elongation by SMS treatment. Furthermore, SMS treatment facilitated neurite elongation of dorsal root ganglion (DRG) sensory neurons that were co-cultured with non-neuronal cells prepared from injured spinal cord. Phospho-Erk1/2 was strongly induced in both spinal cord and motor cortical areas after spinal cord injury (SCI), and it was further unregulated in the motor cortex by SMS treatment. In contrast, upregulation of cell division cycle 2 (Cdc2) production by SMS treatment was limited to a local, SCI area. These data suggest that SMS may play an active role in regenerative responses and facilitate axonal regrowth after SCI.

Keywords: Shengmai-san, spinal cord injury, axonal regeneration, Erk1/2, cell division cycle 2 (Cdc2)

Introduction

While the injured axons in the peripheral nervous system (PNS) have the ability to regenerate and innervate to their original targets, damaged axons in the central nervous system (CNS) generally fail to regenerate and lead to permanent functional deficit in affected body parts. Myelin-associated molecules such as Nogo, myelin-associated glycoprotein (MAG), oligodendrocyte myelin glycoprotein (OMgp), semaphorin 4D (Sema4D/CD), and chondroitin sulphate proteoglycan (CSPG) are a few examples of glial cell proteins interfering with CNS axonal regeneration (1). Besides environmental influences, some CNS neurons do not produce intrinsic, growth-promoting molecules that are inducible from regenerating peripheral neuron after axonal injury. In this case, molecular supply to injured CNS neurons can induce axonal regeneration. For instance, over-expression of axonal growth-associated protein GAP-43, which was reported to facilitate spontaneous or lesion-induced sprouting of peripheral motor axons to target muscle (2), effectively induced axonal elongation of the central branch of dorsal root ganglion (DRG) sensory neurons that were co-cultured with non-neuronal cells prepared from injured spinal cord. Phospho-Erk1/2 was strongly induced in both spinal cord and motor cortical areas after spinal cord injury (SCI), and it was further unregulated in the motor cortex by SMS treatment. In contrast, upregulation of cell division cycle 2 (Cdc2) production by SMS treatment was limited to a local, SCI area. These data suggest that SMS may play an active role in regenerative responses and facilitate axonal regrowth after SCI.
be considered for therapeutic strategies to induce axonal growth and functional recovery after SCI.

In Chinese traditional medicine, Shengmai-san (SMS) has been used for treating symptoms related to cardiovascular diseases such as heart failure, stroke, and shock, and recent studies have begun to provide evidence showing that SMS has protective effects against oxidative damages in the cells or tissues of the cardiovascular and nervous systems (14 – 16). Suggested potential of SMS for neuroprotection and neurite outgrowth further implicates the applicability of SMS for treatment of axon-degenerative diseases such as amyotrophic lateral sclerosis (ALS) in which the deficiency of superoxide dismutase (SOD) is thought to be linked to neuronal damage (17).

Here we investigated whether SMS has a supportive role in axonal growth and cell survival after SCI, which would otherwise undergo axonal die-back and degeneration. Throughout the primary screening procedure of selected herbal decoctions, SMS was identified to induce enhanced axonal regeneration of the injured sciatic nerve in the rat (TB Seo et al., unpublished data). To examine the hypothesis that one or more molecular component(s) of SMS responsible for growth-promoting activity in the injured peripheral nerve might have a supportive role for improved regenerative responses in CNS axons, we generated a contused rat model and determined the effects of SMS on the regrowth of spinal cord axons. We have found that SMS induced changes in neural tissue responses that may have a positive effect on axonal growth, as demonstrated by enhanced axonal elongation of the corticospinal tract (CST) after SCI. Our data further show that SMS induces Cdc2 and phospho-Erk1/2 protein production in spinal cord axons after injury.

