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Original Article

Down-regulation of GAP-43 by inhibition of caspases-3 in a rat model of neuropathic pain

Feixiang Wu[1], Xuerong Miao[1], Jiaying Chen[1], Yuming Sun[1], Zhiqiang Liu[2], Yong Tao[1], Weifeng Yu[1]

[1]Department of Anesthesiology, Eastern Hepatobiliary Hospital, the Second Military Medical University, No. 225 Changhai Road, Shanghai 200433, China; [2]Department of Anesthesiology, Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, No. 536 Changle Road, Shanghai 200126, China

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Abstract: Background Neuropathic pain remains a prevalent and persistent clinical problem due to incomplete understanding of its pathogenesis. Objective The present study aimed to investigate the role of caspase-3 in the neuropathic pain in rats with chronic constriction injury (CCI). Methods SD rats were randomly assigned four groups (n=18 per group): sham group, normal saline group (NS group), Z-DEVD-FMK group (DEVD group) and RNA interference group (siRNA group). Z-DEVD-FMK (1 U/30 μl), siRNA targeting caspase-3 (10 μg/30μl) and NS of equal volume were intrathecally administered once daily for 5 days starting 1 day before surgery in the DEVD, siRNA and NS group, respectively. Thermal hyperalgesia was assessed at one day before and 1, 2, 4, 5, 6, 7 and 10 days after surgery. The mRNA and protein expressions of caspase-3 were measured by real time PCR and immunofluorescence assay. Apoptosis was detected by TUNEL staining. GAP-43 expression was measured by immunofluorescence and western blot assays. Results The right paw withdrawal latency (PWL) was decreased after CCI (P<0.05). TUNEL-positive neurons and the mRNA and protein expressions of caspase-3 in the spinal cord were increased significantly. After Z-DEVD-FMK or siRNA treatment, TUNEL-positive neurons were decreased, PWLs increased (P<0.05) and the mRNA and protein expressions of caspase-3 decreased. The expression of GAP-43, a sprouting related protein, was decreased in the DEVD and siRNA group as compared to NS group (P<0.05). Up-regulation of GAP-43 following CCI was decreased following caspase-3 inhibition. Following sciatic nerve ligation, the gene expression, translation and transcription are significantly changed in the neurons which finally results in neuron apoptosis. The neuron apoptosis induce the up-regulation of GAP-43 expression leading to hyperalgesia. Conclusion Caspase-3 mediated neuron apoptosis is probably responsible for the neuropathic pain in CCI rats. Inhibition of caspase-3 may serve as a treatment of neuropathic pain.

Keywords: Apoptosis, caspase-3, neuropathic pain, GAP-43, RNA interference

Introduction

Neuropathic pain, characterized by hyperalgesia, allodynia and spontaneous pain, often occurs as a result of injuries to the peripheral nerves, dorsal root ganglion (DRG), spinal cord or brain [1]. Neuropathic pain remains a prevalent and persistent clinical problem due to incomplete understanding of its pathogenesis.

In recent years, studies in the neurobiological fields have found important clues showing apoptosis may be responsible for cell damage and neuropathic pain. De Novellis et al [2] revealed that apoptotic pathways were activated in rat spinal cord following chronic constriction injury (CCI) of the sciatic nerve, and blockade of glutamate mGlu5 receptors may prevent early over-expression of pro-apoptotic genes and morphological changes in dorsal horn. Kimberly et al [3] demonstrated that the excitotoxic death of inhibitory neurons in the laminas I-III of the spinal dorsal horn contributed to the neuropathic pain. The study of Joseph et al [4] indicated that in the latent phase, before the presence of apoptotic cell death, the caspase signaling pathway contributed to the pain in the small-fibre peripheral neuropathy, and inflammatory / immune mediators involved in the activation of these pathways.

Pathological pain is an expression of neuronal plasticity. Growth associated protein 43 (GAP-43) is considered as an internal decision factor

in the nerve regeneration and serves as a marker of neural plasticity [5]. In the L5 spinal nerve transection model of mechanical pain hypersensitivity, the spinal GAP-43 was found to be up-regulated in the superficial L5 dorsal horn from 5 up to 10 days after injury [6]. Whether the up-regulation of GAP-43 is involved the caspase signaling pathway leading to the neuropathic pain is still unknown.

In present study, caspase-3 siRNA and inhibitor were intrathecally administered to rats with CCI to investigate the role of caspase-3 and GAP-43 in the caspase signaling pathway related neuropathic pain.

