Original Article

MORPHOMETERIC STUDY OF PRIMARY SENSORY NEURONS FOLLOWING SURGICAL REPAIR OF SCIATIC NERVE

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ABSTRACT
Objective: Peripheral nerve transection causes neuonal cell death. Effects of surgical nerve repair on survival of primary sensory neurons was to be studied.
Methodology: This is a morphometeric study of dorsal root ganglion neurons of adult rats by Hoechst staining conducted at Neuroscience laboratory and Cellular and Molecular Center, Medical faculty, Iran University of Medical Sciences. Models of direct epineurial suture and using of nerve guidance channel of polyvinilydin flourid (PVDF) were used and neuronal number and volume of dorsal root ganglion of L5 were evaluated by morphometeric method.
Results: Direct epineurial suture technique decreased sensory neuronal loss and both direct epineurial suture & using of nerve guidance channel decreased reduction of the ganglion volume.
Conclusion: Present study showed distal segment of peripheral nerve is essential for neuronal survival, but surgical nerve repair could not completely prevent from neuronal cell death.

KEY WORDS: Dorsal root ganglion neurons, Sciatic nerve, Surgical nerve repair, Morphometery.

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INTRODUCTION

Nerve transection induces neuronal cell death. It is reflected in a decline of neuronal number of dorsal root ganglion in the neonatal and adult rat.[1-4] Between 7% and 50% of primary sensory neurons die after peripheral nerve injury.[4-6] Although various factors are implicated in the poor sensory outcome,[7,8] the single most important factor is probably the death of primary sensory neuron itself.[4] Indeed, the first prerequisite for axonal regeneration is survival of the neuron following injury.[7] There are various techniques of surgical nerve repair such as nerve graft, direct epineurial suture, and using of nerve guidance channels.[9,10] but poor recovery of function is a frequent complaint following even the most successful peripheral nerve repair, with many patients suffering lack of sensation and impaired coordination.[11] It is important to know the fate of primary sensory neurons after the surgical repair of their peripheral process to test the hypothesis that the extent of functional recovery is dependent on the survival of neurons.To date, no long-term comparative studies have been undertaken to assess the changes that occure in the primary sensory neurons after the repair of a specific peripheral nerve by means of a clinically appropriate surgical technique. The aim of this study is to investigate

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number of dorsal root ganglion neurons and volume of dorsal root ganglion after the nerve has been repaired, using two techniques of surgical nerve repair.

METHODS

The experimental (repair) and control (axotmoy and sham) groups each consisted of four male Wistar rats weighing between 200-300g. The animals were anesthetized with ketamine (100mg/kg) and xylazine (10mg/kg), both given intraperitonealy and their left sciatic nerves were exposed in midthigh. In axotomy groups, 9- to 10-mm portion of nerve excised. Spontanous regeneration was prevented by ligating both nerve stumps (5-0 nylon). In direct epineurial suture groups, the sciatic nerve divided and then repaired immediately, using 10-0 nylon sutures.

In NGC (Nerve guidance channel) groups, 9- to 10-mm portion of nerve excised and the transected nerve ends were secured approximatly 1mm into the ends the sterile poled PVDF (Polyvinylidine flouride) tube with 10-0 nylon epineural sutures at each end creating an intervening 10- mm gap. The PVDF tube was 14mm in length with an internal diameter of 1.6mm (Harvard Apparatus Ltd). After placement, the chambers were filled with 1.28mg/ml,[12] collagen gel (Roche**)**. For polling of PVDF tube, a tin wire inserted in the lumen of the PVDF tube served as an inner electrode and a circumferential array of steel needles served as the outer electrode. The outer needle electrodes were connected to the positive output of a voltage supply and the inner electrode was grounded. The voltage output was gradually increased to 21kV over a 2-h period and was maintained at that level for 12h,[13] For sham groups, left sciatic nerves were exposed in midthigh but were not cut.

