ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Combination sciatic nerve graft and fibroblastic growth factor 2 promotes tissue regeneration for NF-200 and 5-HT in spinal cord injury.

Aline Samaya Santos Fernandes[a], Aline Brito Ferreira de Castro[a], Amália Cinthia Meneses do Rêgo[a], Irami Araújo Filho[a], Eudes Euler de Souza Lucena[b], José Rodolfo Lopes de Paiva Cavalcanti[b], Ianara Mendonça da Costa[b], Jeferson de Souza Cavalcante[c], Mirian Stela Maris de Oliveira Costa[d], and Fausto Pierdoná Guzen[a,b]*.

[a]Postgraduate Program in Biotechnology, Health School, Potiguar University (UnP), Natal-RN, Brazil.
[b]Laboratory of Experimental Neurology, Health Science Center, State University of Rio Grande do Norte, Mossoró-RN, Brazil.
[c]Laboratory of Neurochemical Studies, Department of Physiology, Bioscience Center, Federal University of Rio Grande do Norte, University Campus, Natal-RN, Brazil.
[d]Laboratory of Neuroanatomy, Department of Morphology, Bioscience Center, Federal University of Rio Grande do Norte, University Campus, Natal-RN, Brazil.

ABSTRACT

Traumatic injury to the spinal cord results in a rapid and significant loss of function. One barrier to successful regeneration in the adult CNS is the diminished axonal growth capacity after maturation. Therefore, strategies that seek to promote the restoration of function to the chronically injured spinal cord have high therapeutic value. Neurotrophic factors and peripheral nerves are known to be good substrates for bridging the lesions associated with CNS trauma. The role of fibroblast growth factor-2, when added to the sciatic nerve, was examined following spinal cord injury in a rat. We evaluated whether FGF-2 added to a sciatic nerve graft placed in a gap promoted nerve recovery following a complete transection of the spinal cord and if it could enhance neuronal plasticity. Rats underwent a transection at the thoracic level, which was repaired with saline or a fragment of the sciatic nerve. In another group, FGF-2 was added immediately after the lesion. The effects of FGF-2 and the fragment of the sciatic nerve graft on neuronal plasticity were investigated at the epicenter of the injury using NF-200 and 5-HT immunoreactivity after 8 weeks. A high number of NF-200 and 5-HT immunoreactive fibers were observed in the treated groups with sciatic nerve graft in the presence or absence of FGF-2 when compared to the saline group. However, a small number of NF-200 (★p=0.03) and 5-HT fibers were observed in the epicenter of the graft when FGF-2 was added, when compared to the group that received sciatic nerve graft. These results indicate that sciatic nerve grafting favors the growth of fibers in the traumatized spinal cord, an effect that is slightly influenced by the addition of FGF-2 to the NF-200 and 5-HT immunoreactive fibers.

Keywords: Fibroblastic growth factor-2; plasticity; regeneration; sciatic nerve graft and spinal cord.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Central nervous system (CNS) injuries in humans usually result in crippling conditions due to their limited regenerative capacity. Recovered locomotor activity results from the functional regeneration of some of the lesioned brainstem and spinal fibre tracts following transection.[1,2]

The weakened axonal growth capacity after maturation constitutes an important obstacle to the successful regeneration in the adult CNS.[3-5] This difficulty of regrowth that was observed in acutely injured adult axons is more evident in cases of chronic injury.[6,7] Chronically injured axons require additional stimulation (e.g., exogenous neurotrophins) to induce regeneration.

Several studies have shown that the administration of exogenous growth factors following spinal cord injury (SCI) promotes functional recovery.[8-14]

Heparin-binding growth factors include the family of fibroblast growth factors (FGFs) and may stimulate mitogenesis in a variety of cells with mesodermal or neuroectodermal origin. To date, 20 distinct FGFs have been discovered and numbered consecutively from 1 to 20.[15-17]

Among the factors that are believed to have a significant role in spinal cord regeneration is fibroblast growth factor-2 (FGF-2), a member of a large family of small peptide growth factors with multiple biological activities.[18] This factor is involved in regulating developmental processes,[19,20] as well as mediating outcomes in response to various types of injury.[21,22] FGF-2 plays a role in the neuronal development during prenatal life and also influences survival and plasticity of neurons in the mature central nervous system.[23] Both FGF-1 and FGF-2 treated cultures promoted a significant increase in neurite outgrowth of ventral spinal cord neurons, suggesting that they can influence neuronal development.[24]