Materials and methods

Animals

Male Sprague-Dawley rats (200-250g, Shanghai Experimental Animal Center of Chinese Academy of Sciences) were housed were housed in a SPF laboratory with a 12/12 h light /dark. All animal experiments were approved by the Administrative Committee of Experimental Animal Care and Use of Shanghai, and conformed to the National Institute of Health guidelines on the ethical use of animals. Animals were randomly assigned four groups (n=18 per group): sham group, normal saline group (NS group), Z-DEVD-FMK group (DEVD group), and RNA interference group (siRNA group). Z-DEVD-FMK (1 U/30 μl), siRNA targeting caspase-3 (10 μg/30 μl) and NS of equal volume were administered intrathecally once daily for 5 days starting 1 day before surgery in the DEVD, siRNA and NS groups, respectively. The siRNA (GAUACCAGUGGAGGCCGAC) targeting caspase-3 gene (siRNA-caspase-3) was screened and tested in our previous study [7].

Chronic constriction injury

The CCI model was established as previously described [8]. Briefly, rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The common sciatic nerve was exposed at the mid-thigh level. The nerve was ligated loosely with a 4-0 chromic gut thread at 4 sites with an interval of 1 mm, so that the nerve diameter was only slightly reduced. Meanwhile, a sham surgery was performed with the sciatic nerve exposure but absence of ligation. Upon recovery from anesthesia, animals were housed individually in clear plastic cages.

Lumbar subarachnoid catheterization

One week prior to CCI, a chronic indwelling catheter was implanted into the subarachnoid space of each rat. Briefly, rats were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). A PE-10 catheter (Becton Dickinson, Sparks, MD, USA) was inserted into the lumbar subarachnoid space between 5th and 6th lumbar vertebrae (L5 and L6) [9]. The catheter was slowly implanted and the external portion was protected according to Milligan's method [10].

Evaluation of thermal hyperalgesia

Thermal hyperalgesia was assessed by the paw withdrawal latency (PWL) to radiant heat according to the described by Hargreaves et al [11]. Rats were placed in an inverted clear plexiglas cage (23×18×13 cm) on a piece of 3-mm-thick glass plate and allowed to acclimate to their environment for 30 min before testing. After acclimation, the radiant heat source was positioned under the glass floor directly beneath the hind paw. The radiant heat source consisted of a high-intensity projection lamp bulb (8 V, 50 W) and located at 40 mm below the glass floor projecting through a 5×10-mm aperture on the top of a movable case. A digital timer automatically recorded the duration between the stimuli and the paw withdrawal which was defined as PWL. Experiment was carried out thrice in each rat with a 5-min interval. The heat exposure was confined to 20 sec to avoid tissue damage.

Real time PCR

The caspase-3 mRNA expression was detected by real time PCR using primers 5'-TATGGAATTGATGGATAGT-3'and5'-CTGAAGAA-ACTAGTTAGTT-3'. A Real-Time PCR Detection System (Roche, Switzerland) was used to continually monitor the fluorescence intensity which was directly proportional to the PCR product.

Western blot assay

Nuclear extracts were prepared from lumbar spinal cord (L4-L5) as previously described [12]. Proteins were separated on 8% SDS-PAGE

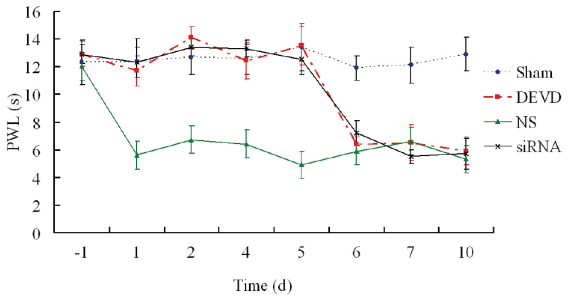

Figure 1. Antinociceptive effect of intrathecal injection of siRNA-Caspase-3 or Z-DEVD-FMK on chronic neuropathic pain. Rats were administered with siRNA-caspase-3 or Z-DEVD-FMK one day before CCI, and then nociception response was monitored during and after treatment. Following administration of caspase-3 inhibitor or siRNA, PWL was not significantly reduced in the siRNA group or DEVD group and similar to that in the sham group (P>0.05) but markedly different from that in the NS group (P<0.01).

gel and transferred onto a nitrocellulose membrane which was blotted with primary antibody against GAP-43 (1:100, Santa Cruz, CA, USA) and then with horseradish peroxidase conjugated secondary antibody. Protein signals were detected with an ECL system (Amersham Pharmacia Biotech, Uppsala, Sweden). β-actin (Sigma, St. Louis, MO, USA, 1:500) served as an internal control.