After 12 weeks, two animals from each groups anaesthetized with intraperitoneal ketamine (100mg/kg) and xylazine (10mg/kg) and pre-fixation achieved by transcardiac perfusion with 150ml normal saline then 200ml 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (PB). The left and right L5 DRG were removed and post-fixed in 4% PFA then 30% sucrose, both for 24 h at 4ºC. The ganglia were blocked in OCT and stored at -80ºC. Each entire ganglion was cut into serial 15-Î¼m cryosections & 1 from 4 sections mounted onto gelatine-coated glass slides and dried overnight.

Neuron counts were performed using Hoechst 33342 *(Sigma,B2261)*. H33342 is a nonspecific nuclear stain allows excellent determination of nuclear morphology.[14] For the staining, at first the slides washed 3 times for 5 min each in 0.1 M PBS at room tempreature. Then the slides were placed in Hoechst 33342 (1Î¼g ml^{-1} in PBS) for 15 min at room temperature. At the end, the slides were washed 3 times for 5 min each in 0.1M PBS at room temperature, mounted under glass coverslips, and veiwed by Fluorescence microscopy *(Olympus, AX70 Japan)*. From each section prepared images *(DP11 camera)* with X100 and X400 magnification, and normal nuclei were counted.

Estimation of the ganglion performed using Cavalieri Principle,and direct measurements in a systemic random sample of 10 or more sections from each ganglion to permit calculation of the mean thickness and cross-sectional area (using Olysia softwear). The volume of the ganglion (V) is calculated thus: V= a.t.s [4] where V is volume of ganglion, a = mean area section, t = mean section thickness and s is the total number of sections. Neuron loss was calculated by subtracting the number of neurons in in ipsilateral ganglion from that in their contralateral controls. Loss was then expressed as a percentage of the neuron count in the control ganglia. Comparison between groups was performed using ANOVA and students *t-test*. $P<0.05$ was adopted for rejection of null hypothesis.

RESULTS

Twelve weeks after nerve repair the mean number of neurons present in the unoperated contralateral control ganglion (L5) of untraeted was 248.6 ± 21.9. This was similar to the equivalent count in sham treated (241.8 ± 18.06; $P < 0.8$), but lower than that in the axotomy group (144.2 ± 11.2; P =<0.001). How-

ever by 12 weeks after direct epineurial suture repair, the mean number of neurons in the ipsilateral ganglion (L5) was 189.8 ± 15.5 and in the comparison with axotomy group it was significant ($P < 0.02$), while in nerve guidance channel group, it was not significant (163.9 ± 5.6; $P < 0.8$) (Figure-1). The mean number of neurons in the ipsilateral ganglion (L5) both repair groups were lower than the contralateral ganglion significantly ($P < 0.04$ and $P < 0.001$ respectivly), but it wasnot significant between epineurial suture group and NGC group ($P < 0.6$).

In the axotomised animals a loss of 42% of L5 neurons occurred during the 8 weeks after axotomy. At 12 weeks neuron loss was not affected by sham treatment (2.84%), while in epineurial suture group and in NGC group were 23.7% and 34.1% respectively.

After12 weeks the mean volume in the contralateral ganglion of L5 (unoperated side) was 2963 ± 306Î¼m^3. This was similar to sham group (2870 ± 259Î¼m^3; $P<0.8$), but it was lower than axotomy group (1558 ± 163Î¼m^3; $P<.000$). The volume reduction in sham group and axotomy group were 3% and 47.7% respectivly. After epineurial suture repair, the mean volume of the ipsilateral ganglion of L5 was 2084 ± 100Î¼m^3 while in NGC group it was 2071 ± 48Î¼m^3. In comparison with intact and axotomy groups (Figure-2),these data are significant ($P<0.01$ compared with contralateral side; $P<0.005$ and $P<0.004$ compared with axotomy group). The volume reduction in epineurial suture group was 29.7% and in NGC group was 33.9%.