FGF-2 is involved in locomotor recovery after SCI. Indeed, in adult rats, a significant and rapid up-regulation of FGF2 expression was observed both above and below the epicentre of a compression injury.[25-27] This up-regulation in the distal spinal cord is thought to have a prominent role in motor neuron survival below the injury and in partial recovery of locomotor function over time.[28]

It is well known that adult neurons survive after an injury of their fibres, and it is likely that multiple sources of trophic support are required to maintain those neurons following axotomy.

Primary injury is immediate and irreversible, but the secondary injury evolves over time and provides a window of opportunity for treatment. It is important to emphasize that therapeutic interventions that are designed to foster regeneration of the injured spinal cord must primarily counteract the sequel of secondary tissue damage following trauma. To evaluate the potential regenerative role of FGF-2 in the CNS, we have analysed the expression of markers for neuronal cell body and fibre growth after spinal cord lesion.

MATERIALS AND METHODS

Animal treatment

Pathogen-free adult male Wistar rats (*n* = 30) from the Federal University of Pernambuco, Pernambuco, Brazil (body weight [b.w.] 180–200 g) were used in the present study. The Wistar rats were taken from a long-established colony that has been maintained using the San Poiley outbreeding method.[29] Rats were kept under a controlled temperature with free access to food pellets and tap water. The study was conducted according to protocols that were approved by the Research Ethic Committee at the Federal University of Rio Grande do Norte and in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the National Institutes of Health.

Microneurosurgery

Rats were pre-anaesthetized with ketamine chloridrate 10% (Agener União, Brazil, 0.01 m/100 g b.w.) and xylazine 2% (Agener União, Brazil, 0,01 ml/100 g b.w.) and then anesthetized with isoflurane inhalation (Isoflorine®) (Cristália, Brasil). A small laminectomy at the tenth/eleventh thoracic levels was performed. The

dura mater was incised longitudinally and pulled laterally. Complete transections created a 4 mm-long gap on the eleventh/twelfth spinal cord levels. A gelfoam soaked in 10 µl of saline was left at the bottom of the gap close to the vertebral body in 10 rats, while another group of 10 rats received a gelfoam filled with two sciatic nerve fragments. Additionally, 10 rats received sciatic nerve fragments that was added to 10 µl of a FGF-2 (Santa Cruz Biotechnology, USA) solution diluted in PBS (1 µg/10 µl/animal).[14] We did not considered further concentrations because higher doses may be toxic to neurons. Four mm-long sciatic nerve fragments were carefully grafted close to the edges of the spinal cord stumps, thus maintaining in a position that was rostral and adjacent to the filled gelfoam. Muscular layers and skin were then sutured. All animals received sodium cephalexin (40 mg/kg/day b.w., im, Eurofarma, Brasil) prior to surgery. The animals were allowed to recover from anaesthesia and were returned to their home cages. Sciatic nerve fragments were obtained from a separate group of adult Wistar rats, as described previously.[30] Just before the spinal cord injury procedures, fragments were bilaterally removed from the rat sciatic nerves by being trimmed out in small 4 mm-long pieces. Fragments were then washed several times and maintained in a 100 mm dish with Leibovitz's 15 medium (L-15, Gibco) at room temperature for about 30 minutes while cord injuries were performed in the host rats.

Tissue preparation, sectioning and sampling

Animals were deeply anaesthetized with Isoforine® (Cristália, Brasil) and euthanized 2 months after surgery by a transcardiac perfusion with 100 ml of 0.9% saline at room temperature followed by 500 ml of fixation fluid (4°C) over a period of 6 minutes, as previously described.[31] The fixative consisted of 4% paraformaldehyde (w/v) in 0.1 M phosphate buffer, pH 7.4. The spinal cord epicentre was removed and kept in the fixative solution at 4°C for 90 minutes, and then it was rinsed in 10% sucrose (Merck, Germany) that was dissolved in 0.1 M phosphate buffered saline (PBS), pH 7.4, for 48 hours. The adjacent DRG was also removed and stored at -70°C until use. Serial 20 µm thick transversal frozen sections were obtained from the spinal cord with a microtome (Leica, SM 2000R, Germany). The sections were carefully evaluated for visualization of the spinal cord. Every 10th section was sampled in series and immunolabeled.