Immunofluorescence assay

After defined survival times, rats were anesthetized and perfused through the ascending aorta with saline, followed by 4% paraformaldehyde in 1.5% picric acid in 0.16 M phosphate buffer (pH 7.2-7.4). After perfusion, the L5 spinal cord segments were removed and postfixed in the same fixation solution for 3 hr and then in 15% sucrose overnight. Transverse spinal sections (30 μm) were obtained on a cryostat and processed for immunostaining with immunofluorescence assay [13]. All of the sections were blocked with 2% goat serum in 0.3% Triton X-100 for 1 hr at room temperature and incubated over two nights at 4°C with anti-caspase antibody (1:400; Santa Cruz, USA). The sections were then incubated for 1 hr at room temperature with Cy3-conjugated secondary antibody (1:300; Santa Cruz, USA), followed by a mixture of Alexa Fluor 555-conjugated secondary antibodies for 1 hr at room temperature. These sections were examined under an Olympus (Olympus, Japan) fluorescence microscope, and representative images were captured with a camera.

TUNEL analysis

The sections of L5 were incubated with proteinase K for 15 min at room temperature. After rinsing in PBS-Tween 20 for 2 min twice, sections were incubated in 3% H2O2 in PBS for 10 min to block endogenous peroxidase activity. After washing with PBS twice, sections were labeled (60 min; 37℃) with fluorescein TUNEL reagent mixture according to the manufacturer's instructions [14]. After rinsing with PBS thrice, these sections were visualized under an Olympus (Olympus, Japan) inverted microscope, and representative images were captured with a camera.

Statistical analyses

Data were expressed as mean ± standard deviation (SD). Statistical analyses were performed using Student's t-test or multiple ANOVA followed by least-significance difference post-hoc comparison. A value of P<0.05 was considered statistically significant.

Results

Detection of thermal hyperalgesia

Before ligation of the sciatic nerve, PWL remained unchanged following administration of caspase-3 inhibitor or siRNA targeting caspase-3(siRNA-caspase-3) (P>0.05). However, PWL was significantly reduced in the NS group following ligation when compared with sham group (P<0.01) (**Figure 1**). Following administration of caspase-3 inhibitor or siRNA-caspase-3, PWL was not significantly reduced in the siRNA group or DEVD group and similar to that in the sham group (P>0.05) but markedly different from that in the NS group (P<0.01). After treatment discontinuation, PWL was dramatically reduced in the siRNA group or DEVD group and significantly different from that in the sham group (P<0.01). However, no marked difference was found between siRNA group and DEVD group (P>0.05). These findings suggest that inhibition of caspase-3 attenuates the thermal hyperalgesia in CCI rats.

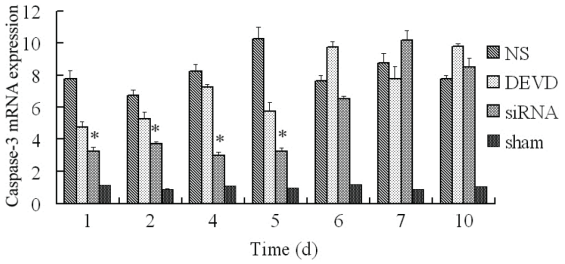

Figure 2. Real-time quantitative RT-PCR analyses of Caspase-3 mRNA expression in rat lumbar spinal cord. *P<0.01VS NS group.

Detection of caspase-3 mRNA expression by real-time PCR

To confirm that siRNA-caspase-3 interfered the caspase-3 expression, real time quantitative PCR was employed to detect the mRNA expression of caspase-3 in the L4~6 (**Figure 2**). Results showed, the mRNA of caspase-3 at 1, 2, 4, and 5 days after siRNA treatment was significantly lower than in the NS group (P<0.01) suggesting that siRNA targeting caspase-3 markedly inhibited the mRNA expression of caspase-3.

