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## The Effect of Exogenous Testosterone Administration on Peripheral Nerves Regeneration after Sciatic Nerve Compression in Rat

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**Abstract:** There are extensive evidences those axonal processes of the nervous system (peripheral and/or central) may degenerate after nerve injuries. Wallerian degeneration and chromatolysis are the most conspicuous phenomena that occur in response to injuries. In this study the effects of exogenous testosterone administration on peripheral neurons regeneration after sciatic nerve compression in rat was investigated. Thirty-two male Wistar rats divided to 4 group (control, compression, compression+castrated, compression+subcutaneous administration testosterone n = 8). After 4 weeks the lumbar segments of spinal cord were sample, processed, sectioned serially and stained with toluidine blue (pH = 4.65). By using stereological quantitative technique (physical dissector), the number of alpha motoneurons in the right ventral horns of spinal cord were counted and compared with each other. Statistical analysis showed that the number of motor neurons in compression group reduced significantly. In castration animal compression induced remarkable reduction in compare with control group. Treatment of testosterone (100 mg kg<sup>-1</sup>) at 4 week significantly (p<0.05) reduced neuronal damage and testosterone has a neuroprotective effect on motor neurons degeneration after sciatic nerve injury.

**Key word:** Neuronal degeneration, testosterone, numerical density

### INTRODUCTION

When a motor axon in a peripheral nerve is severed, a characteristic sequence of changes occurs (Ro *et al.*, 2007). The distal of portion of the axon degenerates, as dose a short length of the proximal portion. Certain effects of axotomy-chromatolysis, atrophy and cell death-result from the less of trophic substance produced by the target tissue and transported retrogradely along the axon to the cell body (Wang *et al.*, 2007).

Neurodegenerative diseases are characterized by progressive dysfunction and death of neurons. Neurodegeneration may occur by apoptosis, necrosis or both. It is believed that there are many different mechanisms and neurochemical modulators responsible for the central nervous system damage, which may overlap temporarily (Bialek *et al.*, 2004).

Among the most important factors contributing to neuronal cell death are: genetic factors, glutamate mediated excitotoxicity leading to disturbances in intracellular calcium and sodium metabolism, mitochondrial dysfunction, oxidative stress, growth factor withdrawal, cytokines and toxins (Singh *et al.*, 2008).

Neurons have androgen receptors. Androgens alter the morphology, survival and axonal regeneration (Kulkarni *et al.*, 2008) of both sexually and nonsexually dimorphic motor neurons (Fargo *et al.*, 2008; Huppenbauer *et al.*, 2005). The molecular mechanism responsible for the effect of androgens on motor neurons is poorly understood (Stein, 2008; Gibson *et al.*, 2008; Sayeed *et al.*, 2007).

Testosterone acts via Androgens Receptor (AR<sub>c</sub>). Regulation of AR protein and AR mRNA by androgens has been observed in mammals in multiple androgen-responsive tissue, such as the brain, prostate, testes, ovary and adrenal glands (Bialek *et al.*, 2004).

Testosterone, the gonadal sex steroid hormone has various effects on numerous body tissues, including the brain. Numerous biological effects of (testosterone) within the brain support the argument that their influence is not restricted to the reproductive system (Rudzinski and Krejza, 2002; Billeci *et al.*, 2008) following crush injury to the sciatic nerve in rats, treatment with androgens enhances axonal regeneration rats and decreases time to recovery (Fargo *et al.*, 2008).

Therapeutic effects of gonadal steroid encompass neuroprotection from axotomy induced cell death is the focus of the present study.

## MATERIALS AND METHODS

All experiment was conducted in faculty of science, Islamic Azad University of Mashhad, Iran (2008-2009).

**Animal subjects:** Thirty-two male, Wistar rats weighting between 300-350 g served as subjects for these experiments. All animals were housed individually and maintained on a 12/12 light/dark cycle, with lights for 6 h. Ambient temperature in the animal facility was kept at  $22\pm 2^{\circ}\text{C}$ . Food and water was given *ad libitum*.

**Surgery:** Animals were anesthetized under interaperitoneal injection of 0.24 cc of a mixture (1:2) of 10% ketamin and 2% xylazine. Right sciatic nerve was exposed through a gluteal muscle splitting incision. At this location the nerve trunk was crushed for 30 sec period between prongs of No. 5 clamp forceps. The muscle and skin were then closed with 14 mm stainless steel sutures (Behnam-Rasouli *et al.*, 2000).