Immunohistochemical procedures for NF-200 and 5-HT

Immunoreactivity was developed by the avidin-biotin peroxidase technique.[32,33] Series of sections were washed 2 × 10 minutes in 0.1 M PBS, pH 7.4, at room temperature and incubated with 5% normal goat serum (NGS, Sigma) for 30 minutes at room temperature. Series were then incubated for 24 hours at 4°C with one of the following antisera: mouse monoclonal antisera against neurofilament 200 (NF-200) (Abcam, diluted 1:1200) or rabbit monoclonal antisera against 5-hydroxytryptamine (5-HT) (Sigma, diluted 1:3000). The antibodies were diluted in PBS containing 0.3% Triton X-100 (Sigma) and 1% bovine serum albumin (Sigma). The series of sections were washed again in PBS (2 × 10 minutes) and incubated with either biotinylated goat anti-mouse or anti-rabbit immunoglobulins diluted 1:1000 (Jackson Labs, USA) for 1 hour, depending upon the host species in which the primary antiserum was raised. The sections were washed again in PBS and incubated with an avidin-biotin peroxidase complex (both diluted 1:100, Vectastain, Vector, for 90 minutes). Immunoreactivity was visualized using 3-3'-diaminobenzidine tetra hydrochloride (DAB, Sigma) as a chromogen and H_2O_2 (0.05%, v/v, Sigma) for 4 minutes.

Morphometric/microdensitometric image analysis

The NF-200 and 5-HT immunoreactivity was measured in fibres in one section per rat in the central regions. We did this to obtain the means per group. An optical microscope (Olympus BX41) in a bright field was used. The counts and measurements were made using the software Image J. Digital images of representative sections were obtained using a digital video camera (Nikon DXM1200).

Statistical analyses

Statistical analyses were performed using the analysis of variance (ANOVA), and significant interactions were followed-up with Tukey and Bonferroni post-test comparisons. All statistical analyses were performed using SPSS 22, and significance was considered as $P < 0.05$.

ISSN: 0975-8585

RESULTS

NF-200 immunoreactive fibers (arrows) were seen on sections from the injury epicenter that was treated with sciatic nerve graft and sciatic nerve graft plus FGF-2 (Fig. 1A and B). NF-200 immunoreactivity attained values of 259.3 in the nerve plus FGF-2 group and 248.1 in the nerve group (★p=0.03). Sciatic nerve graft and the sciatic nerve graft plus FGF-2 treatment groups showed significant effect compared with saline group (★★★p<0.0001) (Fig. 2).

5-HT immunoreactive fibers were seen on sections from the injury epicenter that was treated with sciatic nerve graft and sciatic nerve graft plus FGF-2 (Fig. 3A and B). The values for 5-HT immunoreactivity were 48.0 in the nerve plus FGF-2 group and 46.0 in the nerve group, indicating no statistical difference. Both treated groups showed a significant effect when compared to the saline group (★★★p<0.0001) (Fig. 4).

Figure 1. Photomicrographs of NF-200-labelled coronal sections at the epicenter of injury in the spinal cord of animals that were treated with sciatic nerve graft (A) and sciatic nerve graft in addition to FGF-2 (B). Illustrations show NF-200-immunoreactive fibers at the epicenter of the lesion (arrows). Bars: 200μm.

