European Review for Medical and Pharmacological Sciences 2015; 19: 4434-4442

Protective role of selenium-enriched supplement on spinal cord injury through the up-regulation of CNTF and CNTF-Rα

X.-B. CHEN[1,3], H. YUAN[2], F.-J. WANG[3], Z.-X. TAN[3], H. LIU[3], N. CHEN[4]

[1]Key Laboratory of Biological Resource Protection and Utilization of Hubei Province, Enshi, Hubei, China
[2]Graduate School, Wuhan Sports University, Wuhan, Hubei, China
[3]School of Medicine, Hubei University For Nationalties, Enshi, Hubei, China
[4]College of Health Science, Hubei Exercise Training and Monitoring Key Laboratory, Hubei Provincial Collaborative Innovation Center for Exercise and Health Promotion, Wuhan Sports University, Wuhan, Hubei, China

Xianbing Chen and *Hong Yuan* contributed equally to this project

Abstract. – OBJECTIVE: Spinal cord injury (SCI), as one of the common serious spine disorders, often leads to severe neurological dysfunction and even permanent disability, which will cause heavy economical burden for family and society. Currently, selenium-enriched products have an obvious role in the protection and recovery of SCI; however, its protective mechanism is still unclear.

MATERIALS AND METHODS: In order to explore the protective effect of selenium-enriched supplement (SES) on SCI, the adult rats were randomly divided into sham operation control (SC) group, ischemia-reperfusion model (IM) group and SES pretreatment (ST) group to investigate the change of ciliary neurotrophic factor (CNTF) and its receptor-α (CNTF-Rα) during SCI in the presence of SES. The rats in IM and ST groups were subjected to the blockage of their abdominal aorta to establish the model of SCI; but the rats in SC group were subjected to sham operation without the blockage of abdominal aorta. The rats in ST group were fed with foods containing SES at the dose of equivalent 5 mg/L selenium in water before blocking their abdominal aorta. After 7 days, the rats were sacrificed to observe the structure of nerve cells through HE staining and the expression of CNTF and CNTF-Rα by Western blot, immunohistochemical, and RT-PCR methods, respectively.

RESULTS: Both protein and mRNA of CNTF and CNTF-Rα were positively expressed in rats from SC group. The mRNA expression levels of CNTF and CNTF-Rα in ST group were much higher than SCI model group.

CONCLUSIONS: SES can execute a protective role in SCI through up-regulating the expression of CNTF and CNTF-Rα.

Key Words:
Spinal cord injury, Ciliary neurotrophic factor (CNTF), Ciliary neurotrophic factor receptor-α (CNTF-Rα), Selenium-enriched supplement.

Introduction

Spinal cord injury (SCI) is one of the common serious spine disorders. It often leads to severe neurological dysfunction and even permanent disability, which will cause heavy economical burden for family and society. SCI is characteristics of high incidence, high disability and high expenditure for the recovery and treatment[1]. Therefore, SCI has been gained great attention to explore its mechanisms and effective treatment strategies. Although the continuous development of modern medicine has made marked progress in many diseases, the recovery or treatment of SCI is still a difficult medical issue worldwide. The necrosis and apoptosis of nerve cells play an important role in the progression of SCI[2]. Due to the limited capacity of spinal cord regeneration, the interventions for reducing neuronal loss may improve the prognosis of SCI, reduce secondary injury after SCI, and promote the growth and regeneration of axons, thus correspondingly inhibiting the damage of spinal signal pathways and the apoptosis of neuron and glial cells[3]. All of these are critical to improve the therapeutic efficacy of SCI.

Ciliary neurotrophic factor (CNTF) is a biologically active factor with multiple functions. The most prominent function is to promote the sur-

Corresponding Author: Ning Chen, Ph.D; e-mail: nchen510@gmail.com; Hong Liu, MD; e-mail: 2548449651@qq.com

vival of central and peripheral motor neurons, prevent the degeneration of damaged neurons, delay the occurrence of neuronal injury, and execute protective effect on damaged neurons especially motor neurons. All of these functions are significance in the regeneration and repairing after SC[4]. CNTF is widely distributed in central nervous system and peripheral nervous system. Its receptor (CNTF-Rα) is a specific receptor-binding protein. After nerve injury, the specific changing pattern and adjustment mechanism of CNTF-Rα play an important role in nerve regeneration[5].

Selenium as an essential trace element is an important foodborne antioxidant. In mammals, selenium mainly presents as the form of selenocysteine protein[6] and bioactive selenoproteins play an important role in many physiological processes[7,8]. Due to its strong antioxidant capacity, selenium can inhibit lipid peroxidation induced by free radicals, and block or reduce lipid peroxidation chain reaction[9]. In the present study, the blockage of infra-renal segment of abdominal aorta in rats was conducted to establish spinal cord ischemia-reperfusion model. The change of CNFT and CNTF-Rα in spinal cord ischemia-reperfusion model rats, and the protective effect of SES intervention on SCI caused by spinal cord ischemia were investigated. Meanwhile, the possible mechanisms of SCI caused by spinal cord ischemia-reperfusion were explored, which may provide the references for prevention and clinical treatment of spinal cord ischemia-reperfusion injury.