DISSCUSION

The present study shows that after 12 weeks from surgical nerve repair, sensory neuronal loss and reduction of ganglion volume decrease, but cannot prevent completely. Although several previous studies have evaluated morphometric aspects of primary sensory neurons after axotomy, the conclusions of surgical nerve repair still remain controversial.[4,11,15] At 42% the peak neuronal loss after nerve transection was in keeping with results in the literature,[4,5,16] although smaller losses have been reported.[1,2]

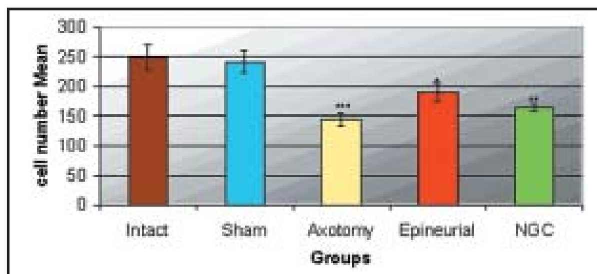

Fig-1: Comparison of neuronal count in DRG neurons of L5, 12 weeks after surgery
***P=.000 **P<0.001 *P<0.04 NGC: Nerve guidance channel , DRG: Dorsal root ganglion

Variations may be due to different animal models, counting techniques or levels of axotomy. Following peripheral nerve injury, there is a disruption in the supply of retrogradly transported neurotrophic factors, leading to neuronal cell death.[6] In adult animals, this is particularly true for sensory neurons, where a peripheral nerve axotomy induces a substantial cell loss varying between 25%-50% depending on the type and proximo-distal level of the lesion.[4,16]

Our study showed that repair of the proximal nerve stump with its distal stump will reduce the sensory neuronal loss from 42% to 23.7%. Ma et al. showed this reduction is 50%.[15] Other results have indicated that sensory neurons retain their ability to regenerate for at least 8 weeks after nerve injury,[17] but early[15] and delayed[17] reconstruction of injured with peripheral nerve grafting fail to rescue neurons in dorsal root ganglia.After nerve repair the Schwann cells surrounding the distal nerve stump will produce trophic molecules that are retrogradely transported to the cell bodies and

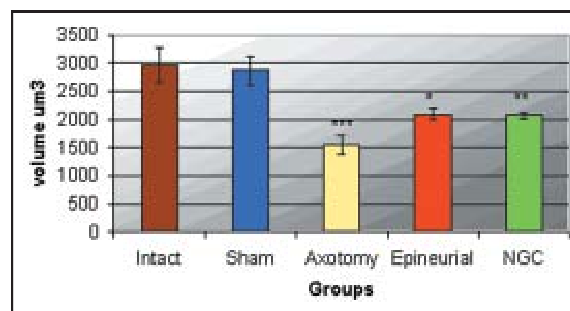

Fig 2. Comparison of volume in DRG of L5 , 12 weeks after surgery
***P=.000 **P<0.01 *P<0.01 NGC: Nerve guidance channel , DRG: Dorsal root ganglion

will prevent neuronal atrophy and death. Many growth factors such as nerve growth factor, neurotrophin-3 and brain-derived neurotrophic factor act directly on sensory neurons to support their development, maintenance and regeneration.[18] In recent years, a number of studies have showen that exogenous administration of neurotrophic factors will significantly improve neuronal survival and prevent neuronal atrophy.[6] In the present study the importance of peripheral neurotrophic support was clearly demonestrated.

Axotomy caused a reduction in volume of L5 ganglion when compared with controls, and matches with the previously studies.[5] This commenced one week after axotomy and plateaued after 2 month.[4] The 18% and 13% rise in L5 ganglion volume after 12 months following direct epineurial nerve repair and using of nerve guidance channel respectively show that connection of proximal and distal segment directly and indirectly will decrease reduction of the volume of ganglion. Loss of volume may reflect both the reduction in volume of surviving neurons[5] and the actual loss of neurons. Reanastomosis between proximal and distal segment of the nerve can prevent neuronal atrophy[16] and subsequent death by providing early trophic support.

In conclusion, the present study showed that surgical nerve repair can have an effect on maintanance of primary sensory neurons after direct epineurial suture. Such an effect, however, could not be demonestrated in the using of nerve guidance channel of PVDF.

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