

Panax notoginseng saponins improve recovery after spinal cord transection by upregulating neurotrophic factors

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Abstract

Saponins extracted from *Panax notoginseng* are neuroprotective, but the mechanisms underlying this effect remain unclear. In the present study, we established a rat model of thoracic (T₁₀) spinal cord transection, and injected *Panax notoginseng* saponins (100 mg/kg) or saline 30 minutes after injury. Locomotor functions were assessed using the Basso, Beattie, and Bresnahan (BBB) scale from 1 to 30 days after injury, and immunohistochemistry was carried out in the ventral horn of the spinal cord at 1 and 7 days to determine expression of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Our results show that at 7–30 days post injury, the BBB score was higher in rats treated with *Panax notoginseng* saponins than in those that received saline. Furthermore, at 7 days, more NGF- and BDNF-immunoreactive neurons were observed in the ventral horn of the spinal cord of rats that had received *Panax notoginseng* saponins than in those that received saline. These results indicate that *Panax notoginseng* saponins caused an upregulation of NGF and BDNF in rats with spinal cord transection, and improved hindlimb motor function.

Key Words: nerve regeneration; *Panax notoginseng* saponins; spinal cord injury; nerve growth factor; brain-derived neurotrophic factor; traditional Chinese medicine; neural regeneration

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Introduction

Spinal cord injury (SCI) often results in devastating motor and/or sensory dysfunction (Anderson and Hall, 1993; Galuppo et al., 2015). This is often accompanied by other complications such as inflammation, insufficient microcirculation, ischemic reperfusion, elevation of excitatory amino acids, Ca²⁺ overload and cell apoptosis (Fernandez et al., 1993). A number of exogenous and endogenous substances aid functional recovery after SCI (Yang et al., 2014; Zhang et al., 2015). *Panax notoginseng* saponins (PNS), extracted from *Panax notoginseng*, a perennial herb of the Acanthopanax gracilistylus family, inhibit neuronal apoptosis, inflammation, and focal ischemia (Happel et al., 1981; Friedman et al., 1995; Hu et al., 1996, 1997, 2005), and may therefore be beneficial in the treatment of SCI (Jakeman et al., 1998; Ikeda et al., 2002).

Nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) are members of the neurotrophin family. They are important for neuronal survival and neurotransmission (Hao and Yang, 1986; Lessmann et al., 2003; Li et al., 2007) and enhance sprouting of corticospinal axons (Liu et al., 1991; Lykissas et al., 2007). BDNF also protects damaged neurons by increasing the expression of myelin basic protein and promoting Schwann cell and peripheral myelin formation, as well as enhancing regenerative activity (Ma et al., 1997, 1999). However, whether NGF and BDNF are involved in the neuroprotective effect exerted by PNS af-

ter SCI remains unclear.

Therefore, in the present study, we examined whether PNS would improve functional recovery in rats after spinal cord transection, and if so, whether this would be associated with increased expression of NGF and BDNF.

Materials and Methods

Animals

A total of 60 specific-pathogen-free adult male and female Sprague-Dawley rats, weighing 200–220 g, were provided by the Experimental Animal Center of Kunming Medical University, China (license No. SCXK (Dian) 2005-0008). The care and treatment of the animals were in accordance with the guidelines provided by the National Institutes of Health, USA, and all protocols were approved by the Ethics Committee of Kunming Medical University, China. All efforts were made to minimize animal discomfort and reduce the number of rats used.

The rats were equally and randomly divided into four groups: sham-operated, SCI alone, SCI + normal saline (NS), and SCI + PNS.

Establishment of spinal cord transection model

The rats were anesthetized intraperitoneally with a mixture of ketamine (50 mg/kg) and diazepam (2.5–5 mg/kg). Skin and muscle were separated, and a laminectomy was

performed. The dura mater was cut, and the spinal cord was completely transected with a pair of microscissors at the level of the T₁₀ vertebra. A piece of Gelfoam (Pfizer Inc., New York, NY, USA) was placed at the cut ends of the cord to ensure completeness of the transection, and the muscles and skin were then sutured. In the sham-operated group, the skin was incised only.

PNS administration

Thirty minutes after SCI, rats in the SCI + NS group received a single intraperitoneal injection of normal saline (0.5 mL/kg), and rats in the SCI + PNS group received intravenous PNS (100 mg/kg; aqueous solution, 25 mg/mL; approval No. GYZZ Z53020662; KPC Pharmaceuticals, Kunming, Yunnan Province, China) through the tail vein.