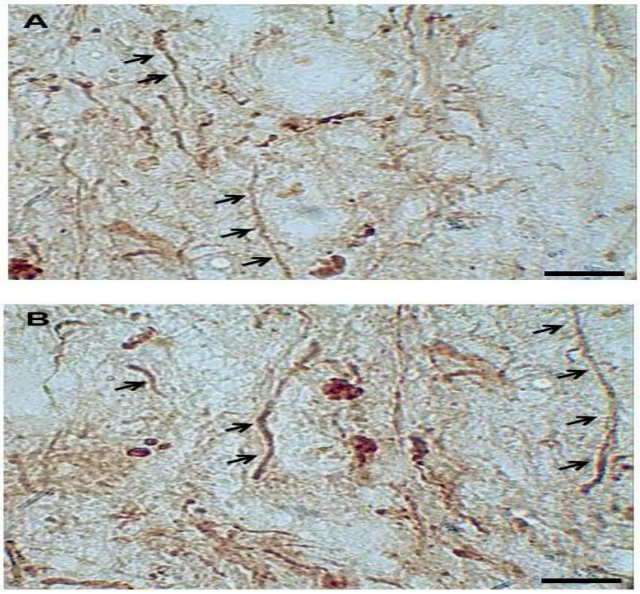
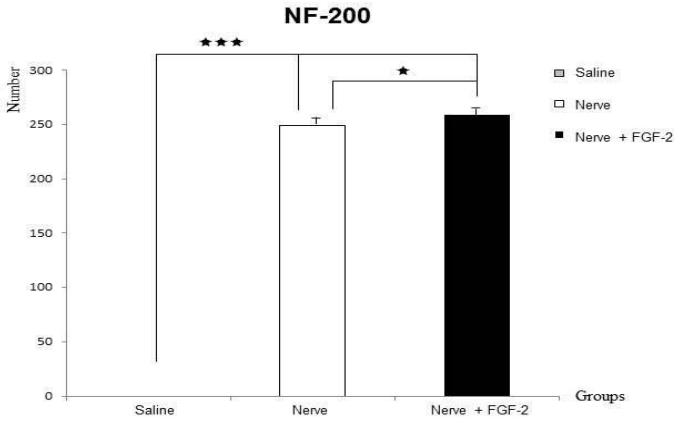


Figure 2. Distribution of NF-200-immunoreactivity at the epicenter of spinal cord injury of animals that were treated with saline, sciatic nerve graft (Nerve) and sciatic nerve graft added FGF-2 (Nerve + FGF-2). Means ± S.E.M. ★p<0.05, ★★p<0.01, ★★★p<0.001, according to ANOVA – Tukey and Bonferroni.



ISSN: 0975-8585

Figure 3. Photomicrographs of 5-HT-labelled coronal sections at the epicenter of injury of the spinal cord of animals that were treated with sciatic nerve graft (A) and sciatic nerve graft in addition to FGF-2 (B). Illustrations show fibers at the epicenter of the lesion (arrows). Bars: 200μm.

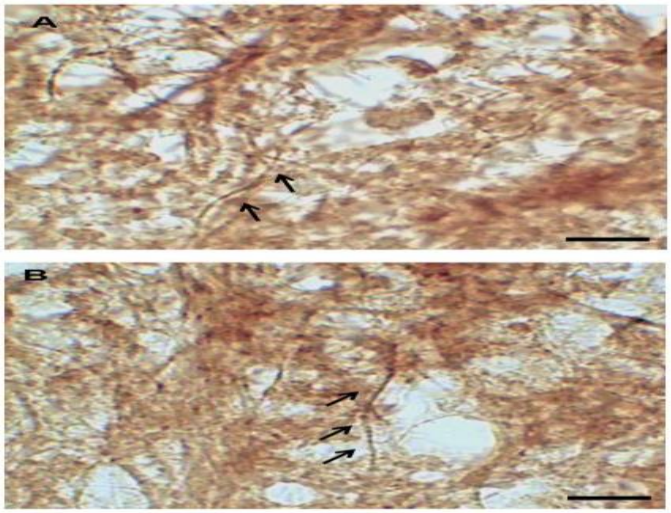
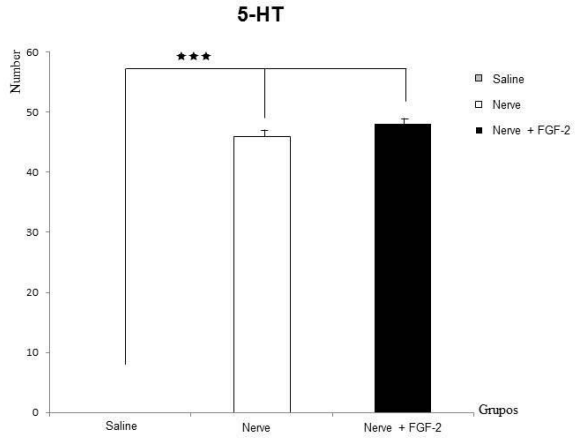


Figure 4. Distribution of 5-HT-immunoreactivity at the epicenter of spinal cord injury of animals that were treated with saline, sciatic nerve graft (Nerve) and sciatic nerve graft added FGF-2 (Nerve + FGF-2). Means ± S.E.M. *p<0.05, **p<0.01, ***p<0.001, according to ANOVA – Tukey and Bonferroni.