Materials and Methods

Experimental animals and surgery

Sprague-Dawley rats (male, 200 – 250 g; Samtago, Seoul, Korea) were maintained in an animal room with regulated temperature (22°C), 60% humidity, and a 12-h light/dark cycle (lights on 0700 to 1900 h). They were allowed to eat commercial pellet chow (Samyang Co., Seoul, Korea) and drink water ad libitum. Rats were anesthetized by injecting intraperitoneally a single dose of a mixture of ketamine (80 mg/kg) and xylazine (5 mg/kg). Using aseptic technique, a laminectomy was performed to expose the dura mater of the spinal cord at thoracic levels 9 – 10. A contusion injury was given to the exposed dura using the NYU compactor by dropping a 10-g impactor (a cylindrical metal rod tapered to a tip diameter of 2 mm) from 2.5-cm height (18). After the suture of the wound in anatomical layers, rats were maintained in standard plastic cages until further treatment. Rat care and all experimental procedures were in accordance with the Animal-use Statement and Ethics Committee approval statement for animal experiments at Daejeon University. A total of 36 rats (3 – 4 animals per group) were used in this study.

SMS preparation and administration

Dried SMS, which consists of three herbal components, Panax Ginseng (specimen numbers: 06100501, production area: Geumsan, Chungnam Province, Korea), Ophiopogon Japonicus (specimen numbers: 06190101, production area: Miryang, Gyeongnam Province, Korea), and Schisandra Chinensis (specimen numbers: 06020301, production area: Muju, Jeonbuk Province, Korea) (1:2:1 in dry weight) were obtained from Daejeon University Oriental Medicinal Hospital. Twenty-three grams of dried SMS were suspended in 1 liter of distilled water for 2 h, boiled for 3 h, and filtered with Whatman filter paper (Grade 1; Whatman Inc., Clifton, NJ, USA) three times. The extract was frozen at −70°C for 4 h and then freeze-dried for 24 h. The yield of SMS was 2 g for 23 g of the initial raw materials. Purified material was stored at −20°C and used for the experiments after dissolving it in physiological saline solution (1 mg of extract residue/ml in 0.9% NaCl solution). For efficient drug delivery into the nervous system, 10 μl of SMS solution or an equal volume of saline was applied into the area of SCI for a 1-min period followed by intraperitoneal supplementation of 0.1 ml solution every other day until the animal’s sacrifice.

HPLC analysis

HPLC analysis of SMS was performed by the previously reported procedures (9). Briefly, SMS solution was centrifuged at 12,000 rpm for 10 min, and standard solutions of Schisandrin (Chromadex, Irvine, CA, USA) and Gomisin A (Chromadex) were diluted to a concentration range of 0 – 50 μg/ml for standard curves. The supernatant of SMS and standard solutions were analyzed by LC-10AD HPLC system (Shimadzu, Kyoto). The analysis system includes a Thermo Hypersil reverse phase C18 column (250 × 4.6 mm) with linear gradient elution at 5 – 40 min with CH3CN and aqueous solution at a flow rate at 1 ml/min. Aliquots (20 μl each) of SMS and standard solutions were injected to the column. Temperature of the column oven was set to 30°C and UV detection was at 214 nm.
**Primary cell culture**

For DRG sensory neuron culture, DRG at lumbar 4–5 of the intact rat was dissociated by treatment with 125 U/ml type XI collagenase (Sigma, St. Louis, MO, USA) in DMEM for 80 min at 37°C and then washed twice with DMEM. Cells were treated with 0.5 mg/ml type SII trypsin for 15 min, followed by an inhibition reaction for 5 min in 1 mM EDTA, 100 μg/ml of soybean trypsin inhibitor, and 40 μg/ml of DNase I. Cells (1 × 10^5 cells) were plated onto 12-mm coverslips (Bellco Glass Inc., Vineland, NJ, USA) precoated with 0.01% poly-l-ornithine (Sigma) and laminin (0.02 mg/ml; Collaborative Research, Bedford, MA, USA) and 12 h later, changed to DMEM containing 5% fetal bovine serum (GIBCO, Melbourne, Australia) plus 5% horse serum, 2 mM glutamine, and 1% penicillin-streptomycin. Cells were treated with 0.5–2.0 μg/ml of SMS, 1–50 μg/ml of α-tocopherol (Sigma), or ethanol vehicle (0.02%) and then incubated for 48 h before harvesting them for immunofluorescence staining with anti-neurofilament-200 antibody (NF-200, mouse monoclonal, 1:200; Sigma).