Caspase-3 expression in rat spinal cord

The caspase-3 expression in the spinal cord was determined by immunofluorescence assay (**Figure 3**). In the sham group, the caspase-3 expression was low in ipsilateral side of ligation and similar to that contralateral side(**Figure 3A**). Following ligation, the caspase-3 expression was dramatically increased in the NS group (**Figure 3B**). After treatment with caspase-3 inhibitor or siRNA, the caspase-3 expression was significantly reduced when compared with NS group, but similar to or slightly higher than that in the sham group (**Figure 3C** and **3D**). These findings suggest that ligation of sciatic nerve may result in increase of caspase-3 expression, and treatment with Z-DEVD-FMK or siRNA targeting caspase-3 can significantly inhibit the up-regulation of caspase-3 following ligation.

TUNEL staining of apoptotic cells in the spinal cord

As shown in **Figure 4**, the apoptotic neurons were seldom found in the dorsal horn of sham group. In the NS group, a large number of apoptotic neurons were found which were characterized by yellowish brown granules in the cells. Following treatment with caspase-3 inhibitor or siRNA, the apoptotic neurons were markedly reduced when compared with NS group but similar to or slightly higher than in the sham group. These findings imply that ligation of sciatic nerve may induce the neuron apoptosis, and treatment with Z-DEVD-FMK or siRNA targeting caspase-3 can significantly inhibit the neuron apoptosis and blockage of caspase-3 can reduce the apoptotic neurons.

Detection of GAP-43 expression by immunofluorescence and western blot assay

As shown in **Figure 5**, the GAP-43 was massively expressed in the ipsilateral dorsal horn (red) following ligation, but less found in the contralateral side without ligation. Western blot assay revealed the GAP-43 expression in the siRNA group and DEVD group was significantly lower than that in the NS group (**Figure 6**). These results indicate that inhibition of caspase-3 signaling pathway can reduce the GAP-43 expression.

Discussion

Chronic pain refers to the persistent pain after the cause of acute pain is removed or the pain accompanied by the chronic disease and it usually lasts for more than 3 months [15]. The therapeutic efficacy of neuropathic pain is still unsatisfactory due to the complicated pathogenesis. Apoptosis is similar to the neuronal plasticity in pathology [16], and can explain some problems difficult to explain in the pain theory. For example, neuropathic pain is less responsive to calcium channel blocker, which may be attributed to the pre-existing apoptosis resulting in poor response to blocking of upstream of apoptosis signaling pathway. Apoptosis is a self-destructive programmed biochemical process. Morphologically, apoptosis is characterized by karyopyknosis and cytoplasmic concentration and breakage of chromatins. Apoptosis is often mediated by the caspase related protein cleavage in which caspase-3 plays an important role [17].

To further confirm the association between neuron apoptosis and neuropathic pain, caspase-3 inhibitor (Z-DEVD-FMK) and siRNA targeting caspase-3 were used to block the apop-

