THE EFFECTS OF NERVE GROWTH FACTOR ON MYELINATION OF REGENERATED FIBERS IN RAT

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Abstract- The effect of nerve growth factor (NGF) on regeneration of rat sciatic nerves in adult rat was studied. The sciatic nerve was cut out across a 6-mm gap, then the proximal and distal stumps were inserted into the silicone tube chamber. 7s NGF was extracted from submaxillary gland and then was injected into the silicone in experimental group. After seven months nerve was transected and stained with toluidine blue. Semithin sections (1 µm from middle of silicone (control group, without NGF) showed that regenerated axons (mostly unmyelinated) were dispersed randomly, and they were not grouped into bundles. In this group some of the myelinated fibers were degenerated and macrophages or in other word, schwann cells contained a large amount of these degenerated sheaths. Semithin section of experimental group (with NGF) showed numerous regenerated axons (myelinated) that were grouped into small bundles. Schwann cells in experimental group were large and eucromatin and some of them were divided. These data indicate that NGF causes myelinated axons, regenerate and making new myelinated sheaths.
Acta Medica Iranica, 41(3): 183-187; 2003

Key Words: Peripheral nerve regeneration, schwann cell, NGF, myelination, rat

INTRODUCTION

A large gap in the continuity of a peripheral nerve is filled, in clinical practice, by autologous nerve grafting. This however, requires the sacrifice of a healthy nerve, usually by sural nerve, with permanent function impairment. A variety of strategies have therefore been used in attempt to develop alternative repair methods, including artificial grafts like silicone tubes or the use of biological allografts of different tissues (1-4). From these studies it apears that it is sufficient to provide the regenerating nerve fiber with a structure to grow on, and/or favour conditions by which such a structure can be formed between the nerve stumps to promote regeneration. Another approach for repair could be the use of nerve growth factor (NGF). NGF stimulates aspects of neuronal development as diverse as proliferation, survival and neurite outgrowth (2). The notion that NGF can act to guide growing axons was introduced over 20 years ago with the demonstration that injection of NGF into the brain of neonatal rodents causes the aberrant growth of axons of peripheral sympathetic neurons into the spinal cord to reach the site of injection (3). Later, culture experiments supported this idea by showing that NGF induces a chemotactic response from sensory neurons.

Received: 8 July 2002, accepted: 16 April 2003

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Recent studies from several groups, however, suggest that a possible function for growth factors in guiding axons should be re-examined (2). In this study we have investigated the influence of NGF on growing axons in which a gap between cross-anastomosed rat sciatic nerves was encased by a cylindrical silicone between chambers.

MATERIALS AND METHODS

Fourteen adult albino rats weighing 250-300 g were anesthetized by intraperitoneal injection of a solution containing nembutal (50 mg/ml), normal-saline and diazepam (5 mg/ml) in 1:1:2 volume proportions (5). The left and right sciatic nerves were all exposed and a 6 mm segment of the midthigh (nerves) was removed surgically. The two proximal and distal stumps of the nerves were inserted into either end of a silicone rubber tube leaving an interstump gap of about 6 mm. 7s NGF was extracted from submaxillary glands of 100 mature male mice (6), and assayed by Lowry's method (7). Bovine serum albumin (BSA) at concentrations of 0, 20, 40, 60, 80 ng was used for standard curve determination. The extracted NGF showed a concentration of 5.7mg/ml. Bioassay of NGF was done using E8 chicken dorsal root ganglia (8) and optimized concentration of 100 ng/ml NGF. NGF was injected into the silicone in the interstump gap region (experimental) and another

sciatic nerve was used as control. After 7 month (when rats seemed to look normal and natural from movement point of view) the remaining rats (6 rats) were killed, then the silicone together with the adjacent nerve segments (proximal, medial and distal) were intersected for histological analysis. In order to have microscopic images, silicone chambers (proximal, medial and distal) were immersed in glutaraldehyde for 3 hours at room temperature and in 2% of osmium tetroxide for 1 hour. Once fixed, the segments were dehydrated in a series of graded ethanol solutions and finally in absolute ethanol. To mold the specimens the nerves were then placed into molds and after toping up with Epon were incubated in an oven at 6 °C On a ultramicrotome, cut semithin (1 µm) was sectioned. Semithin sections were stained with toluidine blue and viewed by a light microscopy (Olimpus AH-2).