Evaluation of hindlimb locomotor function

Locomotor function was evaluated using the 21-point Basso, Beattie, and Bresnahan (BBB) open-field locomotor scale. Three independent observers conducted the test, and the mean of the three scores was used for each trial. Rats were tested for 4 minutes at a time. The evaluation was conducted before surgery and 1, 3, 7, 14, 21 and 30 days after surgery.

Immunohistochemistry

At 1 and 7 days after surgery, five rats in each group were anesthetized intraperitoneally with sodium pentobarbital (50 mg/kg), then perfused with 0.1 M PBS through the ascending aorta until the outflow was colourless, followed by 300 mL of 4% paraformaldehyde. Spinal cord was obtained from the injury site, postfixed in 4% paraformaldehyde for 24 hours, and stored in 20% sucrose solution at 4°C until the specimens sank to the bottom of the container. The spinal cords were frozen and cut into sections 12 µm thick in a freezing microtome (CM1900; Leica, Nussloch, Germany).

Every tenth section was processed for immunohistochemistry. Sections were washed in PBS three times for 30 minutes each time, and incubated for 30 minutes in 0.3% hydrogen peroxide at room temperature to block any endogenous peroxidase activity. After three more PBS washes, sections were incubated in PBS containing 0.3% Triton X-100 and 5% normal goat serum at 37°C for 30 minutes, then incubated for 48 hours at 4°C with rabbit anti-rat NGF monoclonal antibody (1:500; Chemicon, Santa Cruz, CA, USA) and rabbit anti-rat BDNF monoclonal antibody (1:500; Chemicon) containing 2% normal goat serum and 0.3% Triton X-100. All sections were then washed with PBS as before and incubated with biotinylated goat anti-rabbit IgG (1:200; Chemicon) at 37°C for 1.5 hours. Following three further washes in PBS for 30 minutes, the sections were incubated with an avidin-biotin-peroxidase reagent (1:100; ABC Elite, Vector Laboratories, Burlingame, CA, USA) for 2 hours at room temperature, and the antibodies were visualized in a staining solution containing 0.04% 3,3'-diaminobenzidine, 0.06% nickel sulfate and 0.06% hydrogen peroxide, for 5 minutes. After washing, sections were mounted, dehydrated, coverslipped, and observed under a light microscope,

and analyzed with the HPIAS-1000 Image Analysis System (Olympus, Tokyo, Japan). As a negative control, 0.05 M PBS was used instead of primary antibodies. The numbers of NGF- and BDNF-immunoreactive cells in the ventral horn of the spinal cord were counted as described in our previous reports (Li et al., 2008). The total number of immunoreactive neurons in each section was counted at 100× magnification (Panter et al., 1990; McTigue et al., 1998; Namiki et al., 2000).

Statistical analysis

Data are expressed as the mean ± SD. One-way analysis of variance was performed using SPSS17.0 software (SPSS, Chicago, IL, USA). A value of $P < 0.05$ was considered statistically significant.

Results

PNS improved hindlimb locomotor function in rats after spinal cord transection

All rats had a BBB score of 21 before surgery. After surgery, at all time points, the BBB score was significantly lower in all three groups that underwent spinal cord transection than in the sham-operated group ($P < 0.01$ or $P < 0.05$). The BBB score was higher in the PNS group than in the SCI group from 7 to 30 days after injury ($P < 0.05$). There was no difference in BBB score between the SCI and SCI + NS groups ($P > 0.05$; **Table 1**).

NGF and BDNF upregulation in the ventral horn of rats after spinal cord transection

Immunohistochemistry revealed large BDNF-immunoreactive neurons distributed mainly in the spinal cord gray matter. NGF immunoreactivity was distributed largely in the neuronal cytoplasm and in glial cell nuclei, whereas that of BDNF was present only in the neuronal cytoplasm. The sham-operated group showed few NGF- and BDNF-immunoreactive neurons in the ventral horn of the spinal cord. There were more NGF- and BDNF-immunoreactive neurons in the SCI group than in the sham-operated group 7 days after injury ($P < 0.01$), and more at 7 days than at 1 day after injury in the SCI group ($P < 0.01$). A greater number of NGF- and BDNF-immunoreactive neurons were observed in the SCI + PNS group than in the SCI or SCI + NS groups ($P < 0.01$ or $P < 0.05$; **Table 2**).

Discussion

We have shown here that, compared with saline, administration of PNS to rats after spinal cord transection resulted in elevated NGF and BDNF expression in the transected cords, and that this correlated with improved hindlimb motor function.