For spinal cord non-neuronal cell culture, a dorsal half of spinal cord covering 1-cm length of the rostral and caudal region from the injury site was dissected from a rat that had been given SCI for 3 days. For DRG sensory neuron culture, DRG at lumbar 4–5 were prepared from rats that had undergone sciatic nerve injury for 3 days. The procedures of primary DRG neurons and spinal cord non-neuronal cells are essentially the same as described previously (13). Spinal cord non-neuronal cells (1 × 10^5 cells per 12-mm coverslip) were incubated for 24 h before the addition of freshly prepared DRG sensory neurons (1 × 10^5 cells per 12-mm coverslip). Cocultured cells were treated for 48 h with 1 μg/ml of SMS, a mixture of 5 mM minocycline plus 0.5 μM rolipram, or the equivalent volume of saline and used for immunofluorescence staining with anti-NF-200 antibody (mouse monoclonal, Sigma) and anti-glial fibrillary acidic protein (GFAP) antibody (rabbit polyclonal; Chemicon, Temecula, CA, USA).

Digital images of neuronal process were captured and transferred to the Adobe Photoshop program (version 5.5; Adobe). The number and length of neurite processes exhibiting clear outgrowth (longer than cell body size) from the cell body were analyzed by the i-Solution software program (Image and Microscope Technology, Goleta, CA, USA). Mean neurite length was determined by analyzing at least 30 sensory neurons grown on the coverslip.

**Immunohistochemistry**

Dissected spinal cord tissues were frozen immediately at −80°C and embedded into the OCT medium. The sections (20 μm) were cut using a cryostat and mounted on positively charged slides (Fisher, Pittsburgh, PA, USA). Pretreatment, primary and secondary antibody reactions, and microscopic analysis of sections were performed as described previously (13). The primary antibodies used in the present study were anti-GFAP antibody (rabbit polyclonal, Chemicon), anti-CSPG antibody (mouse monoclonal, CS56; Sigma), anti-NF-200 antibody (mouse monoclonal, 1:200; Sigma), and anti-CD11β antibody (mouse monoclonal; BD Bioscience, San Jose, CA, USA); and the secondary antibody was fluorescein-goat anti-mouse (Molecular Probes, Eugene, OR, USA) or rhodamine-goat anti-rabbit secondary antibodies (Molecular Probes). Cellular nuclei were stained with 2.5 μg/ml of Hoechst 33258 dye (bis-benzimide, Sigma) for 10 min before the final washing with 0.1% Triton X-100 in PBS, and the sections were coverslipped with gelatin mount medium. Samples were viewed with a fluorescence microscope (Nikon Eclipse E-600; Nikon, Tokyo) equipped with a digital camera, and the captured images in the computer were analyzed by Adobe Photoshop (Version 5.5). Average number of CD11β-stained cells or Hoechst-stained nuclei in individual images (area: 650 × 500 μm^2) were counted from 4 nonconsecutive spinal cord sections per animal.

**Western blot analysis**

The dorsal half of the spinal cord tissue covering approximately 1 cm of rostral and caudal spinal cord at the injury area and the motor cortex area covering 1–4 mm posterior to the bregma, 1–4 mm from the midline with a depth to 1.8 mm were dissected. The tissues were washed with ice-cold PBS and sonicated under 400–600 μl of Triton lysis buffer (20 mM Tris, pH 7.4, 137 mM NaCl, 25 mM β-glycerophosphate, pH 7.14, 2 mM sodium pyrophosphate, 2 mM EDTA, 1 mM Na_2VO₃, 1% Triton X-100, 10% glycerol, 5 μg/ml leupeptin, 5 μg/ml aprotinin, 3 μM benzamidine, 0.5 mM DTT, and 1 mM PMSF); and 25 μg protein of each sample was used for western analysis. Quantitative analysis of protein bands in the autoradiographic images was determined using the i-Solution software. The antibodies used in the present study were anti-Cdc2 antibody (mouse monoclonal, Santa Cruz Biotech), anti-Erk1/2 antibody (rabbit polyclonal, Cell Signaling), anti-phospho-Erk1/2 antibody (rabbit polyclonal, Cell Signaling), and anti-actin antibody (rabbit polyclonal; ICN Biomedicals, Cleveland, OH, USA) as primary antibodies, and horseradish peroxidase (HRP)–conjugated secondary antibodies (goat anti-rabbit, from Santa Cruz Biotech or sheep anti-mouse, from Amersham
Anterograde tracing
Fluorescent lipophilic carbocyanine dye 1,1'-dioctodecyl-3,3',3',3'tetramethylindocarbocyanine perchlorate (DiI) (Molecular Probes), an anterograde tracer, was injected into the motor cortical area at the time when the SCI was given. The rats were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (5 mg/kg) and placed in a stereotaxic instrument (Harvard Apparatus, Holliston, MA, USA). The rat’s head skin was incised, a hole was drilled in the skull, a glass capillary filled with DiI (5 μl of 3% in DMSO) was inserted, and DiI was injected at a flow rate of 1.6 μl/min for 3 min into the motor cortex (2.0-mm posterior to the Bregma, 2.3-mm lateral to the mid-line, 1.5-mm ventral to the dura surface) on the left side of the brain by using a picoinjector (Harvard Apparatus PLI-100). DiI-labeled CST axons of the spinal cord sections (20 μm) were analyzed 2 weeks later under Nikon fluorescence microscope (Eclipse E-600).