### Groups

- **Controls (N = 8):** For baseline measurement in this group on the right side an operation was performed which exposed the sciatic nerve but did not disturb it. (Just for induced stress effect of operation)
- **Compression or Sham-operated controls groups (N = 8):** In this group after operation the right sciatic nerve was crushed
- **Castrated male rat (N = 8):** In this animal Coordinatetd with sciatic nerve crush were castrated
- **Subcatenus injection testosterone (N = 8 L):** After sciatic nerve crush were given one subcutaneous injection ( $100\text{ mg kg}^{-1}$ ) in each week

At the selected post-operative time (4 weeks), rats were anesthetized and intracardially perfused with formaldehyde. Immediately following perfusion a block of the spinal cord segments L4 to L6 (approximately 8mm length) was removed while sciatic nerve roots of both sides were still attached it. The spinal blocks were placed in the same fixative for post sampling fixation overnight and then processed and embedded in paraffin. The blocks were sectioned serially at 7 mm. A uniform random sampling scheme was employed so that about 10 sections

from each block were sampled. With each section thus selected its immediately preceding neighbor was also collected.

Sections were stained with toluidine blue staining method with special buffer of acetic acid, sodium acetate and distilled water (pH = 4.65). After permanent mounting the number of motoneurons in right sides of ventrolateral regions of the spinal cord ventral horns (L4 to L6) were determined, using stereological counting technique; physical dissector (Behnam-Rasouli *et al.*, 2000).

The dissector principle was used to determine the umbers of motoneurons in each section. Form each section and it's adjacent neighbor two photos were taken, one from each section with a final magnification of 100. A two-dimensional unbiased counting frame was overlaid in a uniform, random manner on to regions of any two photos taken from right sides of both sections. Those cell nuclei selected by the frame on the reference plane but not appearing on the adjacent look-up frame section were deemed to have their tops in the volume described by the product of the area of the counting frame and the distance between sections. These nuclei were counted (Q) to provide the numerical density of cells (NV) in the ventral horns of spinal cord according to the Equation:

$$NV = \frac{\sum a}{\sum \text{frame} \times V_{\text{dissector}}}$$

where,  $\sum a$  is the sum of counted neurons,  $h$  is the depth of the dissector equal to the section thickness ( $7\ \mu$ ) and  $a$  (frame) is the scaled area of the dissector frame.

**Statistical analysis:** The ratio of numerical density of neurons in samples of spinal cord was then used as an index of neuronal death. All quantitative data were analyzed using ANOVA and t-test.

## RESULTS

The effects of testosterone treatment on the numbers of intact neurons in the right ventral horn of spinal cord region at 28 days after sciatic nerve compression in rats are shown in Fig. 1 and 2. The control group revealed healthy neuronal cells (Fig. 1a, b) amounted by  $11000 \times 10^{-9} \pm 400$  intact neurons Fig. 2. Sciatic nerve crush resulted in 112 massive neuronal damage manifested as a significant ( $p < 0.05$ ) 25% decrease in the number of normal appearing neurons (Fig. 1, 2). In castrated group this reeducation increases (50%).

Animal treated with testosterone immediately after compression (Sciatic nerve injury) and continued for 4 week resulted in a significant ( $p < 0.05$ ) increase in the

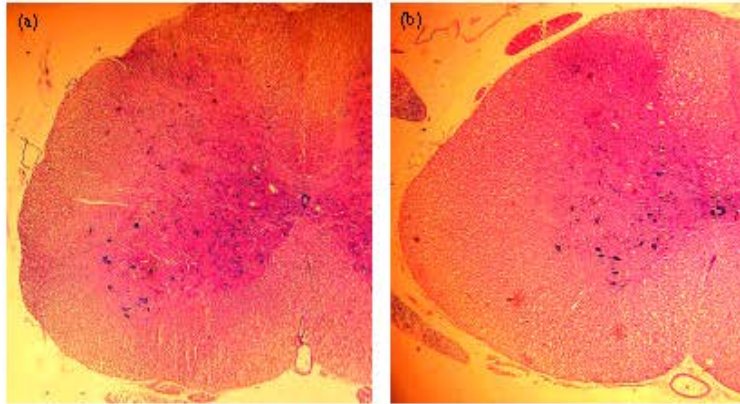


Fig. 1: Photomicrographs illustrate neurons within the anterior horn of spinal cord stained with toluidine blue and eosin at magnification of (20x) 28 days after injury. ((a) Left: Compression + testosterone, (b) Right: Compression)

