Neuroscience Letters 493 (2011) 49–54

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Contralateral neuropathology in dorsal root ganglia in a rat model of noncompressive disc herniation

Yanjing Li[a,1], Chunyang Xi[a,1], Ming Niu[b], Xiaoqi Liu[a], Zhiyong Chi[a], Xintao Wang[a], Jinglong Yan[a,*]

[a] Department of Orthopedic Surgery, The First Affiliated Hospital, Harbin Medical University, Harbin 150001, PR China
[b] Department of Mammary Surgery, The Third Affiliated Hospital, Harbin Medical University, Harbin 150001, PR China

ARTICLE INFO

Article history:
Received 7 December 2010
Received in revised form 20 January 2011
Accepted 8 February 2011

Keywords:
Nucleus pulposus
Disc herniation
Activating transcription factor 3
Glial fibrillary acidic protein

ABSTRACT

Some cases of lumbar disc herniation with contralateral radiculopathy have been reported. Many investigators explained this clinical syndrome from several aspects. However, to our best knowledge, there is no explanation on the basis of molecular changes in the contralateral dorsal root ganglia (DRG) until now. We firstly explored the expression of activating transcription factor 3 (ATF3, a marker of nerve injury), glial fibrillary acidic protein (GFAP, a marker of satellite cells activation) in bilateral L5 DRG and spinal cord using immunohistochemistry after nucleus pulposus (NP) application onto the left L5 DRG exposed by unilateral facetectomy. Immunoblotting was used to detect the expression of tumor necrosis factor-alpha (TNF-alpha) in bilateral L5 DRG. We tested that ATF3-immunoreactive (IR) neurons, GFAP-IR satellite cells and TNF-alpha expression in the contralateral DRG increased significantly after NP application. In the spinal cord, ATF3-IR motor neurons increased significantly after surgery, but GFAP-IR astrocytes were not significant. These results suggested that NP application on the unilateral DRG could induce nerve injury, satellite cells activation and upregulation of TNF-alpha expression in the contralateral DRG. In addition, our results indicated that motor neurons injury might play a significant role in contralateral changes.

In clinical situations, radiculopathy is usually ipsilateral in the common lateral type of lumbar disc herniation, but contralateral radiculopathy exists in some patients with lumbar disc herniation [1,2,7,18]. Some authors thought that these contralateral symptoms were the result of prominent spondylotic changes and stenosis contralateral to the side of disc herniation associated with anatomical anomalies of lumbar nerve roots, whereas others thought that in the case of disk herniation, the rigid cord or conus medullaris transmitted the pressure to the other side of the vertebral canal as a result of increased pressure in a closed space [1,2,7]. In a recent report, some authors suggested that because of the absence of the dural attachments to the posterior longitudinal ligaments that fixed the lumbar nerve roots at some levels, the ipsilateral nerve root could simply be displaced posteriorly while the contralateral nerve root was shifted laterally into the lateral recess to be compressed [18].

The phenomenon of contralateral mechanical allodynia has been shown in some animal models of neuropathic pain [4,14]. In addition, unilateral nerve injuries or inflammation induced molecular changes in the contralateral dorsal root ganglia (DRG), and these molecular changes have been demonstrated to contribute to the induction of neuropathic pain [3,4,15]. Recently, in a rat model of disc herniation, the contralateral limb exhibited greater mechanical sensitivity in nucleus pulposus (NP)-treated animals compared with sham animals, but differences between NP-treated and sham animals were not observed in the contralateral limb [15]. However, to our best knowledge, particular attention has been paid to molecular changes in the DRG ipsilateral than contralateral in experimental lumbar disc herniation, and little has been reported on molecular changes in the contralateral DRG [8,10].

We hypothesized that molecular changes might occur in the contralateral DRG in experimental lumbar disc herniation. This study paid attention to the expression of activating transcription factor 3 (ATF3), glial fibrillary acidic protein (GFAP), tumor necrosis factor-alpha (TNF-alpha).