Statistical analysis
Data were presented as the mean ± S.E.M. The mean values in individual groups were compared by one-way ANOVA with Tukey’s post hoc test (SPSS version 14.0; SPSS Korea, Data Solution Inc., Seoul, Korea), and statistically significant difference was reported as P<0.05.

Results
To validate the chemical profile of SMS extract, we performed HPLC analysis for two of ingredients in SMS, Schisandrin and Gomisin-A. The HPLC chromatogram of SMS solution showed the peaks of Schisandrin and Gomisin-A at 33.0 and 34.1 min, respectively, which coincided with those of the standard solutions (Fig. 1A). We evaluated the potential of SMS to increase neurite outgrowth of DRG sensory neurons using α-tocopherol as a positive control. Cultured DRG neurons revealed the highest neurite outgrowth with treatments of SMS at 1.0 μg/ml and of α-tocopherol at 10 μg/ml (Fig. 1: B and C). Comparison

Fig. 1. SMS facilitates neurite outgrowth of DRG sensory neurons. A: HPLC profile of SMS extract. Two peaks of the SMS solution at 33.0 min and 34.1 min coincide with those of Schisandrin and Gomisin-A standard solutions, respectively. B and C: Dose-dependent responses in the neurite outgrowth of DRG sensory neurons. Cells were cultured for 2 days at different concentrations of SMS and α-tocopherol as indicated in the figure. Mean ± S.E.M. (N = 4). D and E: Comparison of mean neurite length among cultured cells treated with SMS (1 μg/ml), α-tocopherol (α-Toco, 10 μM), and 0.02% of ethanol solution as a vehicle. Mean neurite length in SMS- or α-tocopherol–treated cells was significantly longer than the vehicle control (**P<0.01, N = 4, one-way ANOVA). No significant difference in mean neurite length was found between SMS- and α-tocopherol–treated groups. Representative fluorescence images of NF-200–stained neurons are shown in photo E.
of mean neurite length showed significant increase in cells treated with 1.0 μg/ml SMS or 10 μg/ml α-tocopherol compared to the vehicle control (Fig. 1: D and E).

To examine the effects of SMS extract on injury cavity formation after SCI, we determined in vivo distribution of reactive astrocytes and CSPG in the spinal cord tissue. GFAP-labeled astrocytes were clearly observed with demarcating cell layers around the lesion cavity (Fig. 2A). The lesion cavity in SMS-treated tissue was confined to a more limited area compared to the saline control, and the trabecular meshwork within the cavity, which was identified by CSPG immunostaining, was seen more clearly in the SMS-treated group (Fig. 2A). Signals of CSPG, one of the major glial scar ingredients, were detected in the injury cavity of saline- and SMS-treated animals, but not in the intact control. Merged image analysis showed that a majority of CSPG signals were localized within the injury cavity (Fig. 2: B and C). Immunohistochemical analysis further showed that the number of cells positive for CD11β protein and Hoechst nuclear staining in the close vicinity of the lesion cavity were significantly higher in saline- or SMS-treated groups compared to the untreated control (Fig. 3: A and B). SMS treatment increased significantly the number of cells positive for CD11β protein- or Hoechst staining above the saline controls. Merged image analysis showed complete overlapping of all the signals of CD11β-positive cells with those of Hoechst-stained nuclei, but many of Hoechst-stained nuclei were devoid of CD11β signals.