DISCUSSION

Our findings show that, at the time of tissue removal, atrophy was observed at the injury site in the group that received saline injection, as indicated by an absence of continuity between the regions of damaged tissue. The regions above and below the site of the lesion displayed scar tissue, thereby resulting in a spinal cord cavity. Tissue removal in the groups that were treated with fragments of the sciatic nerve displayed a greater number of fibers that were immunoreactive for NF-200 and lower number of 5-HT immunoreactive fibers at the epicenter of the graft, when compared to the saline group.

Thus, the regenerative potential of peripheral nerve graft (PNG) to injured spinal cord is associated with an ability to induce branching and axonal sprouting within the graft[14], which stimulates the production of neurotrophic factors including FGF-2, the factor used in this study that mitigates the formation of glial scar at the site of trauma.[34,35]

Although the combination of PNG and FGF-2 was effective in promoting regeneration of chronically injured axons after a high-level injury, it is not known whether this strategy would be as effective in

encouraging regrowth of chronically injured axons that were damaged at thoracic levels of the spinal cord. The growth potential of lesioned axons is greater when the injury is closer to the cell body, which is likely attributable to the induction of regeneration associated genes (RAGs), such as GAP-43 and cytoskeletal proteins, when axotomy occurs at more proximal levels than at distal levels.[36,37] Therefore, it would be expected that axons that were injured at a higher level would have a greater regenerative capacity than axons that were injured further down the spinal cord. Indeed, axons severed at cervical levels regenerate into PNGs better than those that were injured at thoracic or lumbar levels.[38] It would be interesting to determine whether treating an acute or chronic thoracic injury site with FGF-2 before grafting a PNG successfully promotes axonal regeneration into and beyond the transplant.

Studies have shown that implants of FGF-2 can increase the expression of GAP-43, a molecular marker of axonal sprouting to the injury site, which was then followed by the restoration of hindlimb function.[39-45] It is plausible that interactions between FGF-2 and glial cells strongly influence the microenvironment of the injury, thereby restoring metabolic homeostasis, providing protection to undamaged cells and possibly rescuing damaged neurons.

However, we observed a small number of NF-200 and 5-HT fibers were observed in the epicenter of the graft when FGF-2 was added, when compared to the group that received sciatic nerve graft. Possibly the regenerative effects on descending pathways in the spinal cord such as eg. serotonergic is somewhat more complex to exert plastic action when compared to GAP-43 expression.

Kawamata[46] reported an increase in GAP-43 immunoreactivity (a molecular marker of axonal sprouting) in the intact sensorimotor cortex contralateral from the location of cerebral infarcts that were induced by a middle cerebral artery occlusion following FGF-2 treatment. This suggests that FGF-2 enhances functional improvement after stroke via the stimulation of neuronal sprouting in the intact brain. FGF-2 also significantly increases both the sprouting of the cholinergic septodentate pathway following entorhinal cortex injury[47] and the production of astrocyte-associated fibronectin[48], an axonal growth-promoting molecule.[49,50]

Our data further indicates that the distal spinal cord stump, the target for the regenerated descending fibres, also responded to FGF-2 inoculation. This action could facilitate the reestablishment of the functional innervation of spinal locomotor networks below the site of injury by regenerated brainstem and intraspinal descending neurons. In line with this assumption, the expression of FGF-2 mRNA and protein was also shown to be upregulated in the distal cord after a compression injury or a complete spinal cord transection in infant rats but not in adult rats.[25,51] This differential expression of FGF-2 has been related to the higher regenerative capacity of some descending tracts and functional recovery in spinally transected infant rats.[52] Moreover, FGF-2 could facilitate the regeneration of damaged axons from descending and ascending intraspinal neurons. The regeneration of damaged axons from intraspinal neurons is thought to have an important role in the reestablishment of the intersegmental locomotor coordination across a spinal cord transection.[53-55,02]

CONCLUSION

In conclusion, the addition of sciatic nerve fragment that was grafted in a gap of the rat spinal cord that was submitted to complete transections was able to improve neuroprotection in the spinal cord. Sciatic nerve grafting favors the growth of fibers in the traumatized spinal cord, an effect that is slightly influenced by the addition of FGF-2 to the NF-200 and 5-HT immunoreactive fibers.