Previous studies have demonstrated the beneficial effects of PNS on aging, central nervous system disorders, and neurodegenerative diseases (Qin et al., 2006). However, few studies to date have addressed the effects of PNS on SCI, and the mechanisms underlying these effects remain poorly understood. We therefore addressed these questions in the

Table 1 Effect of PNS on Basso, Beattie, and Bresnahan scores of hindlimb locomotor function in rats after spinal cord transection

Group	Days after surgery					
	1	3	7	14	21	30
Sham-operated	15.77±0.37	18.03±0.46	21.00±0.00	21.00±0.00	21.00±0.00	21.00±0.00
SCI	0.00±0.00**	0.00±0.00**	0.80±0.27**	2.43±0.74**	3.57±0.52**	4.37±0.36**
SCI+NS	0.00±0.00**	0.00±0.00**	0.78±0.19**	2.77±0.56**	3.68±0.51**	4.36±0.48**
SCI+PNS	0.00±0.00**	0.00±0.00**	0.91±0.11*#	3.53±0.49*#	4.46±0.69*#	6.22±0.77*#

In the sham-operated group, the skin was incised but the spinal cord was not damaged. In the SCI, SCI + NS and SCI + PNS groups, the spinal cord was completely transected at T₁₀. Rats in the SCI + NS and SCI + PNS groups were injected with saline or PNS, respectively, 30 minutes after injury. Data are expressed as the mean ± SD; n = 5 rats per group per time point. *P < 0.05, **P < 0.01, vs. sham-operated group; #P < 0.05, vs. SCI group (one-way analysis of variance). NS: Normal saline; PNS: *Panax notoginseng* saponins; SCI: spinal cord injury.

Table 2 Effects of PNS on numbers of NGF- and BDNF-immunoreactive neurons in the ventral horn of rats 1 and 7 days after spinal cord transection

Group	NGF-immunoreactive neurons		BDNF-immunoreactive neurons	
	1 day	7 days	1 day	7 days
Sham-operated	6.40±1.47	6.44±1.44	8.20±1.05	8.48±1.32
SCI	5.04±1.76	9.72±1.83***§§	8.04±1.37	11.04±1.49***§§
SCI+NS	4.92±1.75	9.84±2.03***§§	8.16±1.56	11.16±1.04***§§
SCI+PNS	10.12±1.33***††	12.76±1.51***††§	11.08±1.24***††	13.84±1.35***††§

In the sham-operated group, the skin was incised but the spinal cord was not damaged. In the SCI, SCI + NS and SCI + PNS groups, the spinal cord was completely transected at T₁₀. Rats in the SCI + NS and SCI + PNS groups were injected with saline or PNS, respectively, 30 minutes after injury. Data are expressed as the mean ± SD; n = 5 rats per group per time point. *P < 0.05, **P < 0.01, vs. sham-operated group; #P < 0.05, ##P < 0.01, vs. SCI group; †P < 0.05, ††P < 0.01, vs. SCI + NS group; §P < 0.05, §§P < 0.01, vs. 1 day (one-way analysis of variance). BDNF: Brain-derived neurotrophic factor; NGF: nerve growth factor; NS: normal saline; PNS: *Panax notoginseng* saponins; SCI: spinal cord injury.

present study.

Antioxidant, anti-inflammatory, anti-apoptotic, immunostimulatory, and anti-excitotoxic activities have previously been suggested as mechanisms underlying the therapeutic effects of PNS (Qin et al., 2006). Indeed, such activities are important in functional recovery, inhibiting apoptotic and inflammatory reactions, decreasing free radicals and excitatory amino acids, and lowering calcium deposits (Tuszynski et al., 1997; Yao and Li, 2002; Wang et al., 2005; Wu et al., 2005; Radad et al., 2006). Our results indicate that PNS administration after spinal cord transection increases the number of NGF- and BDNF-immunoreactive cells in the cord. NGF and BDNF promote robust axonal sprouting, which may provide neuroprotection and enhance regenerative activity after SCI (Yoon et al., 1998; Zhao et al., 2001; Zhu et al., 2003; Zhang et al., 2007). Therefore, PNS-induced increases in NGF and BDNF expression may help restore hindlimb motor function.

In summary, our results show that administration of PNS to rats after spinal cord transection improves motor function, and suggest that the underlying mechanism involves an increase in the expression of two essential neurotrophins, NGF and BDNF. The present study will serve as a platform from which to launch more collaborative projects between clinicians and basic scientists to develop a concrete strategy for the use of PNS in the treatment of SCI. Future studies comparing the effects of PNS with other drugs used in the treatment of SCI, such as methylprednisolone (Albayrak et al., 2015), are warranted.