To examine axonal outgrowth into the lesion cavity, elongated axons into the cavity zone was identified by immunofluorescence staining of neuron-selective NF-200 protein. In the saline-treated group, NF-200 signals were observed as scattered and fragmented entities, whereas most of the NF-200-positive signals were seen as elongated fibers with intense staining (Fig. 4).
further investigated the regrowth of CST axons around the lesion cavity by tracing DiI-labeled axons. While DiI-labeled CST axons were clearly observed within the dorsomedial white matter area, collateral arborization extending into the gray matter zone was observed as descending axons became closer to the injury site (Fig. 5A). Although the extension of the DiI-labeled CST axon bundle was disrupted mostly at the injury boundary, axonal elongation was observed as a detour path around the injury cavity (Fig. 5B). This process was much more intense in the SMS-treated group, and particularly stronger labeling was noted at the border of the injury cavity. DiI-labeling of CST axons at the caudal spinal cord was much lower in both saline-treated and SMS-treated groups compared to the rostral spinal cord sections prepared from the corresponding animals. Yet, overall staining intensity was stronger in the SMS-treated group than in the saline-treated group (Fig. 5C).

Enhanced neurite outgrowth of DRG sensory neurons in culture can be potentiated by nerve injury pretreatment (19). Here, we investigated the effects of SMS treatment on neurite outgrowth of DRG neurons that were co-cultured with non-neuronal cells prepared from injured spinal cord in adult rats. As shown in Fig. 6 (A and B), SMS treatment improved neurite outgrowth in cultured cells. Addition of a mixture of the antibiotic minocycline and the selective cAMP phosphodiesterase (PDE) inhibitor rolipram, known to facilitate regrowth of injured CNS axons (20, 21), similarly improved neurite outgrowth. It was noted that the number of non-neuronal cells and their clustered populations, as identified by Hoechst nuclear staining, were elevated by SMS treatment compared to the saline control (Fig. 6A). Furthermore, a dense population of GFAP-stained astrocytes and Hoechst-stained nuclei was seen around the soma and neurites of DRG neurons (Fig. 6C).

We have recently found that Erk1/2 and Cdc2 pathways are activated during axonal regeneration in peripheral sciatic nerves (9, 13). Here, regulation of phospho-Erk1/2 and Cdc2 proteins by SMS treatment was investigated in the dorsal spinal cord and in the motor cortex where the cell bodies of CST axons are located. Phospho-Erk1/2 was barely detected in the intact spinal cord and motor cortical tissues, but greatly increased 1 week after injury. SMS treatment significantly elevated phospho-Erk1/2 protein levels in the motor cortex but not in the injury area (Fig. 7A). Similarly, Cdc2 protein was induced in the spinal cord after injury, and further increased by SMS treatment (Fig. 7B). However, Cdc2 protein in the motor cortical...
Discussion

Growing evidence shows that herbal drugs can function to repair damaged neurons. Extracts of Hominis placenta and ginsenosides have been shown to promote peripheral axonal regeneration (22, 23), and neuroprotective effects of herbal drugs such as ginkgo biloba, danshen, and ginseng components in the injured spinal cord tissues or ischemic brain were reported (24 – 26). According to the theory of traditional Chinese medicine, SMS is believed to be effective for invigorating the vital energy and producing and converging the vital essence, which is critical for physiological regulation of the circulatory system. Recent studies have provided evidence that the protective effect of SMS is mediated by regulating the damage caused by free radicals such as...
reactive oxygen species or nitric oxide in diverse tissues and organs including the nervous system (14, 27, 28).

As an initial step to explore the potential growth-promoting activity of SMS on injured axons, neurite outgrowth of DRG sensory neurons was investigated in the presence of SMS and with \( \alpha \)-tocopherol treatment as a positive control. \( \alpha \)-Tocopherol is a well-known antioxidant and has been examined for the treatment of diverse cardiovascular and neurological disorders such as stroke, Alzheimer’s disease, amyotrophic lateral sclerosis, and SCI (29 – 32). At the molecular level, \( \alpha \)-tocopherol appears to attenuate LPS-induced production of inflammatory cytokines and NF-\( \kappa \)B and decrease TNF-\( \alpha \) expression in activated macrophages (33, 34). It was shown that \( \alpha \)-tocopherol treatment improved neurite outgrowth of cultured retinal ganglion cells (35).