Materials and Methods

Reagents and Instruments

Anti-rabbit CNTF and CNTF-Rα antibodies were purchased by Santa Cruz Biotechnology (Santa Cruz Biotechnology Inc., CA, USA). The primers for RT-PCR were synthesized by Boster Company (Wuhan, China). Immunohistochemistry kit was purchased from Shanghai Biotechnology Company (Shanghai, China). RT-PCR was conducted for the gene amplification in a Mastercycler (Eppendorf Company, Hamburg, Germany), and analyzed by Gel Imaging Analysis System (Bio-Rad Laboratories, Hercules, CA, USA).

Experimental Grouping

Totally 15 Wistar adult male rats (190-250 g, 4 months old) were ordered from the Experimental Animal Center of Hubei Province. The rats were randomly divided into three groups including sham operation control group (SC, n = 5), ischemia-reperfusion model group (IM, n = 5), and selenium-enriched supplement (SES) pretreatment group (ST, n = 5). The rats from SC group were subjected to regular accessibility of feeds and water, as well as the sham operation with exposure of abdominal aorta and then the suture of abdominal cavity without the blockage of abdominal aorta. The rats from IM group were provided with regular feeding and water, as well as the blockage of the abdominal aorta for 30 minutes and then reperfusion. The rats from ST were administered with regular feeds containing 5 mg/L SES (equal to 5 mg/L sodium selenite in water) for 2 weeks prior to experiments, and then sequentially subjected to the blockage of the abdominal aorta for 30 minutes and reperfusion. Five rats from IM and ST groups were used for sample harvest and analysis at Day 7 after operation, respectively.

Establishment of Spinal Cord Ischemia-Reperfusion Animal Model

Experimental animals were subjected to intraperitoneal anesthesia using 2% sodium pentobarbital at the dose of 40 mg/kg body weight[10]. The rats were fixed on the stage in a spine position, and the hair of the abdomen was removed. The disinfection and toweling were conducted. Under the sterile conditions, the abdominal incision was conducted to expose the abdominal aorta and bilateral renal arteries. Below approximately 1 cm location of the right renal artery, the abdominal aorta artery was clamped with atraumatic clamp. The clamp was removed after arterial ischemia for 30 min, the abdominal cavity was sutured, and the erythromycin ointment was applied in the wound. Then, the spinal cord ischemia-reperfusion injury model was established. The rats from SC group were subjected to the exposure alone of abdominal aorta and, then, the abdominal cavity was closed. Intramuscular injection with conventional penicillin treatment at the dose of 400,000 units was conducted once a day. Rats were housed separately.

Specimen

After the experimental rats reached the designated time point, the anesthesia was performed again, and the sampling for different experiments was conducted as follows:

For immunohistochemical analysis: Totally 200 mL of saline was used for aortic perfusion

Table I. The primer sequences for RT-PCR.

Genes	Primers	Sequences (5'–3')
CNTF	Forward	CTTTCGCAGAGCAAACACCTC
	Reverse	ACTGTGAGAGCTCTTGAAGGAC
CNTF-Rα	Forward	AGGAGGCACCCCATGTTCAG
	Reverse	CATGTCACCTCCAGTCGACG
GAPDH	Forward	ACCCCTTCATTGACCTCAACTA
	Reverse	ATTGGGGGTAGGAACACGGAA

through the heart until the outflow of liquid was clear. Then, 400 mL of 4% neutral paraformaldehyde was used for infusion for 30 min. The L3-5 lumbar spinal segments were harvested, and fixed by 4% neutral paraformaldehyde.

For RT-PCR analysis: The rats were sacrificed, and the L3-5 lumbar spinal cord segments were subjected to washing with 0.01 mol/L phosphate buffered saline (PBS). Under the observation by a light microscope (Olympus, Tokyo, Japan), the anterior horn of the spinal cord gray matter was isolated and stored in liquid nitrogen.

HE and Nissl staining

Conventional paraffin slices with the thickness of 5 μm were subjected to HE staining. In addition, during Nissl staining, the specimens were fixed with 30% sucrose, the slices were frozen, and sectioned at the thickness of 20 μm. After the slices were washed with water and incubated in working solution for 15 min, the conventional dehydration was conducted.

Immunohistochemical Analysis of CNTF and CNTF-Rα

The spinal cord sample slices with the thickness of 10 μm from the rats in each group were stained by immunohistochemical streptavidin complexes (streptavidin peroxidase, SP). In the control group, PBS was used as the substitute of primary antibody. The pathological anterior horn of the spinal cord motor neurons was analyzed by HPIAS-1000 high-resolution color image system (Qianping Imaging Engineering Company of Wuhan Tongji Medical University, Wuhan, China).