Author contributions: BW performed the experiment. YL designed study and modified the paper. XPL was responsible for data analysis. YL wrote the paper and aid experiments. All authors approved the final version of the paper.

Conflicts of interest: None declared.

References

- Albayrak S, Atci İB, Kalaycı M, Yılmaz M, Kuloglu T, Aydin S, Kom M, Ayden O, Aydin S (2015) Effect of carnosine, methylprednisolone and their combined application on irisin levels in the plasma and brain of rats with acute spinal cord injury. *Neuropeptides* doi: 10.1016/j.npep.2015.06.004.
- Anderson DK, Hall ED (1993) Pathophysiology of spinal cord trauma. *Ann Emerg Med* 22:987-992.
- Fernandez E, Pallini R, Lauretti L, Mercanti D, Serra A, Calissano P (1993) Spinal cord transection in adult rats: effects of local infusion of nerve growth factor on the corticospinal tract axons. *Neurosurgery* 33:889-893.
- Friedman B, Kleinfeld D, Ip NY, Verge VM, Moulton R, Boland P, Zlotchenko E, Lindsay RM, Liu L (1995) BDNF and NT-4/5 exert neurotrophic influences on injured adult spinal motor neurons. *J Neurosci* 15:1044-1056.
- Galuppo M, Rossi A, Giacompo S, Pace S, Bramanti P, Sautebin L, Mazzon E (2015) Use of Mometasone furoate in prolonged treatment of experimental spinal cord injury in mice: a comparative study of three different glucocorticoids. *Pharmacol Res* 99:316-328.
- Hao CQ, Yang F (1986) Anti-inflammatory effects of total saponins of *Panax notoginseng*. *Zhongguo Yao Li Xue Bao* 7:252-255.
- Happel RD, Smith KP, Banik NL, Powers JM, Hogan EL, Balentine JD (1981) Ca²⁺-accumulation in experimental spinal cord trauma. *Brain Res* 211:476-479.
- Hu Y, Lam BS, Chang CQ, Chan FH, Lu WW, Luk KD (2005) Adaptive signal enhancement of somatosensory evoked potential for spinal cord compression detection: an experimental study. *Comput Biol Med* 35:814-828.