All of the handling of the animals was carried out in accordance with the National Institute of Health Guide for the Care and Use of

Abbreviations: ATF3, activating transcription factor 3; DRG, dorsal root ganglia; GFAP, glial fibrillary acidic protein; IR, immunoreactive; NP, nucleus pulposus; NGF, nerve growth factor; PBS, phosphate buffered saline; TNF-alpha, tumor necrosis factor-alpha; VRT, ventral root transaction.

* Corresponding author at: Department of Orthopedic Surgery, The First Affiliated Hospital, Harbin Medical University, 23 Youzheng St, Nangang District, Harbin 150001, PR China. Tel.: +86 451 85555512; fax: +86 451 85555155.
E-mail address: yanjing-1985@hotmail.com (J. Yan).
[1] These authors contributed equally to this work.

0304-3940/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.neulet.2011.02.018

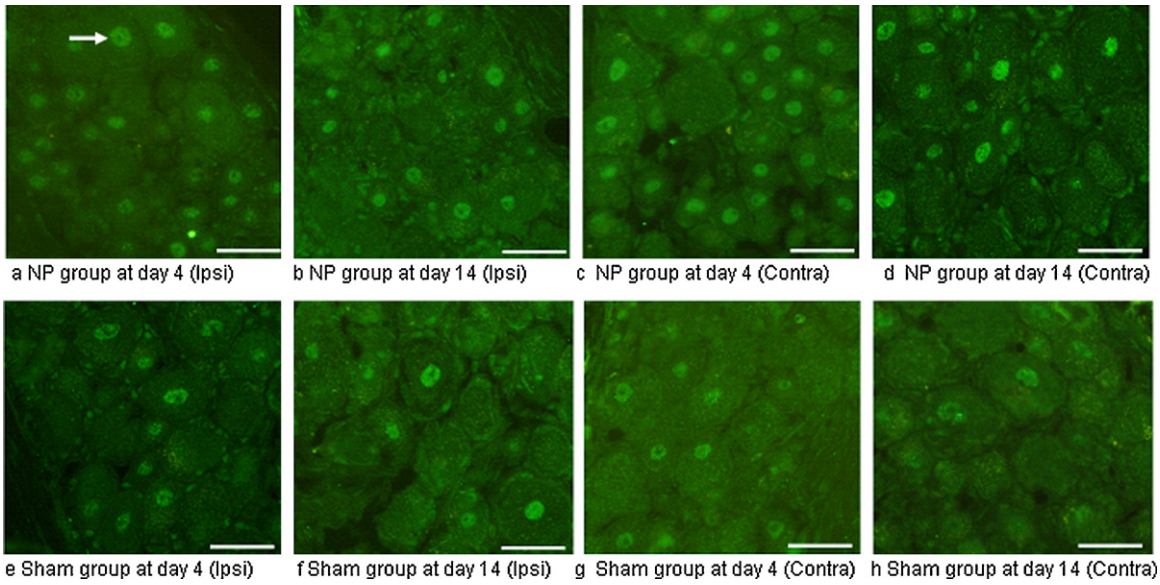

Fig. 1. ATF3 expression in DRG. ATF3-IR neurons increased significantly in the NP group (a–d) compared with the sham group (e–h) at the same side at the same time point ($P < 0.05$). The arrow (in a) indicates the ATF3-IR nucleus.

Laboratory Animals (1996 revision), and was conducted with the permission of the ethics committee of our institution.

Thirty-seven adult female Sprague–Dawley rats weighing 200–250 g were divided into NP group ($n = 16$), sham group ($n = 16$) and control group ($n = 5$). All surgical procedures were performed as described previously [16]. In NP group, the obtained NP was gently spread to the left L5 DRG. In sham group, the NP was resected but not implanted. The animals without any procedure were used as control group.

At 4 and 14 days after surgery, twenty-five rats from NP group ($n = 10$), sham group ($n = 10$) and control group ($n = 5$) were anesthetized and the bilateral L5 DRG and corresponding spinal cord were resected. Then tissue sections were made as described previously [3].