Extraction of Total Spinal Cord RNA and RT-PCR Amplification

Five rats in each group were sacrificed, and L3-5 lumbar spinal segments were harvested and stored in phosphate buffer. Then, the spinal cord was isolated under a microscope (Olympus, Tokyo, Japan). The mRNA was extracted using TRIzol kit (Invitrogen, Carlsbad, CA, USA). Primers were synthesized by Wuhan Boster Company (Wuhan, China). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal reference. The sequences of the designed primers were listed in Table I. Gel image analyzer was used for the ratio analysis of electrophoretic band density, and the absorbance ratios of CNTF mRNA/GAPDH mRNA and CNTF-Rα mRNA/GAPDH mRNA were measured.

Extraction of Spinal cord Protein and Western Blotting Analysis

The spinal cords was harvested from the rats, and washed with PBS (pH 7.2) twice. Approximately 1 mL of RIPA lysis buffer was added to the spinal cord sample, and homogenized on ice bath. The homogenous mixture was centrifuged at 10000×g for 10 min. After the removal of the precipitate, the supernatant was collected and the protein concentration was determined by BCA method. Approximately 60 mg protein sample after boiled for 5 min was separated on SDS-PAGE, and then transferred to polyvinylidene fluoride (PVDF) membrane. The membrane with protein was blocked with 5% skim milk in PBS buffer for 2 h at room temperature. Then, the membrane was incubated with primary antibody such as CNTF (200 μg/mL, 1:200) or CNTF-Rα (200 μg/mL, 1:200) at 4°C overnight. The membrane incubated with primary antibody was washed with PBS for three times with 15 min for each time. Sequentially, the secondary antibody (HRP-labeled anti-rabbit IgG, 1:7500) was added for incubation at 37°C for 60 min. After washing the membrane with PBS for three times with 10 min each time, the chemiluminescence agent was incubated with the membrane in darkroom for 2 min and

the protein probed by primary antibody in membrane was imaged. The GAPDH was used as an internal reference.

Statistical Analysis

Statistical analysis was performed using SPSS16.0 software package (SPSS Inc., Chicago, IL, USA), and the experimental data were expressed as mean ± standard deviation (M ± SD). The analysis of variance was used to evaluate the difference between groups. $p < 0.05$ was considered statistically significant.

Results

Morphological Change of Spinal Cord Tissue

As shown in Figure 1A, a clear spinal structure of adult rats from SC group was observed after sham operation for 7 days, which revealed no obvious cavities, bleeding, inflammatory cell infiltration and edema neuron degeneration. In contrast, in the IM group, the infiltration of inflammatory cells in spinal cord tissue was obvious, but no significant bleeding area was observed. The gaps between neuronal cells re-