RESULTS

At the middle of the silicone, there were differences in the pattern of nerve regeneration between the control and experimental group. In the silicone not treated with NGF, the fibers mostly were unmyelinated, not grouped and rather separated and dispersed individually. Indeed, transverse semithin sections of control group showed both unmyelinated and immature myelinated axons. The sheath of myelin arround the immature myelinated axons was thin. Mostly, these were surrounded by endoneurial capillaries. In some of areas, the myelin was partially or completely broken up into scroll formations or globoids, with complete or partial obliteration central space normally occupied by axons. It seemed that the immature myelinated fibers before maturating become degenerated.

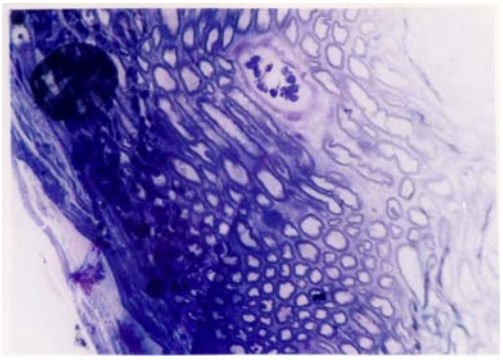

Fig. 1. Normal intact sciatic nerve from mature rat, Semithin section (1 µm) stained by toluidine blue.

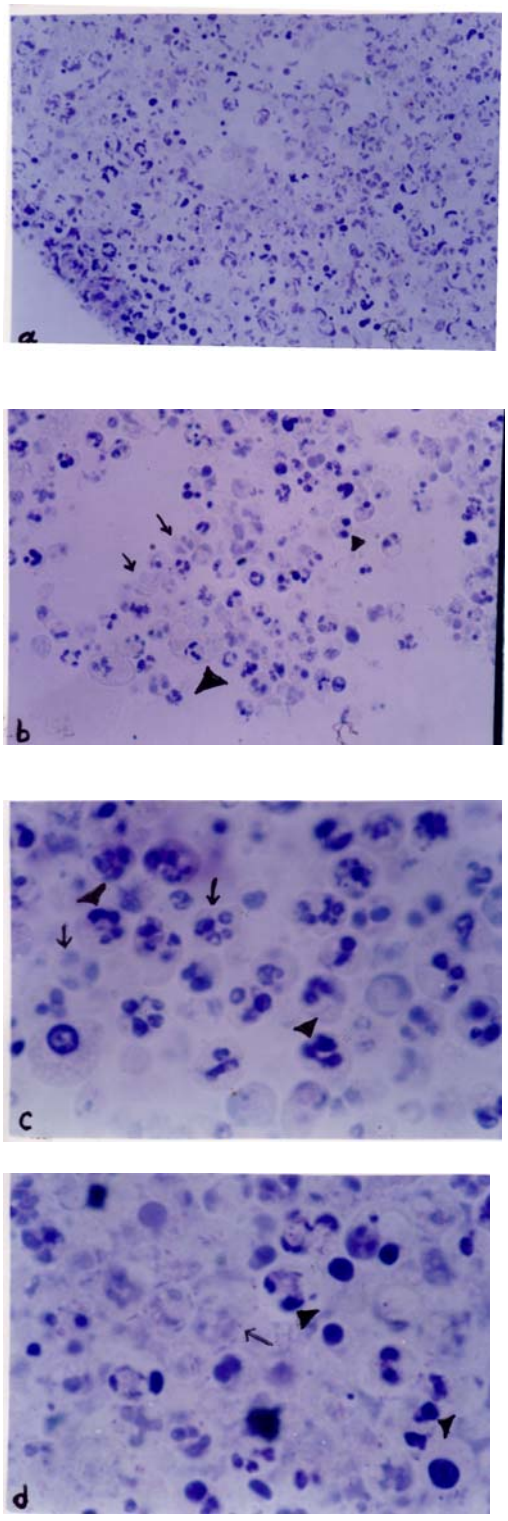

Fig. 2. Seven months after applying silicone chamber, transvers semithin section (1 µm) at the middle level stained by toluidine blue, (control group). a: x200 b: x400 C, D: x 1000 Numerous regenerated axons were found. The axons were dispersted randomly. Also there was an increasing in free endoneurial space. some myelinated axones are marked with arrow and newly made myelinated fibers marked with arrow head and macrophages containing phagocytic material marked with large arrow head.