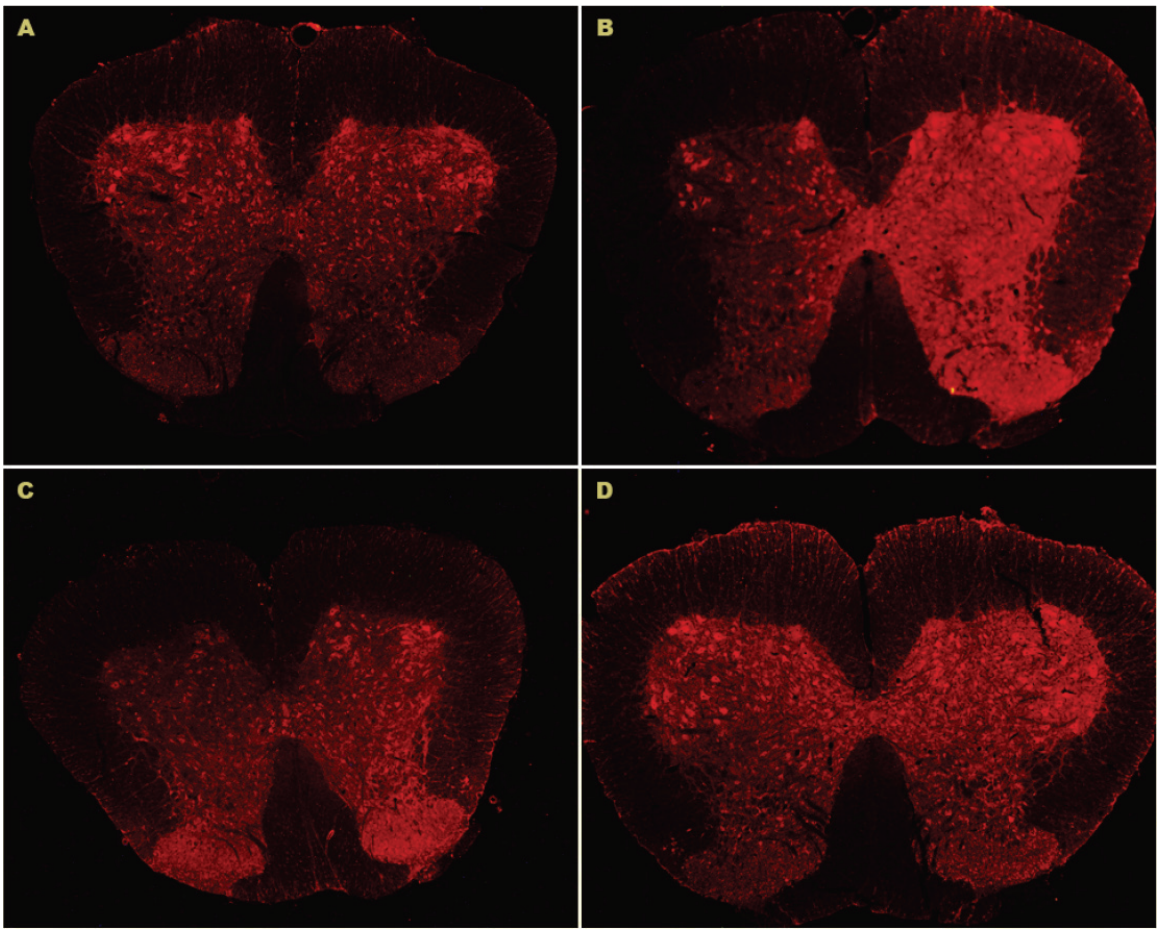

Figure 3. Caspase-3 expression in spinal cord by immunofluorescent assay. In the sham group, the caspase-3 expression was low in ipsilateral side of ligation and similar to that contralateral side (Figure 3A). Following ligation, the caspase-3 expression was dramatically increased in the NS group (Figure 3B). The caspase-3 expression was significantly reduced in the siRNA group or DEVD group (Figure 3C, D).

tosis signaling pathway. Results showed both treatments could significantly inhibit the apoptosis of neurons and the thermal hyperalgesia following sciatic nerve ligation. These findings indicate caspase-3 pathway mediated apoptosis of neurons in the spinal cord plays a critical role in the pathogenesis of neuropathic pain and blockage of caspase-3 pathway may serve as a novel strategy for the treatment of neuropathic pain.

However, it still raises a question that why the apoptotic neurons which execute signal transmission can produce hyperalgesia? In the neuropathic pain, the damage to the neural system may lead to cascade reaction resulting in neuron apoptosis and the axonal degeneration in the injured site. The components of cytoskeleton are degraded and the terminal axoplasm becomes granular. Finally, the cells shrink and are fragmented. The cell debris is swallowed by macrophages and some glial cells are subsequently activated resulting in secretion of a series of molecules which leads to the proliferation and migration of cells to counteract injury [18]. The proximal swelling axons are separated from the distal stump and some axons become degenerated and others sprouting. The peripheral and central mechanisms of sprouting induced neuropathic pain participate in the pathogenesis of hyperalgesia. For peripheral mechanism, the damage to the nerves induces the recruitment of ion channels into the injured sites and DRG which leads to the non-coding ectopic electrical activity. When the damage to axons results in the myelin loss in the myelinated neurons and formation of neuroma, the insulation between nerve fibers is compro-