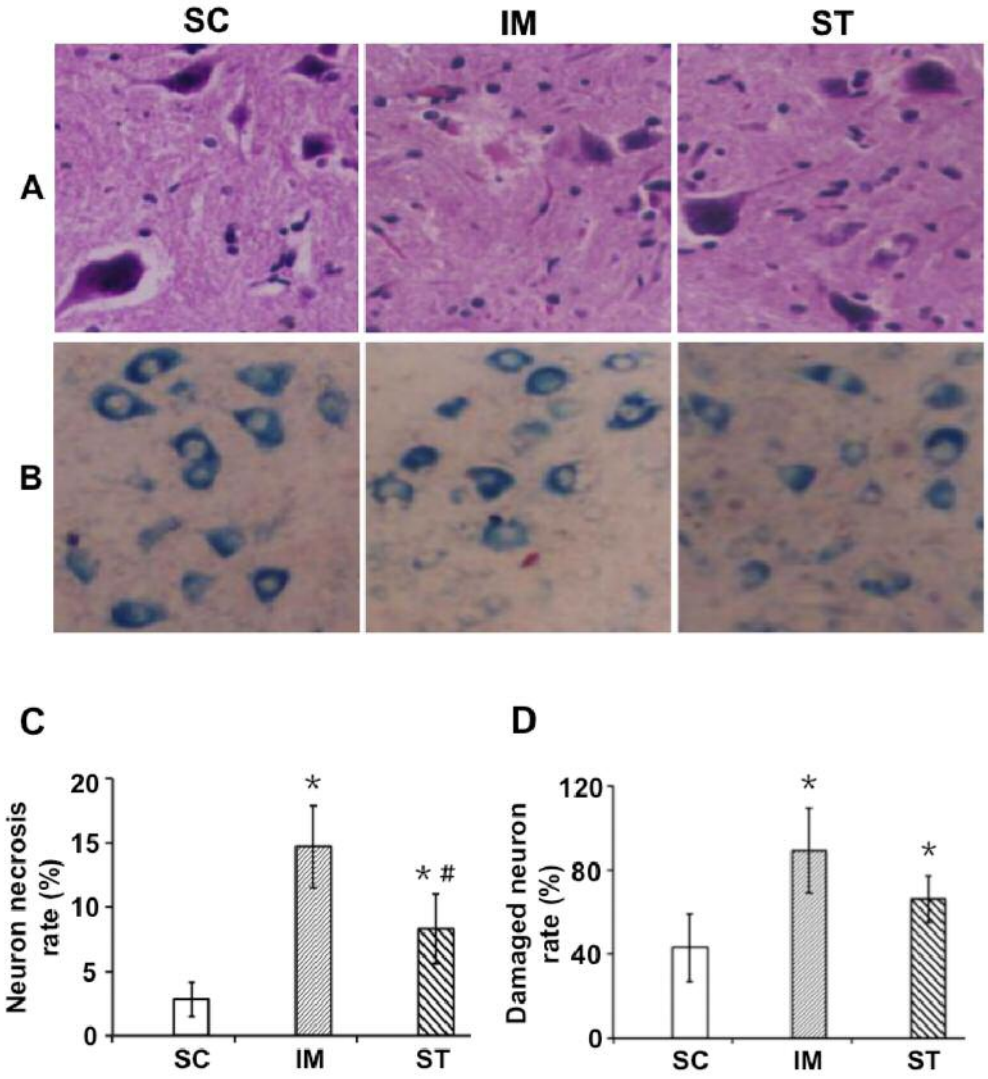

Figure 1. The images of cellular structure of spinal motor neurons with HE staining ***(A)*** and Nissl staining ***(B)*** (200×) from sham operation control group (SC), ischemia-reperfusion model group (IM) and selenium-enriched supplement (SES) pretreatment group (ST) after SCI for 7 days. Meanwhile, the necrosis rate ***(C)*** and damage rate ***(D)*** of neuron cells from SC, IM and ST groups were calculated on the basis of the statistical data from 50 image areas. $^{*}p < 0.05$ when compared with SC group; $^{\#}p < 0.05$ when compared with IM group.

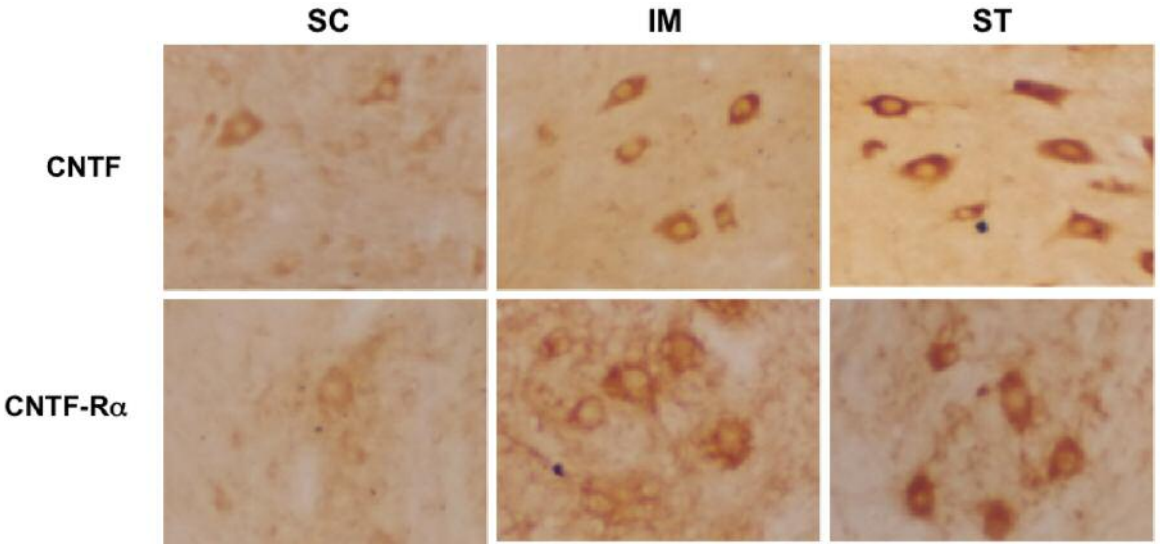

Figure 2. The expression of CNTF and CNTF-Rα in spinal cord anterior horn from sham operation control group (SC), ischemia-reperfusion model group (IM) and selenium-enriched supplement (SES) pretreatment group (ST) after SCI for 7 days evaluated by immunohistochemical analysis.

vealed an increasing trend, or reduced cell number or cell disappear was observed due to nerve cell edema and necrosis. On the other hand, in ST group, the spinal cord structure was clear, more neuronal cells survived, and less neuronal edema or necrosis was observed when compared with the IM group. Moreover, on the basis of the statistical analysis from 50 image areas, the edema or necrosis rate of neurons with was approximately 2 times more than that in the ST groups although the edema or necrosis rate of neurons from both IM and ST groups were significantly more than that in the SC group, as shown in Figure 1C.