Having noted that the protective effects of SMS on oxidative damages in different kind of cells or tissues might be shared mechanistically with those of \( \alpha \)-tocopherol, several studies reported similar protection by \( \alpha \)-tocopherol (14, 16, 36, 37). Our data showed that neurite outgrowth of DRG sensory neurons was enhanced to a similar level by treatment of either SMS or \( \alpha \)-tocopherol in comparison to the untreated control, which implicates physiological significance of the antioxidant activity of SMS for axonal regeneration.

Since we found growth-promoting activity of injured sciatric nerve by SMS treatment (TB Seo et al., unpublished data), we examined whether SMS has a similar effect on the induction of axonal regenerative responses after SCI. The lesion cavity, formed 2 weeks after SCI in the dorsal spinal cord and surrounded by a layer of demarcated reactive astrocytes, was confined to a smaller area in the SMS-treated group than the saline control. CSPG staining within the injury cavity was clearly observed in both saline- and SMS-treated groups. CSPG, a major constituent of glial scar acting as an inhibitory barrier against axonal regrowth, is highly expressed in reactive astrocytes and secreted into the extracellular matrix (1). Thus, its presence in the inside of the injury cavity as well as surrounding glial scar may negatively affect axonal growth in both the saline- and SMS-treated group. Yet, the filamentous pattern of astrocytic extensions, as observed in SMS-treated tissue, may form a trabecular meshwork within the cavity (Fig. 1C), which may function as a bridge for axonal outgrowth through the cavity (38, 39). The slightly enhanced elongation of CST axons in the SMS group could be attributed partly to the trabecular formation (see Fig. 4).

Considering that SMS is a mixture of numerous components extracted from three different galenicals, its effect on regenerative axons is presumably the consequence of diverse interactions at the injured tissue (i.e., direction actions on axons vs. surrounding non-neuronal cells and other components). Direct activity of SMS on injured spinal cord axons was investigated by histochemical analysis. Immunofluorescence staining of neuronal protein NF-200 revealed intense, elongated axons within the lesion cavity in SMS-treated animals, whereas fragmentary and scattered staining was seen in the saline control. Anterograde tracing of DiI-labeled CST axons further supports enhanced axonal regrowth by SMS treatment. We found intense collateral sprouting of rostral CST axons into the gray matter in both SMS- and saline-treated groups. CST axonal arborization developed more intensely within and around the injury boundary by SMS treatment. Furthermore, SMS treatment increased the signals of DiI-labeled CST axons at the caudal spinal cord. These axons might originate from the detour path surrounding the injury cavity or the direct trabecular connection through the lesion cavity. Whether the observed CST axons at the caudal zone were the consequence of \textit{bona fide} axonal regeneration or from collateral sprouting of the spared axons remains to be determined. Measurement of retrogradely-labeled cortical neurons in the motor cortical area after Di injection at the location caudal to the injury site may provide one approach to determine the levels of regenerating axons. Although increases in staining intensity of DiI-labeled axons in the SMS-treated group were subtle compared to the saline control, the accompanying physiological consequence via the correct synaptic targeting to the motor neurons in the central pattern generator (CPG) and in turn, functional recovery of motor activity might be of significance, considering the previous notion that 1% – 2% axonal regeneration could induce functional improvement (40, 41).

To examine the effects of SMS on non-neuronal cells in the injured spinal cord, we prepared a coculture of DRG sensory neurons with glial cells. The extent of neurite outgrowth of DRG sensory neurons was reduced when cocultured with the glial cells prepared from injured spinal cord (unpublished observation). SMS treatment improved neurite outgrowth of DRG neurons in culture, suggesting that SMS might suppress the inhibitory activity mediated by glial cells. Reactive astrocytes are the most abundant among non-neuronal cells in the injured CNS tissue, and thus, inhibitory molecules released from astrocytes such as CSPG could be the potential blocking target of SMS.

We further investigated whether SMS-mediated axonal regeneration is linked to molecular events in the injured neurons. The present data demonstrate robust increases of phospho-Erk1/2 and Cdc2 proteins in the
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References