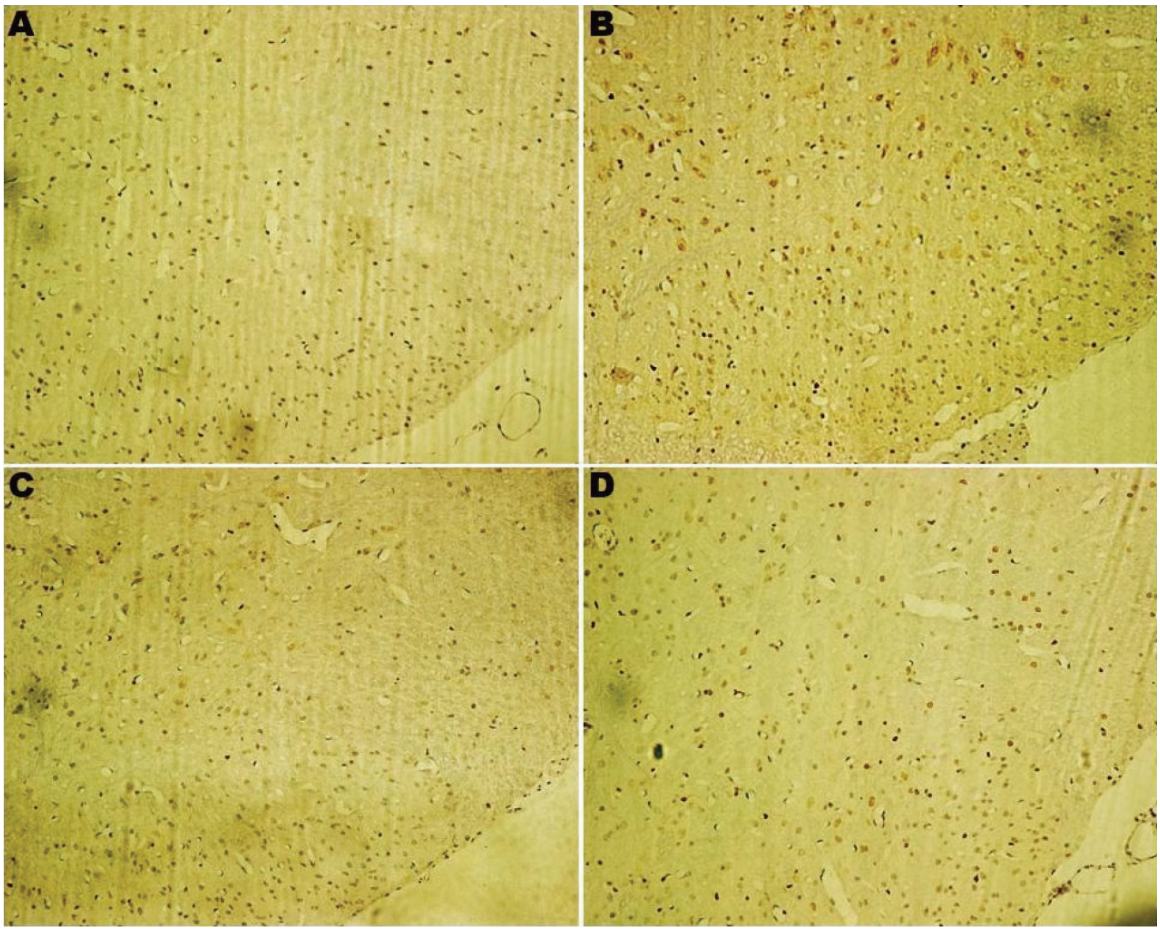

Figure 4. Detection of apoptotic neurons by TUNEL analysis. A large number of apoptotic neurons (yellow brown granules) were found in the NS group. Following treatment with caspase-3 inhibitor or siRNA, the apoptotic neurons were markedly reduced. A: sham group; B: NS group; C: ZDEVD group; D: siRNA group.

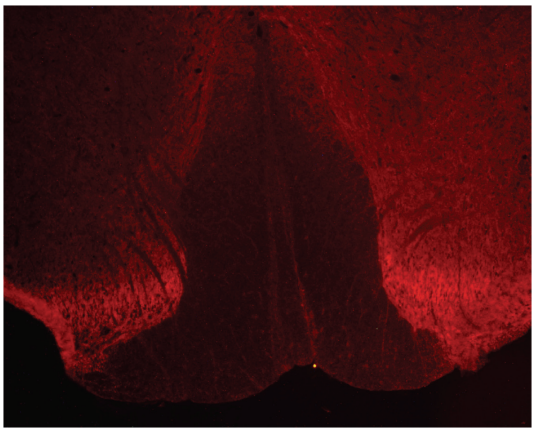

Figure 5. Detection of GAP-43 expression by immunofluorescence staining. The GAP-43 was massively expressed in the ipsilateral dorsal horn (red) following ligation, but less found in the contralateral side without ligation (×100).

mised. When a nerve fiber is activated, the depolarized potential can spread into the adjacent resting fibers and elicit the discharge, which is also known as crosstalk [19]. For the central mechanism, the damage to peripheral nerves may result in morphological, histological and electrophysiological changes in neurons in the dorsal horn: 2 weeks after CCI, a lot of dark neurons were found in the spinal cord [20] which contains numerous wide dynamic range neuron (WDR) and nociceptive specific (NS) neurons. These dark neurons had active metabolism under a light microscope and were closely related to the occurrence of pain. Electrophysiologically, WDR and NS neurons had increased excitability and spontaneous discharge.