Colored tabby-like neuron Nissl bodies in the SC group were observed and also exhibited as an even distribution, as shown in Figure 1B. On the other hand, the colored tabby-like neuron Nissl bodies in the IM and ST groups revealed a decreasing trend due to the damage of neuron cells or more necrosis of neurons. The STS pretreatment intervention could partially inhibit the damage or part recovery of neuron cells after treatment for 7 days. Similarly, according to the analysis from 50 image areas, the STS pretreatment could result in the reduced damage rate of neuron cells by approximately 86% when compared with the IM group although both IM and ST groups still revealed the more obvious damage of neuron cells than SC group, as shown in Figure 1D.

Immunohistochemical Examination of CNTF and CNTF-Rα

In order further explore the protective mechanism of selenium-enrich products on SCI, we have examined the expression of CNTF and CNTF-Rα in anterior horn motor neurons in the presence of pretreatment with SES through immunohistochemical analysis. Results indicated that anterior horn motor neurons in SC group had slight expression of CNTF and CNTF-Rα, but the expression of CNTF and CNTF-Rα in IM and ST group revealed an obvious enhancement after SCI injury for 7 days, as shown in Figure 2, which suggests that SES pretreatment could activate the expression of CNTF and CNTF-Rα for executing their protective functions during SCI.

mRNA Expression of CNTF and CNTF-Rα Evaluated by RT-PCR

Spinal cord anterior horn in SC group had high mRNA expression levels of CNTF and CNTF-Rα. Meanwhile, the mRNA expression of CNTF and CNTF-Rα in spinal cord from the IM group revealed a gradually increasing trend as the extension of injury time. Compared with the rats from SC group, the higher mRNA expression of CNTF was observed after SCI injury for 7 days, and revealed a significant difference ($p < 0.05$). Compared with IM group, SES intervention could result in an increased mRNA expres-

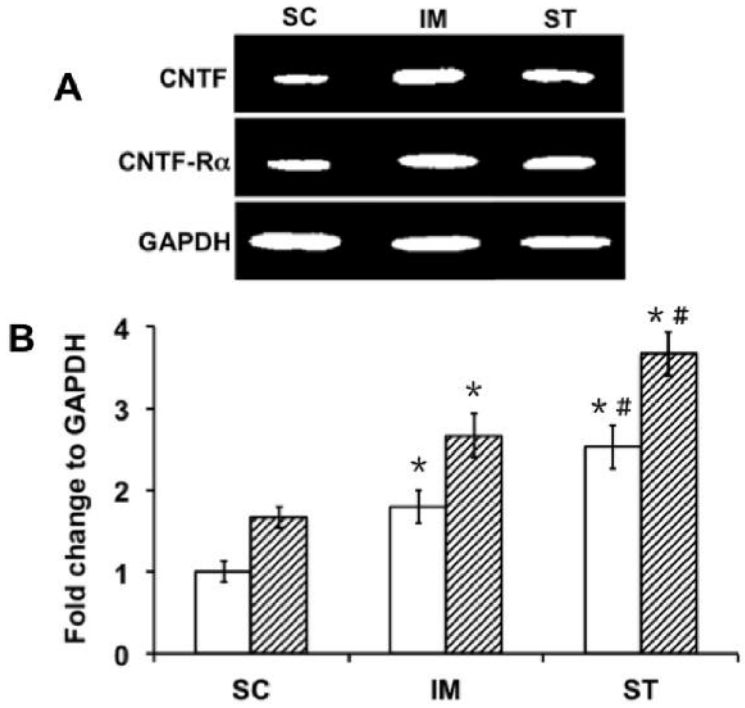

Figure 3. The relative expression levels of CNTF and CNTF-Rα mRNA in spinal cord anterior horn from sham operation control group (SC), ischemia-reperfusion model group (IM) and selenium-enriched supplement (SES) pretreatment group (ST) after SCI for 7 days. $^{*}p < 0.05$ when compared with SC group; $^{\#}p < 0.05$ when compared with IM group.

sion of both CNTF and CNTF-Rα for improving the protective role in neuron cell damage during SCI, as shown in Figure 3.

Protein Expression of CNTF and CNTF-Rα in Spinal Cord

In IM group, the protein expression of CNTF and CNTF-Rα in spinal cord revealed a gradual increase as the development of injury. Compared with SC group, an obviously increased expression of CNTF and CNTF-Rα was observed after SCI injury for 7 days, which also revealed a significant difference ($p < 0.05$). Similarly, compared with IM group, STS intervention could obviously enhance protein expression of both CNTF and CNTF-Rα, as shown in Figure 4, which further confirmed the SES-induced activation of CNTF and CNTF-Rα to promote the protective role in neuron cell damage during SCI.

