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Gene expression alterations of neurotrophins, their receptors and prohormone convertases in a rat model of spinal cord contusion

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ABSTRACT

We have used a semi-quantitative RT-PCR approach to investigate the alterations in the expression of the main regulators of neuronal survival and death, neurotrophins (NTs), NT receptors, and prohormone convertases (PC), in a rat model of spinal cord contusion. Our results revealed that the expression of the members of NT family (Nerve-Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), and Neurotrophin-3 (NT-3)) is significantly declined in the injured spinal cord, as early as 6 h after the induction of the contusion. The expression was recovered afterward to that of the control levels. Furthermore, the expression of all NTs high-affinity Trk receptors decreased severely after the contusion. While the expression of TrkA and TrkC were completely shut down after 6 and 12 h after injury respectively, the expression of TrkB receptor declined at 12 h after injury and remained at this low level thereafter. In contrast to the pattern of Trk receptor expression, p75NTR receptor showed a significant upregulation after contusion. The expression of PC members functioning in the constitutive secretory pathway, i.e. furin, PACE4 and PC7, increased after damage, while the expression of PC members acting in regulated secretory pathway, PC1 and PC2, reduced after spinal cord injury. All together, the down-regulation of NTs, their designated Trk receptors and PC1/PC2 enzymes along with an upregulation of p75NTR promote neuronal death after injury. Our results suggest that either overexpression of NTs, Trk receptors and PC1/PC2 or interfering with the expression of p75NTR in host and/or grafted cells before transplantation could increase the success of the transplantation.

The following gene family members are the main regulators of neuronal survival and death in mammals: (1) neurotrophins (NTs), (2) NT high-affinity receptors (Trk), (3) NT low-affinity/common receptor (p75NTR), and (4) prohormone convertases (PCs) responsible for the proteolytic processing of NT precursors.

NTs are target-derived factors, with critical roles in the survival, differentiation, and the maintenance of the function of different neurons both in peripheral and central nervous system (CNS) [1]. In mammals, the NT family consists of four members: Nerve-Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3) and Neurotrophin-4/5 (NT-4/5). NTs encode structurally related proteins, which are proteolytically processed and secreted in the extracellular space. Their actions on neuronal tissue are thought to be mediated by two types of cell surface receptors: the high-affinity tropomyosin-related kinase receptors (TrkA that preferentially binds NGF, TrkB that binds BDNF as well as NT-4, and TrkC that binds NT-3) and the low-affinity pan-NT receptor p75 (p75NTR), a member of the tumor necrosis factor receptor family [19]. While specific binding of NTs to their designated Trk receptors promote neuronal survival, the binding of NTs to their common p75 receptor, in the absence of Trk receptors, causes neuronal death [22].

NTs are synthesized first in a precursor form and their activation requires a cleavage at specific sites by prohormone convertases enzymes. PC1/PC3 and PC2 are the major forms expressed in the neuroendocrine system and brain, where they act on prohormone and neuropeptide precursors in the regulated secretory pathway. In contrast, furin, PACE4, PC7 and SKI-1 are expressed and functioned ubiquitously within constitutive secretory pathway. In addition, the neuroendocrine protein 7B2 is essential for the activation of proPC2 [24]. While precursors of NGF and NT-3 are primarily processed by furin-like enzymes and released constitutively, proBDNF is primarily processed by PC1 and PC2 enzymes within regulated secretory pathway [7,21]. In contrast to the mature forms, the unprocessed forms of NTs bind with high affinity to the p75NTR receptor and could induce cell death in damaged cells [12].

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In this study, we have analyzed the changes in the expression of the main regulators of neuronal survival and death in a rat model of spinal cord injury. Our data reveals some alterations in the expression of some genes following spinal cord injury. Manipulating the expression of these genes could potentiate the survival of the host and transplanted cells.

Animals and treatment: 8–10-week-old Sprague–Dawley rats weighting between 200 and 250 g, were purchased from Razi Institute (Tehran, Iran). Animals were housed under standard conditions of humidity and temperature with 12-h light:12-h dark cycle and had unlimited access to food and water throughout the experiments. All surgical procedures were performed in an aseptic manner and were approved by the Animal Care and Use Committee of Tarbiat Modares University (TMU). Efforts were made to minimize animal suffering and to reduce the number of animals used.