GAP-43 plays an important role in the sprouting. It is known that neurons with GAP-43

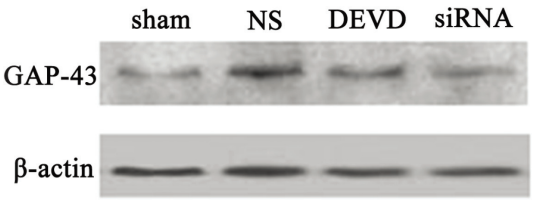

Figure 6. Detection of GAP-43 expression by western blot assay. Western blot assay revealed the GAP-43 expression in the siRNA group and DEVD group was significantly lower than that in the NS group.

expression have high plasticity and the changes in the plasticity may induce the chronic pain. During the neuron sprouting, the GAP-43 is massively synthesized accompanied by the growth of axons and has been applied as a marker of axon growth [21]. In the nerve injury model, the GAP-43 expression is up-regulated accompanied by nerve sprouting. When the pain is improved, the GAP-43 expression is down-regulated. These findings suggest GAP-43 is an important cause of sprouting and neuropathic pain [22, 23]. In the presence study, the rat sciatic nerve was ligated to induce the neuropathic pain. Our results showed the GAP-43 in the ipsilateral dorsal horn of spinal cord with ligation was significantly increased when compared with contralateral side. This result implies that there is axon growth in the dorsal horn of spinal cord following damage to peripheral nerves. At the same time, our results also showed the caspase-3 expression and the number of apoptotic neurons were dramatically increased in the ipsilateral dorsal horn. These findings reveal the potential correlation between neuron apoptosis and sprouting. In addition, caspase-3 inhibitor (Z-DEVD-FMK) and caspase-3 siRNA were applied to block the caspase-3 apoptosis signaling pathway. Results showed the GAP-43 expression was significantly inhibited following inhibition of caspase-3 by its inhibitor or siRNA. This also shows that inhibition of neuron apoptosis can attenuate the sprouting, which further confirms the association between the apoptosis of neurons in the spinal cord and sprouting, and the neuron apoptosis and sprouting may involve the changes in neuron plasticity and hyperalgesia.

Following sciatic nerve ligation, the gene expression, translation and transcription are significantly changed in the neurons which finally results in neuron apoptosis. The neuron apoptosis induce the up-regulation of GAP-43 expression leading to hyperalgesia. Thus, we speculate that the increased expression of caspase-3 following sciatic nerve ligation activates the apoptosis pathway resulting in increase of neuron apoptosis and also elevates the GAP-43 expression. Both caspase-3 and GAP-3 involve the neuropathic pain. Blocking caspase-3 signaling pathway can reduce the apoptotic neurons and down-regulate GAP-43 expression, which may become a promising strategy for the treatment of neuropathic pain.

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Conflict of interest statement

Authors declared no conflict of interests.

Address correspondence to: Dr. Weifeng Yu, Department of Anesthesiology, Eastern Hepatobiliary Hospital, No. 225 Road Changhai, Shanghai 200433, China. Tel: +86-021-81875231; Fax: +86-021-81875231; Email: ywf808@smmu.edu.cn

References

[1] Bouhassira D, Lantéri-Minet M, Attal N, Laurent B, Touboul C. Prevalence of chronic pain with neuropathic characteristics in the general population. Pain 2008; 136: 380-387.

[2] de Novellis V, Siniscalco D, Galderisi U, Fuccio C, Nolano M, Santoro L, Cascino A, Roth KA, Rossi F, Maione S. Blockade of glutamate mGlu5 receptors in a rat model of neuropathic pain prevents early overexpression of pro-apoptotic genes and morphological changes in dorsal horn lamina II. Neuropharmacology 2004; 46: 468–479.

[3] Moore KA, Kohno T, Karchewski LA, Scholz J, Baba H, Woolf CJ. Partial peripheral nerve injury promotes a selective loss of GABAergic inhibition in the superficial dorsal horn of the spinal cord. J Neurosci 2002; 22: 6724–6731.

[4] Joseph EK, Levine JD. Caspase signalling in neuropathic and inflammatory pain in the rat. Eur J Neurosci 2004; 20: 2896-2902.