Discussion

The prevention and treatment of SCI is a hot topic in the field of neuroscience. According to the theory of nutritional factor, neurotrophic factors play a very important role in the development, differentiation and damage repair of nervous system. CNTF is first discovered in eye tissue of chicken in 1976, and its name is originated from its nutritious effect on ciliary neurons. Currently, it is considered as the only neurotrophic factor with dual role for both nerve and muscle as an intracellular protein from hematopoietic factor superfamily. In recent years, a large number of investigations have shown that considerable progresses in experimental treatments of peripheral nerve injury and central nervous system diseases have made except its action mechanisms[11,12]. It not only has promoting effect on the survival of neurons and glial cells, but also has an important role in promoting the regeneration of axons, preventing the degeneration of damaged neurons, maintaining the function of motor neurons and inducing the differentiation of neurons and glial cells[13,14].

Whether neurons can maintain normal physiological function is closely correlated with neurotrophic factors. Previous researches have confirmed that the neurons from newborn rats are easy to result in the degeneration and necrosis after axonal excision, which is associated with a

low level of neural nutrition factors. Therefore, reduced level of neurotrophic factor is one of the major reasons of neuronal degeneration[15-16]. CNTF can reduce the retrogradative injury after spinal cord damage, and play a protective role in ascending motor neurons and the recovery of limb function, suggesting that CNTF can promote nerve regeneration[17]. In the present study, CNTF and CNTF-Rα were expressed in the normal state of spinal cord motor neurons with significantly different contents between different age groups (data no shown). The expression of CNTF and CNTF-Rα in youth group reached the peak level and then revealed a gradual decline, which may be related to the demand of neurotrophic factor in different age stages. Similarly, CNTF and CNTF-Rα protein of spinal cord revealed a transient increase after injury; CNTF and CNTF-Rα protein and mRNA expression revealed a gradual increase as the extension of injury. Therefore, we can speculate that CNTF as an injury factor has a protective function, which is activated after the damage of nerves. A large number of neurons and surrounding cells are readjusted to reveal more secretion of CNTF and its receptor CNTF-Rα. In order to obtain more CNTF for meeting the emergent demand of neurotrophic factor, the high expression of CNTF-Rα in neurons is required.

Previous studies have also demonstrated that the mechanism of spinal cord injury is correlated with the sensitivity of spinal motor neurons to ischemia, but free radicals play an important role in the mechanism of the ischemia-reperfusion injury[18]. Pathological process of ischemic spinal cord injury caused by hypoxia and reperfusion injury in the area of spinal cord injury can cause a large amount of oxygen free radicals, which not only can cause secondary injury of spinal cord tissue directly, but also can initiate, activate or exacerbate the secondary injury, as well as the promoting effect. Selenium as an essential trace element has a strong antioxidant capacity to fight lipid peroxidation caused by free radicals, and to prevent and reduce lipid peroxidation chain reaction. Many investigations have shown that selenium mainly exists in proteins as a form of selenocysteine, and has a very close relationship with human health. Selenocysteine is also an essential amino acid for human, which is first discovered in bacteria, archaea and eukaryotes. Compared with

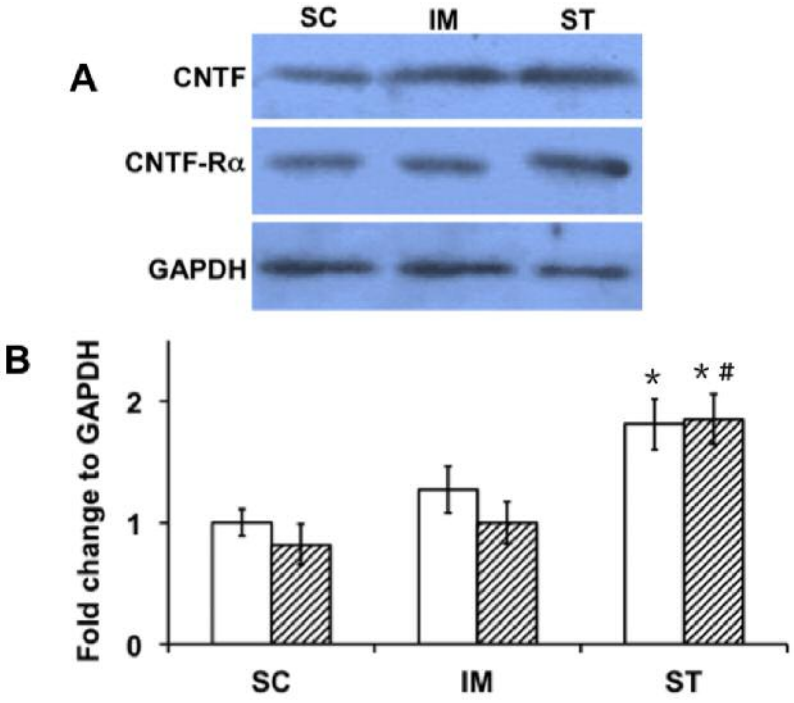

Figure 4. The relative expression levels of CNTF and CNTF-Rα protein in spinal cord anterior horn from sham operation control group (SC), ischemia-reperfusion model group (IM) and selenium-enriched supplement (SES) pretreatment group (ST) after SCI for 7 days. $^{*}p < 0.05$ when compared with SC group; $^{\#}p < 0.05$ when compared with IM group.