Highlights

- Spinal cord transection as a model for regeneration.
- Peripheral nerve grafts as a favorable environment to spinal cord regeneration.
- FGF-2 exerts little effect on the regeneration of immunoreactive fibers to NF-200 and 5-HT.

ACKNOWLEDGMENTS

This study was supported by funding from the National Counsel of Technological and Scientific Development (CNPq) and the Coordination for Improvement of High Level Staff (CAPES). The English version of this text was revised by Sidney Pratt, Canadian, MAT (The Johns Hopkins University), RSAdip (TEFL, University of Cambridge).

REFERENCES

[1] B. M. Davis et al., Time course of salamander spinal cord regeneration and recovery of swimming: HRP retrograde pathway tracing and kinematic analysis, *Exp Neurol*, 108 (1990), 198-213.

[2] S. Chevallier et al., Recovery of bimodal locomotion in the spinal-transected salamander, Pleurodeles waltlii, *Eur J Neurosci*, 20 (2004), 1995-2007.

[3] K. Kalil, J. H. Skene. Elevated synthesis of an axonally transported protein correlates with axon outgrowth in normal and injured pyramidal tracts, *J Neurosci*, 6 (1986), 2563-70.

[4] J. W. Fawcett, Overcoming inhibition in the damaged spinal cord. *J Neurotrauma*, 23 (2006), 371-83.

[5] R. L. Ruff, L. McKerracher and M. E. Selzer, Repair and neurorehabilitation strategies for spinal cord injury, *Ann N Y Acad Sci*, 1142 (2008), 1-20.

[6] W. Tetzlaff et al., Response of facial and rubrospinal neurons to axotomy: changes in mRNA expression for cytoskeletal proteins and GAP-43, *J Neurosci*, 11 (1991), 2528-544.

[7] D. Dolbeare and J. D. Houle, Restriction of axonal retraction and promotion of axonal regeneration by chronically injured neurons after intraspinal treatment with glial cell line-derived neurotrophic factor (GDNF), *J Neurotrauma*, 20 (2003), 1251-261.

[8] L. Schnell et al., Neurotrophin-3 enhances sprouting of corticospinal tract during development and after adult spinal cord lesion, *Nature*, 367 (1994), 170-73.

[9] R. Baffour et al., Synergistic effect of basic fibroblast growth factor and methylprednisolone on neurological function after experimental spinal cord injury, *J Neurosurg*, 83 (1995), 105-10.

[10] Cheng, Y. Cao and L. Olson, Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function, *Science*, 273 (1996), 510-13.

[11] R. Grill et al., Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury, *J Neurosci*, 17 (1997), 5560-72.

[12] D. A. Houweling et al., Collagen containing neurotrophin-3 (NT-3) attracts regrowing injured corticospinal axons in the adult rat spinal cord and promotes partial functional recovery, *Exp Neurol*, 153 (1998), 49-59.

[13] F. P. Guzen et al., Glial cell line-derived neurotrophic factor added to a sciatic nerve fragment grafted in a spinal cord gap ameliorates motor impairments in rats and increases local axonal growth, *Restor Neurol*, 27 (2009), 1-16.

[14] F. P. Guzen FP et al., Sciatic Nerve Grafting and Inoculation of Fgf-2 Promotes Improvement of Motor Behavior and Fiber Regrowth in Rats with Spinal Cord Transection, *Restor Neurol Neurosci*, 30 (2012), 265-75.

[15] B. Weise, K. Unsicker and C. Grothe, Localization of basic fibroblast growth factor in a subpopulation of rat sensory neurons, *Cell Tissue Res*, 267 (1992), 125-30.

[16] C. Oellig et al., Acidic FGF and FGF receptors are specifically expressed in neurons of developing and adult rat dorsal root ganglia, *Eur J Neurosci*, 7 (1995), 863-74.