[5] Verzé L, Paraninfo A, Ramieri G, Viglietti-Panzica C, Panzica GC. Immunocytochemical evidence of plasticity in the nervous structures of the rat lower lip. Cell Tissue Res 1999; 297: 203-211.

[6] Jaken RJ, van Gorp S, Joosten EA, Losen M, Martínez-Martínez P, De Baets M, Marcus MA, Deumens R. Neuropathy-induced spinal GAP-

43 expression is not a main player in the onset of mechanical pain hypersensitivity. J Neurotrauma 2011; 28: 2463-2473.
[7] Wu FX, Yu WF, Yuan Y, Miao X, Xu X, Huang S, Sun Y. Adenoviral vector mediated-expression of caspase-3 siRNA on apoptosis induced by lipopolysaccharide. J Med Coll PLA 2009; 24: 266-273.
[8] Bennett GJ, Xie YK. A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33: 87-107.
[9] Størkson RV, Kjørsvik A, Tjølsen A, Hole K. Lumbar catheterization of the spinal subarachnoid space in the rat. J Neurosci Methods 1996; 65: 167-72.
[10] Milligan ED, Hinde JL, Mehmert KK, Maier SF, Watkins LR. A method for increasing the viability of the external portion of lumbar catheters placed in the spinal subarachnoid space of rats. J Neurosci Methods 1999; 90: 81-86.
[11] Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988; 32: 77-88.
[12] An G, Lin TN, Liu JS, Xue JJ, He YY, Hsu CY. Expression of c-fos and c-jun family genes after focal cerebral ischemia, Ann Neurol. Ann Neurol 1993; 33: 457-464.
[13] Ji RR, Befort K, Brenner GJ, Woolf CJ. ERK MAP kinase activation in superficial spinal cord neurons induces prodynorphin and NK-1 up-regulation and contributes to persistent inflammatory pain hypersensitivity. J Neurosci 2002; 22: 478–485.
[14] Liu B, Wang K, Gao HM, Mandavilli B, Wang JY, Hong JS. Molecular consequences of activated microglia in the brain: over activation induces apoptosis. J Neurochem 2001; 77: 182-189.
[15] Wu FX, Bian JJ, Miao XR, Huang SD, Xu XW, Gong DJ, Sun YM, Lu ZJ, Yu WF. Intrathecal siRNA against Toll-like receptor 4 reduces nociception in a rat model of neuropathic pain. Int J Med Sci 2010; 7: 251-259.
[16] Bomer C. Apoptosis without caspases: an inefficient molecular guillotine. Cell Death Differ 1999; 6: 497-502.
[17] Wu CY, Tang ZH, Jiang L, Li XF, Jiang ZS, Liu LS. PCSK9 siRNA inhibits HUVEC apoptosis induced by ox-LDL via Bcl/Bax-caspase9-caspase3 pathway. Mol Cell Biochem 2012; 359: 347-358.
[18] Csuka E, Hans VH, Ammann E, Trentz O, Kossmann T, Morganti-Kossmann MC. Cell activation and inflammatory response following traumatic axonal injury in the rat. Neuroreport 2000; 11: 2587-2590.
[19] Lehner B, Koeck FX, Capellino S, Schubert TE, Hofbauer R, Straub RH. Preponderance of sensory versus sympathetic nerve fibers and increased cellularity in the infrapatellar fat pad in anterior knee pain patients after primary arthroplasty. J Orthop Res 2008; 26: 342-350.
[20] Jortner BS. The returns of the dark neuron. A histological artifact complicating contemporary neurotoxicologic evaluation. Neurotoxicology 2006; 27: 628-634.
[21] Murata R, Ohtori S, Ochiai N, Takahashi N, Saisu T, Moriya H, Takahashi K, Wada Y. Extracorporeal shockwaves induce the expression of ATF3 and GAP-43 in rat dorsal root ganglion neurons. Auton Neurosci 2006; 128: 96-100.
[22] Macias MY, Syring MB, Pizzi MA, Crowe MJ, Alexanian AR, Kurpad SN. Pain with no gain: allodynia following neural stem cell transplantation in spinal cord injury. Exp Neurol 2006; 201: 335-348.
[23] Kotulska K, LePecheur M, Marcol W, Lewin-Kowalik J, Larysz-Brysz M, Paly E, Matuszek I, London J. Overexpression of copper/zinc-superoxide dismutase in transgenic mice markedly impairs regeneration and increases development of neuropathic pain after sciatic nerve injury. J Neurosci Res 2006; 84: 1091-1097.