Antibodies used were as follows: rabbit anti-GFAP (1:400; Dako, Carpinteria, CA, USA), rabbit anti-ATF3 (1:100) and FITC-labeled donkey anti-rabbit antibody (1:200) were purchased from Santa Cruz Biotechnology. For analysis of immunofluorescence for ATF3 and GFAP immunoreactive (IR) cells, at least three sections selected randomly were used from the DRG, and two observation fields per section were chosen randomly at 200× magnification using a fluorescent microscope (Nikon, Japan). The person who counted cells was blinded to the treatment conditions. The mean numbers of ATF3- and GFAP-IR cells per section per animal were averaged and expressed as mean ± SD.

As described as [6], ATF3 and GFAP were stained in spinal cord sections. We detected ATF3-IR cells in 5 sections selected randomly in each spinal cord, and the number of ATF3-IR cells in ipsilateral and contralateral spinal cord per section was determined. For analysis of GFAP expression in spinal cord dorsal horn, at least five sections selected randomly were used from each spinal cord, and one observation field each side per section was chosen randomly at 200× magnification. The area and mean intensity of positive staining (Integrated Optical Density, IOD) was determined as described previously within a defined intensity threshold applied to all spinal cord sections and analyzed using Image Pro Plus software [13]. The IOD results were expressed per total area of the given section, and the IOD values per side per animal were averaged.

The bilateral L5 DRG in NP ($n = 6$) and sham group ($n = 6$) were removed on postoperative days 4, 14. Standard Western-blot assays were used to analyze protein expression, as described previously [4]. Primary antibodies used were as follows: goat antibody to TNF-alpha (1:200; Santa Cruz Biotechnology, Santa Cruz, California, USA), and β-actin were purchased from Santa Cruz Biotechnology. Band intensity on western blots was quantified by digitalization of the X-ray film and analyzed with Scion Image software (Scion Corporation, Maryland) and normalized to β-actin.

The number of ATF3- and GFAP-IR cells in the DRG, the IOD values of GFAP, and the number of ATF3-IR cells in spinal cord were compared between groups using the one-way analysis of variance. Statistical significance for immunoblotting was analyzed with the Mann–Whitney U test. $P < 0.05$ was considered as statistically significant.

ATF3 expression was not observed in DRG in the control group, while became evident in the nucleus of both small and large neurons in both sides in the NP and sham group (Fig. 1). The mean numbers of ATF3-IR neurons per section were: NP group at day 4, 32.0 ± 7.5 (Ipsilateral), 30.7 ± 6.2 (Contralateral); sham group at day 4, 13.1 ± 3.8 (Ipsilateral), 13.3 ± 3.2 (Contralateral); NP group at day 14, 17.2 ± 3.9 (Ipsilateral), 15.6 ± 5.0 (Contralateral); sham group at day 14, 6.7 ± 3.4 (Ipsilateral), 5.7 ± 2.6 (Contralateral). The number of ATF3-IR neurons was significantly higher in the NP group compared with the sham group at the same side at days 4 and 14 ($P < 0.05$). And the number of ATF3-IR neurons was higher at day 4 compared with day 14 at the same side in the NP and sham group ($P < 0.05$). However, at days 4 and 14 in the two groups, there was no significant difference in the number of ATF3-IR neurons between the ispilateral and contralateral DRG ($P > 0.05$) (Fig. 2a).

GFAP expression was not observed in DRG in the control group, but observed around both small and large neurons in both sides in the NP and sham group (Fig. 3). The mean numbers of GFAP-IR satellite cells per section were: NP group at day 4, 46.6 ± 6.4 (Ipsilateral), 44.6 ± 7.7 (Contralateral); sham group at day 4, 23.3 ± 6.5 (Ipsilateral), 25.7 ± 7.6 (Contralateral); NP group at day 14, 38.5 ± 8.1 (Ipsilateral), 23.6 ± 5.9 (Contralateral); sham group at day 14, 10.9 ± 4.1 (Ipsilateral), 10.2 ± 5.3 (Contralateral). Similarly, GFAP-IR satellite cells increased significantly in the NP group compared with the sham group at the same side at days 4 and 14 (Fig. 2b, $P < 0.05$). GFAP-IR satellite cells increased significantly at day 4 compared with day 14 at the same side in two groups except in the ipsilateral DRG in the NP group. There was no significant difference in the number of GFAP-IR satellite cells between the ispilateral and