[17] C. Grothe, C. Meisinger and P. Claus, In vivo expression and localization of the fibroblast growth factor system in the intact and lesioned rat peripheral nerve and spinal ganglia, *J Comp Neurol*, 434 (2001), 342-57.

[18] G. Szebenyi, J. F. Fallon, Fibroblast growth factors as multifunctional signaling factors, *Int Rev Cytol*, 185 (1999), 45-106.

[19] R. Raballo et al., Basic fibroblast growth factor (Fgf2) is necessary for cell proliferation and neurogenesis in the developing cerebral cortex, *J Neurosci*, 20 (2000), 5012-23.

[20] B. Li, E. DiCicco-Bloom, Basic fibroblast growth factor exhibits dual and rapid regulation of cyclin D1 and p27 to stimulate proliferation of rat cerebral cortical precursors, *Dev Neurosci*, 26 (2004), 197-207.

[21] S. Rowntree, B. Kolb, Blockade of basic fibroblast growth factor retards recovery from motor cortex injury in rats, *Eur J Neurosci*, 9 (1997), 2432-41.
[22] Y. Ganat et al., Chronic hypoxia up-regulates fibroblast growth factor ligands in the perinatal brain and induces fibroblast growth factor-responsive radial glial cells in the sub-ependymal zone, *Neurosci*, 112 (2002), 977-91.
[23] P. A. Walicke, Basic and acidic fibroblast growth factors have trophic effects on neurons from multiple CNS regions. *J Neurosci*, 8 (1988), 2618-27.
[24] Y. Iwasaki et al., Acidic and basic fibroblast growth factors enhance neurite outgrowth in cultured rat spinal cord neurons, *Neurol Res*, 17 (1995), 70-2.
[25] P. Follesa, J. R. Wrathall and I. Mocchetti, Increased basic fibroblast growth factor mRNA following contusive spinal cord injury, *Brain Res Mol Brain Res*, 22 (1994), 1-8.
[26] I. Mocchetti et al., Increased basic fibroblast growth factor expression following contusive spinal cord injury, *Exp Neurol*, 141 (1996), 154-64.
[27] L. J. Zai, S. Yoo and J. R. Wrathall, Increased growth factor expression and cell proliferation after contusive spinal cord injury, *Brain Res*, 1052 (2005), 147-55.
[28] Y. D. Teng, I. Mocchetti and J. R. Wrathall, Basic and acidic fibroblast growth factors protect spinal motor neurones in vivo after experimental spinal cord injury, *Eur J Neurosci*, 10 (1998), 798-802.
[29] S. M. Poiley, In: Proceedings of Animal Care Pane, 1960, **10:** p. 159-66.
[30] [30] G. T. Casella, R. P. Bunge and P. M. Wood, Improved method for harvesting human Schwann cells from mature peripheral nerve and expansion in vitro, *Glia*, 17 (1996), 327-38.
[31] M. DeLeon et al., Subpopulations of primary sensory neurons show coexistence of neuropeptides and glucocorticoid receptors in the rat spinal and trigeminal ganglia, *Brain Res*, 636 (1994), 338-42.
[32] A. Cintra et al., Basic fibroblast growth factor and steroid receptors in the aging hippocampus of the brown Norway rat: immunocytochemical analysis in combination with stereology, *Neurochem Int*, 25 (1994), 39-45.
[33] K. Fuxe et al., Evidence for a regional distribution of hyaluronic acid in the rat brain using a highly specific hyaluronic acid recognizing protein, *Neurosci Lett*, 169 (1994), 25-30.
[34] A. D. Levi et al., Peripheral nerve grafts promoting central nervous system regeneration after spinal cord injury in the primate, *J Neurosurg*, 96 (2002), 197-205.
[35] H. B. Peng et al., Differential effects of neurotrophins and schwann cell-derived signals on neuronal survival/growth and synaptogenesis, *J Neurosci*, 23 (2003), 5050-60.
[36] S. K. Doster et al., Expression of the growth-associated protein GAP-43 in adult rat retinal ganglion cells following axon injury, *Neuron*, 6 (1991), 635-47.
[37] K. J. Fernandes et al., Influence of the axotomy to cell body distance in rat rubrospinal and spinal motoneurons: differential regulation of GAP-43, tubulins, and neurofilament-M. *J Comp Neurol*, 414 (1999), 495-510.
[38] P. M. Richardson, V. M Issa, Peripheral injury enhances central regeneration of primary sensory neurones, *Nature*, 309 (1984), 791-93.
[39] J. R. Wrathall, Y. D. Teng and D. Choiniere, Amelioration of functional deficits from spinal cord trauma with systemically administered NBQX, an antagonist of non-N-methyl-D-aspartate receptors, *Exp Neurol*, 137 (1996), 119-26.
[40] J. R. Wrathall, Y. D. Teng and R. Marriott, Delayed antagonism of AMPA/kainate receptors reduces long-term functional deficits resulting from spinal cord trauma, *Exp Neurol*, 145 (1997), 565-73.
[41] Y. D. Teng, J. R. Wrathall, Local blockade of sodium channels by tetrodotoxin ameliorates tissue loss and long-term functional deficits resulting from experimental spinal cord injury, *J Neurosci*, 17 (1997), 4359-66.
[42] J. R. Madsen et al., Tacrolimus (FK506) increases neuronal expression of GAP-43 and improves functional recovery after spinal cord injury in rats, *Exp Neurol*, 154 (1998), 673-83.
[43] Y. Goldshmit et al., Fgf2 improves functional recovery-decreasing gliosis and increasing radial glia and neural progenitor cells after spinal cord injury, *Brain Behav*, 4 (2014), 187-200.
[44] M. Kasai et al., FGF-2-responsive and spinal cord-resident cells improve locomotor function after spinal cord injury, *J Neurotrauma*, 31 (2014), 1584-98.
[45] D. A. Shin et al., Co-transplantation of bone marrow-derived mesenchymal stem cells and nanospheres containing FGF-2 improve cell survival and neurological function in the injured rat spinal cord, *Acta Neurochir (Wien)*, 156 (2014), 297-303.