Rat model of spinal cord injury (SCI): The contusion model of injuries on rat spinal cord was generated using a standard NYU (New York University) impactor. The whole procedure was performed under sterilized conditions. Briefly, animals were deeply anesthetized with ketamine (0.8 mg/kg, i.p.) and xylazine (0.5 mg/kg, i.p.). The backs of the animals were shaved and cleaned with betadine and 70% ethanol to reduce the risk of infection. Dorsal laminectomy was performed at T9–T10 segments, exposing the underlying dura. Dorsal-vertebral processes were then rigidly clamped to stabilize the spinal cord against displacement during injury and a 10 g weight impact rod (with a 2-mm diameter) was dropped from a height of 25 mm to produce contused SCI model. During surgery, body temperature was kept at 37 °C with a heating pad. After injury the muscles and skin were then closed in layers and rats were placed in a temperature and humidity-controlled chamber overnight. Sham-operated rats receiving laminectomy but not contusion served as controls. After surgery, the rats were allowed to recover from anesthesia before being returned to their cages. The animals were monitored routinely and were given postoperative care on a regular basis or as required until full bladder function was reestablished and no evidence of pain or other discomfort was evident. Details of postoperative animal care and other behavioral measurements were presented elsewhere [2].

Semi-quantitative RT-PCR: Rats were sacrificed at: 3 h, 6 h, 12 h, 24 h, 3 days, 1 week, 2 weeks, and 3 weeks ($n = 4$ per each time-point) after the injury and about 10 mm pieces of spinal cords were removed and frozen immediately in liquid nitrogen and stored at −75 °C until the time of use.

Table 1
The sequences and other features of the primers employed in this study

Gene	GeneBank ID	Primer sequence (5′ to 3′)	Exon	PCR length (bp)
β2m	NM_012512	Forward: CCGTGATCTTTCTGGTGCTT Reverse: TTTTGGGCTCCTTCAGAGTG	1 2	318
Ngfb (NGF)	XM_227525	Forward: GCCCACTGGACTAAACTTCAGC Reverse: CCGTGGCTGTGGTCTTATCTC	3 3	349
BDNF	NM_012513	Forward: GGTCACAGTCCTGGAGAAAG Reverse: GTCTATCCTTATGAACCGCC	2 2	214
Ntf-3 (NT-3)	NM_031073	Forward: TGCAGAGCATAAGAGTCACC Reverse: AAGTCAGTGCTCGGACGTAG	2 2	269
Ntrk1 (TrkA)	NM_021589	Forward: AATGCTCGTCAGGACTTCCATC Reverse: TCTTGACCACTAGTCCCTGACC	14 15	343
Ntrk2 (TrkB)	NM_012731	Forward: AAGTTCTACGGTGTCTGTGTG Reverse: TTCTCTCCTACCAAGCAGTTC	14 15	257
Ntrk3 (TrkC)	NM_019248	Forward: ACCATGGCATCACTACACCTTC Reverse: CTTAGATTGTAGCACTCAGCCAG	12 13	229
Ngfr (p75NTR)	NM_012610	Forward: ACAGTGGCGGATATGGTGA Reverse: AGTCTGCGTATGGGTCTGCT	4 5	291
PC1/PC3	NM_017091	Forward: CTGTTGGCTGAAAGGGAAAG Reverse: TGCTTCATGTGTTCTGGCTG	12 13	206
PC2	NM_012746	Forward: TTGGCTACGGAGTCCTTGAT Reverse: CTGGTTGCGTTGACTGTGAT	11 12	226
Furin	NM_019331	Forward: CCCAACCACATCTCCAGACT Reverse: TGCAGTGAGAAACCTTCCTC	13 16	386
PC4	NM_133559	Forward: TGTACTACTGCACGCTGCTG Reverse: CACTGCTTGCTGGAGATGAG	12 13	179
PC5	XM_342032	Forward: CTGCTGCAAATGGATGACA Reverse: AGTCTTGGTGTGCTCATGGA	31 32	230
PACE4	NM_012999	Forward: GCTCATCAGATGTGGGGAAT Reverse: CGACACACTTTGTGGGGTAA	23 24	174
PC7	NM_019246	Forward: GAGTCCAGTAGACATCAAGG Reverse: TCAGGCACACTTCTAGCAT	16 18	215
PC9	NM_199253	Forward: CCTGAAGAGGAACTGCTGA Reverse: GATGCCATGCTCCTTGAT	9 11	411
SKI-1	NM_053569	Forward: GAAGCTGCTCTCCATTGACC Reverse: GGGATGGTCTGGCCTACTT	22 23	159
7B2	NM_013175	Forward: TAGAAAACGCCCCTGACACT Reverse: TGGCTCCTTCTCCTCTTCTG	5 7	242