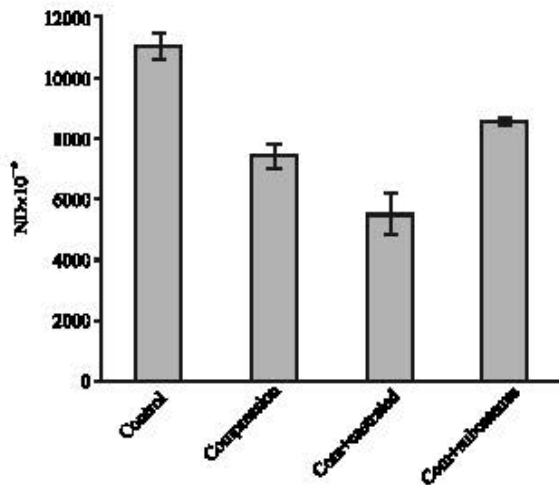


Fig. 2: Effects of compression and testosterone administration on the number of intact neurons right ventral horn of spinal cord in rat. Results are expressed as Mean±SD of 8 rats and data were analyzed by one-way ANOVA followed by Tukey-kramer multiple comparisons test. Significantly different from control, compression, compression+castrated, compression+subcutaneous testosterone groups

number of intact neurons, respectively as compared to compression group (Fig. 1, 2). However, still a significant ( $p < 0.05$ ) decrease in the number of intact neurons when compared to the control group has remained.

## DISCUSSION

Progressive dysfunction and death of neurons characterize neurodegenerative diseases (Baker and

Hagg, 2005). There are some evidences supporting the hypothesis that testosterone may act protectively in some neurodegenerative disorders (Sicotte *et al.*, 2007).

Recent data suggest that gonadal steroids may also exert neurotrophic actions. It provides neuronal differentiation and increase in neurite outgrowth after activation of androgen pathway in the cultured neural cells (Jones *et al.*, 2001).

Other experiments suggest that testosterone is linked to an increase in neuron somal size, neurite growth, plasticity and synaptogenesis in both motoneurons as coordinate to our result of the spinal nucleus (Tanzer and Jones, 2004), there is a remarkable change in the number of Alpha motoneurons in different groups. In Animal treated with testosterone the number of intact neurons was increased, respectively as compared to compression group (Fig. 1).

There is several potential mechanism of testosterone protection against neurodegeneration Based upon the information. One can envision two principal pathways:

- Maintaining neural function
- Protection against damage (Gold and Voskuhl, 2006)

Testosterone such as 17beta-estradiol increases expression of nerve growth factor and mediates promotion of neurite growth and interneuronal communication through branching and arborization (Islamov *et al.*, 2002). Another mechanism includes an increase in rate of axonal regeneration via selective alterations of the neuronal cytoskeleton (Schumacher *et al.*, 2004) and synergistic stimulation of protein synthesis in combinations with other cytokines such as insulin growth factor-1. Some studies show that neurosteroids, which are synthesized within the nervous system by neurons and glial cells, may exert neurotrophic actions (Marian *et al.*, 2007).

Present results showed a decrease in androgen levels after castration. Produced dramatic structural changes, decreasing the dendrite length and soma size and number of these motoneurons ( $p < 0.05$ ) (Fig. 2).

Changes in dendrites morphology that happen after nerve injury may influence synaptic connectivity and neuronal function. Present results showed that, these changes are reversed by androgen testosterone replacement. After 4 week testosterone treatment, dendrites arbors per cell and soma size were restored to normal levels. These results indicate that testosterone may regulate neuronal function in injured rats by influence on length and size of dendrites of motoneuronal somas.

Other protective properties of testosterone within the brain are related to attenuation of the excitotoxic effects of glutamate and to the activation of enzymes scavenging free oxygen radicals. Moreover testosterone can diminish free radicals synthesis and act as free radicals scavengers themselves. Testosterone can also activate synthesis of bcl-2 protein, which prevents cell apoptosis in the injured regions (Prokai-Tatrai *et al.*, 2007), this function is coordinate with our finding to reduced the motoneuron degeneration after peripheral injury.

We have demonstrated that the gonadal steroid testosterone has therapeutic effects in the spinal cord, protecting surviving motor neurons from atrophy after the death of neighboring motor neurons and regulating the expression of receptors for trophic factors, proteins critical for the maintenance of normal structure and function. This study has important implications in that if the appropriate trophic support can be provided to injured motor neurons, then the progression of neurodegenerative diseases could be slowed, or the time required for the recovery of motor function after injury could be reduced.

These findings are important because they demonstrate that testosterone, a powerful hormone long associated with such things as sex, aggression and athletic performance enhancement, could also be playing an important role in the maintenance and repair of the nervous system after injury or disease. These results are directly relevant to clinical application as potential treatment strategies, as well as to basic neuroscience questions on the maintenance of important cellular features.

## CONCLUSION

Present findings demonstrate that testosterone play an important role in the maintenance and repair of the nervous system after injury or disease.

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