- Hu ZM, Lao HC, Zhang BH, Shu J (1996) The effects of Panax Notoginseng Saponins on early experimental spinal cord injury in rats. *Zhonghua Gu Ke Zazhi* 16:384-388.
- Hu ZM, Lao HC, Zhang BH, Liu HJ, Shu J, Li GQ (1997) Histopathologic assessment of panax notoginseng saponins in rat spinal cord injury. *Zhongguo Jizhu Jisui Zazhi* 7:26-28.
- Ikeda O, Murakami M, Ino H, Yamazaki M, Koda M, Nakayama C, Moriya H (2002) Effects of brain-derived neurotrophic factor (BDNF) on compression-induced spinal cord injury: BDNF attenuates down-regulation of superoxide dismutase expression and promotes up-regulation of myelin basic protein expression. *J Neuro-pathol Exp Neurol* 61:142-153.
- Jakeman LB, Wei P, Guan Z, Stokes BT (1998) Brain-derived neurotrophic factor stimulates hindlimb stepping and sprouting of cholinergic fibers after spinal cord injury. *Exp Neurol* 154:170-184.
- Lessmann V, Gottmann K, Malsangio M (2003) Neurotrophin secretion: current facts and future prospects. *Prog Neurobiol* 69:341-374.
- Li XL, Zhang W, Zhou X, Wang XY, Zhang HT, Qin DX, Zhang H, Li Q, Li M, Wang TH (2007) Temporal changes in the expression of some neurotrophins in spinal cord transected adult rats. *Neuropeptides* 41:135-143.
- Li XL, Liu J, Wang XY, Li LY, Ni W, Zheng RY, Yang HJ, Lu YC, Qi JG, Wang TH (2008) Temporal changes in the expression of TGF-beta 1 and EGF in the ventral horn of the spinal cord and associated precentral gyrus in adult Rhesus monkeys subjected to cord hemisection. *J Neurol Sci* 268:163-171.
- Liu D, Thangnipon W, McAdoo DJ (1991) Excitatory amino acids rise to toxic levels upon impact injury to the rat spinal cord. *Brain Res* 547:344-348.
- Lykissas MG, Batistatou AK, Charalabopoulos KA, Beris AE (2007) The role of neurotrophins in axonal growth, guidance, and regeneration. *Curr Neurovasc Res* 4:143-151.
- Ma L, Xiao P, Guo B, Wu J, Liang F, Dong S (1999) Cerebral protective effects of some compounds isolated from traditional Chinese herbs. *Zhongguo Zhong Yao Za Zhi* 24:238-239, 256-inside back cover.
- Ma LY, Xiao PG, Liang FQ, Chi MG, Dong SJ (1997) Effect of saponins of Panax notoginseng on synaptosomal 45Ca uptake. *Zhongguo Yao Li Xue Bao* 18:213-215.
- McTigue DM, Horner PJ, Stokes BT, Gage FH (1998) Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. *J Neurosci* 18:5354-5365.
- Namiki J, Kojima A, Tator CH (2000) Effect of brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 on functional recovery and regeneration after spinal cord injury in adult rats. *J Neurotrauma* 17:1219-1231.
- Panter SS, Yum SW, Faden AI (1990) Alteration in extracellular amino acids after traumatic spinal cord injury. *Ann Neurol* 27:96-99.
- Qin DX, Zou XL, Luo W, Zhang W, Zhang HT, Li XL, Zhang H, Wang XY, Wang TH (2006) Expression of some neurotrophins in the spinal motoneurons after cord hemisection in adult rats. *Neurosci Lett* 410:222-227.
- Radad K, Gille G, Liu L, Rausch WD (2006) Use of ginseng in medicine with emphasis on neurodegenerative disorders. *J Pharmacol Sci* 100:175-186.
- Tuszynski MH, Murai K, Blesch A, Grill R, Miller I (1997) Functional characterization of NGF-secreting cell grafts to the acutely injured spinal cord. *Cell Transplant* 6:361-368.
- Wang TT, Yuan Y, Kang Y, Yuan WL, Zhang HT, Wu LY, Feng ZT (2005) Effects of acupuncture on the expression of glial cell line-derived neurotrophic factor (GDNF) and basic fibroblast growth factor (FGF-2/bFGF) in the left sixth lumbar dorsal root ganglion following removal of adjacent dorsal root ganglia. *Neurosci Lett* 382:236-241.
- Wu X, Yoo S, Wrathall JR (2005) Real-time quantitative PCR analysis of temporal-spatial alterations in gene expression after spinal cord contusion. *J Neurochem* 93:943-952.
- Yang X, Liu J, Liu ZJ, Xia QJ, He M, Liu R, Liu W, Wang W, Liu J, Zhou XF, Zhang YH, Wang TH (2014) Reversal of bone cancer pain by HSV-1-mediated silencing of CNTF in an afferent area of the spinal cord associated with AKT-ERK signal inhibition. *Curr Gene Ther* 14:377-388.
- Yao XH, Li XJ (2002) Protective effects and its mechanism of panaxatriol saponins isolated from Panax notoginseng on cerebral ischemia. *Zhongguo Zhong Yao Za Zhi* 27:371-373.
- Yoon KW, Fuse T, Shah PT, Nguyen S, Klein ML (1998) Indirect glutamate neurotoxicity. *J Neurotrauma* 15:141-147.
- Zhang HT, Li LY, Zou XL, Song XB, Hu YL, Feng ZT, Wang TT (2007) Immunohistochemical distribution of NGF, BDNF, NT-3, and NT-4 in adult rhesus monkey brains. *J Histochem Cytochem* 55:1-19.
- Zhang X, Shi LL, Gao X, Jiang D, Zhong ZQ, Zeng X, Rao Y, Hu X, Li TZ, Li XJ, Li L, Chen JM, Xia Q, Wang TH (2015) Lentivirus-mediated inhibition of tumour necrosis factor- α improves motor function associated with PRDX6 in spinal cord contusion rats. *Sci Rep* 5:8486.
- Zhao FD, Wang KZ, Fan SW (2001) The mechanism of secondary injury after spinal cord injury. *Zhongguo Jiaoxing Waike Zazhi* 8:395-398.
- Zhu LQ, Fan JP, Huang QF, Sun SL, Gao Y, Zou YH, Zhang Z, He LY, Zheng H (2003) Study on the anti-apoptosis induced by hypoxia/hypoglycemia and reoxygenation of panax notoginseng saponins in cultured rat hippocampal neurons. *Zhongguo Zhong Yao Za Zhi* 28:52-55.

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