cysteine-containing selenium mutants, the selenium-enriched protein with selenocysteine in the active center has higher catalytic activity of the enzyme[19]. Similarly, in mammals, 25 selenium-containing protein species have been found, and 24 kinds of selenium-containing protein species have been found in rodents[20]. The biological functions of these selenoproteins are involved in various thiol-dependent catalytic reactions, which is correlation with antioxidant defense, intracellular oxidation-reduction equilibrium and thyroid hormone metabolism[21]. In detail, the biological functions of these selenoproteins focus on: the elimination of excessive reactive oxygen species (ROS) by direct catalytic reduction of hydrogen peroxide and phospholipid hydroperoxide, for example, the amino terminus of glutathione peroxidase (GSH-Px)[22-24]; the elimination of ROS by catalytic oxidation of cysteine and methionine protein residues, for example, thioredoxin reductase and methionine sulfoxide reductase[25,26]; the generation of thyroxine from thyroid hormone through removing iodine and active hormone thyroid iodine or inactivation through further iodine removal, for example, iodinated thyronine deiodinase[27]. After SES is absorbed by the body, the selenium is subjected to metabolism to encode UGA in mRNA into the first 21 amino acids to generate selenocysteine, thus executing its biological functions via the incorporation of protein and selenium protein. For example, selenium-enriched GSH-Px can catalyze the reduction of H_2O_2, and reduce the level of active oxygen to inhibit the generation of excessive free radicals, thereby reducing or delaying the formation of lipofuscin to realize the resistance to cell senescence and cell death[28].

In the present study, compared with the normal group, STS intervention can also induce the expression of CNTF mRNA in adult rats at the 7th day and 14th day after SCI injury, which revealed a significant difference ($p < 0.05$). The mRNA expression level of CNTF-Rα in adult rats at the 7th and 14th day after SCI injury also revealed a significant difference ($p < 0.05$) (data not shown). Compared with the model group, STS intervention could enhance the mRNA expression of CNTF-Rα and CNTF, which may be due to the addiction of selenium-enriched diets through a feedback requirement as the protective mechanism or a potential feedback resistance. The enhanced protein and mRNA expression of CNTF and CNTF-Rα can execute the protective effect on spinal cord injury.

Conclusions

SES can execute a protective role in SCI through up-regulating the expression of CNTF and CNTF-Rα. However, more studies are required to explore its specific mechanism, for example, the correlation between signal pathway of autophagy as the adaptive protection or resistance and CNTF or CNTF-Rα, and whether SES can induce the activation of autophagy for executing the protective role of neuron cells during SCI or how the functional status of autophagy can result in the synergistic effect of selenium-enriched products on the protection of neuron cells should be further explored.

Acknowledgements

We would like to thank the financial supports by Natural Science Foundation of Hubei Province (No. 2014CFB613) to XC from Science and Technology Department of Hubei Province, and Hubei Provincial Collaborative Innovation Center for National Culture and Tourism Industry of Wuling Mountain Area and Key Laboratory of Biological Resource Conservation and Utilization (No. PKLHB1308). Meanwhile, we would also like to thank the financial support by Chutian Scholar Program to NC from Education Department of Hubei Province, and Innovation Program to NC from Wuhan Sports University.

Conflict of Interest

The authors declare no conflict of interests.

References

1) Ackery A, Tator C, Krassioukov A. A global perspective on spinal cord injury epidemiology. J Neurotrauma 2004: 21: 1355-1370.
2) Beattie MS, Hermann GE, Rogers RC, Bresnahan JC. Cell death in models of spinal cord injury. Prog Brain Res 2002: 137: 37-47.
3) Thuret S, Moon LD, Gage FH. Therapeutic interventions after spinal cord injury. Nat Rev Neurosci 2006: 7: 628-643.
4) Ye J, Cao L, Cui R, Huang A, Yan Z, Lu C, He C. The effects of ciliary neurotrophic factor on neurological function and glial activity following contusive spinal cord injury in the rats. Brain Res 2004: 997: 30-39.
5) Sendtner M, Gotz R, Holtmann B, Thoenen H. Endogenous ciliary neurotrophic factor is a lesion factor for axotomized motoneuron in adult mice. J Neurosci 2007: 17: 6999-7006.
6) Huang K, Xu H, Liu Q. The application of selenium in chemistry, biochemistry and life science. The 2nd Edition, Wuhan: Huazhong University of Science and Technology Press, 2009: pp. 217-218.