[46] T. Kawamata et al., Intracisternal basic fibroblast growth factor enhances functional recovery and up-regulates the expression of a molecular marker of neuronal sprouting following focal cerebral infarction, *Proc Natl Acad Sci U S A*, 94 (1997), 8179-84.

[47] J. J. Ramirez et al., Basic fibroblast growth factor enhances axonal sprouting after cortical injury in rats, *Neuroreport*, 10 (1999), 1201-4.

[48] M. Mahler, Y. Ben-Ari and A. Represa, Differential expression of fibronectin, tenascin-C and NCAMs in cultured hippocampal astrocytes activated by kainate, bacterial lipopolysaccharide or basic fibroblast growth factor, *Brain Res*, 775 (1997), 63-73.

[49] P. Liesi, T. Kirkwood and A. Vaheri, Fibronectin is expressed by astrocytes cultured from embryonic and early postnatal rat brain, *Exp Cell Res*, 163 (1986), 175-85.

[50] H. P. Matthiessen, C. Schmalenbach and H. W. Muller, Astroglia-released neurite growth-inducing activity for embryonic hippocampal neurons is associated with laminin bound in a sulfated complex and free fibronectin, *Glia*, 2 (1989), 177-88.

[51] M. L. Qi et al., Changes in FGF-2 expression in the distal spinal cord stump after complete cord transection: a comparison between infant and adult rats, *Spine,* 28 (2003), 1934-40.

[52] Y. Wakabayashi et al., Functional recovery and regeneration of descending tracts in rats after spinal cord transection in infancy, *Spine*, 26 (2001), 1215-22.

[53] A. H. Cohen, S. A. Mackler and M. E. Selzer, Functional regeneration following spinal transection demonstrated in the isolated spinal cord of the larval sea lamprey, *Proc Natl Acad Sci U S A*, 83 (1986), 2763-66.

[54] A. H. Cohen, S. A. Mackler and M. E. Selzer, Behavioral recovery following spinal transection: functional regeneration in the lamprey CNS, *Trends Neurosci*, 11 (1988), 227-31.

[55] A. D. McClellan, Locomotor recovery in spinal-transected lamprey: role of functional regeneration of descending axons from brainstem locomotor command neurons, *Neurosci,* 37 (1990), 781-98.