7) Moghadaszadeh B, Beggs A. Selenoproteins and their impact on human health through diverse physiological pathways. Physiology (Bethesda) 2006: 21: 307-315.

8) Papp L, Lu J, Khanna K, Khanna KK. From selenium to selenoproteins: synthesis, identity and their role in human health. Antioxid Redox Signal 2007: 9: 775-806.

9) Short MD, Xie Y, Li L, Cassidy PB, Roberts JC. Characteristics of selenazolidine prodrugs of selenocysteine: toxicity and glutathione peroxidase induction in v79 cells. J Med Chem 2003: 46: 3308-3313.

10) Zivin J, DeGirolami U, Hurwitz E. Spectrum of neurological deficits in experimental CNS ischemia. Arch Neurol 1982: 39: 408-412.

11) Kasahra K, Nakagawa T, Kubota T. Neuronal loss and expression of neurotrophic factors in a model of rat chronic compressive spinal cord injury. Spine 2006: 31: 2059-2066.

12) Talbott J, Cao Q, Bertram J, Nkansah M, Benton RL, Lavik E, Whittemore SR. CNTF promote the survival and differentiation of adult spinal cord-derived oligodendrocyte precursor cells in vitro but fail to promote remyelinnation in vivo. Exp Neurol 2007: 204: 485-489.

13) Jakeman L, Wei P, Guan Z, Stokes BT. Related articles brain-derived neurotrophic factor stimulates hindlime stepping and sprouting of cholinergic fibers after spinal cord injury. Exp Neurol 1998: 154: 170-184.

14) Xu B, Li J, Jia L, He C, Wu H. Protective effect of ciliary neurotrophic factor on spinal neurons. J Sec Milit Med Univ 2002: 21: 409-411.

15) Albrecht P, Enterline J, Cromer J, Levison SW. CNTF-activated astrocytes release s soluble trophic activity for oligodendrocyte progenitors. Neurochem Res 2007: 32: 263-271.

16) Masu Y, Wolf E, Holtmann B, Sendtner M, Brem G, Thoenen H. Disruption of the CNTF gene results in motor neuron degeneration. Nature. 1993: 365: 27-32.

17) Geng X, Zhang Q, Liu J, Shi J, Wang S, Sun Z. Protective effect of ciliary neurotrophic factor after peripheral nerve injury on motor neurons. Chin J Clin Rehab 2004: 8: 864-865.

18) Takahashi G, Sakuri M, Abe K, Itoyama Y, Tabayashi K. MCI186 prevents spinal cord damage and affects enzyme levels of nitric oxide synthase and Cu/Zn superoxide dismutase after transient ischemia in rabbits. Thorac Cardiovasc Surg 2003: 126: 1461-1466.

19) Johansson L, Gafvelin G, Arner E. Selenocysteine in Proteins: Properties and Biotechnological application. Biochim Biophys Acta 2005: 1726: l-13.

20) Kryukov G, Castellano S, Novoselov S, Lobanov AV, Zehtab O, Guigó R, Gladyshev VN. Characterization of mammalian selenoproteomes. Science. 2003: 300: 1439-1443.

21) Shchedrina V, Zhang Y, Labunskyy V, Hatfield DL, Gladyshev VN. Structure-function relations, physiological roles, and evolution of mammalian ER-resident selenoproteins. Antioxid Redox Signal 2010: 12: 839-849.

22) Toppo S, Flohe L, Ursini F, Vanin S, Maiorino M. Catalytic mechanisms and specificities of glutathione peroxidases: variations of a basic scheme. Biochim Biophys Acta 2009: 1790: 1486-1500.

23) Novoselov SV, Kryukov GV, Xu XM, Carlson BA, Hatfield DL, Gladyshev VN. Selenoprotein H is a nuclear thioredoxin-like protein with a unique expression pattern. J Biol Chem 2007: 282: 11960-11968.

24) Takebe G, Yarimizu J, Saito Y, Hayashi T, Nakamura H, Yodoi J, Nagasawa S, Takahashi K. A comparative study on the hydroperoxide and thiol specificity of the glutathione peroxidase family and selenoprotein. J Biol Chem 2002: 277: 41254-41258.

25) Arner E. Focus on mammalian thioredoxin reductases: important selenoproteins with versatile functions. Bioehim Biophys Acta 2009: 1790: 495-526.

26) Kim H, Gladyshev V. Different catalytic mechanisms in mammalian selenoeysteine and cysteine-containing methionine-R-sulfoxide reduetases. PLoS Biol 2005: 3: 2080-2089.

27) Kuiper GG, Kester MH, Peeters RP, Visser TJ. Biochemical mechanisms of thyroid hormone deiodination. Thyroid. 2005: 15: 787-798.

28) Chen X, Deng M. Selenite alleviates spinal motor neuron injury induced by global cerebral ischemia and reperfusion in rabbits. Basic Clin Med 2008: 28: 501-503.