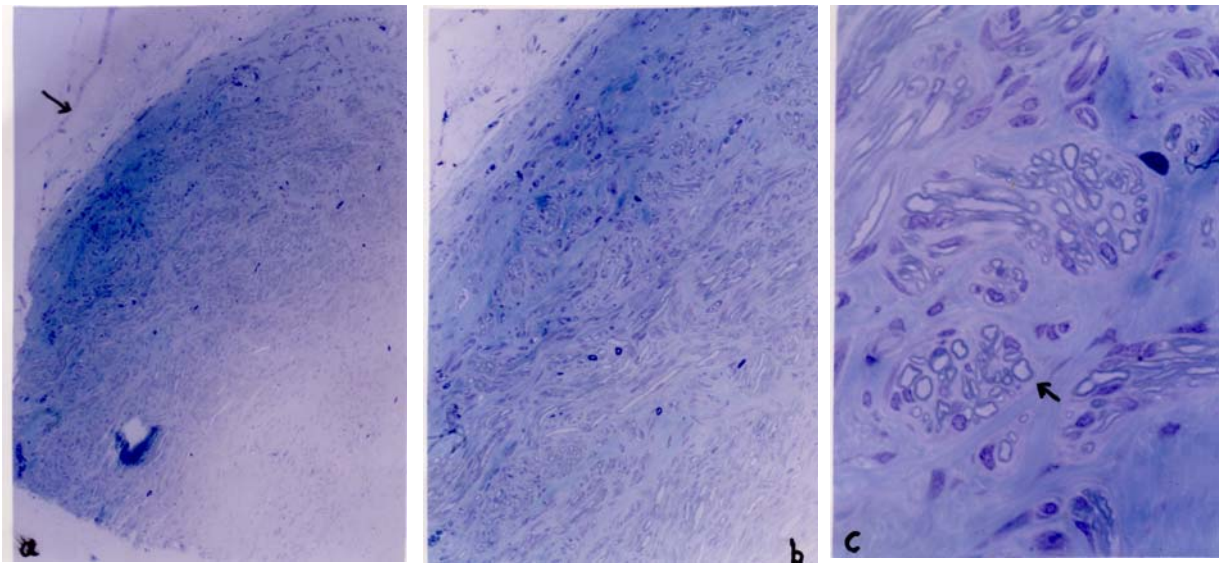

Fig. 3. Seven months after applying NGF into the silicone chamber (experimental group), transvers sernithin section (1 µm) at the middle of silicone stained by toluidine blue. a: x100 epineurium and perineurium marked with arrow b: x200 Numerous regenerated axons were found. This myelinated axons are grouped into newly small bundles (arrow). c: x 400 schwann cells seem to have high activity (arrow).

These myelinated fibers were surrounded by common schwann cells. It means that in the control group multiple regenerating axons, while being accompanied by schwann cells migrating from the proximal stump, had grown through the common schwann cell basal lamina tubes but not yet separated into individul fibers. However, the numerous, relatively small diameter, thinly myelinated fibers were seen as closely packed in clusters and associated with large schwann cells. Also schwann cells contained a large amount of phagocytic material. Numerous macrophages and fibroblasts were observed. Some of schwann cells were without any axons (Fig. 2). In contrast, in the group treated with NGF side myelinated axons were found grouped into small and large bundles consisting of a few to several fibers. These findings demonstrate that the effects of NGF were distinct in the silicone treated with NGF and suggest that NGF can facilitate the extention of regenerating axons (Fig. 3). The axons in control group were dispersed randomly (Fig. 2), where as in the experimental group were grouped into small bundles (Fig. 3) indicating that they had grown along the bundles of proximal stump (Fig. 1).

DISCUSSION

The present study demonstrated the effect of NGF on nerve regeneration. It is reported that nerve growth factor other than its influence on the distal segment of a trasected nerve is essential for successful regeneration.

This study showed that in the experimental group in which NGF was applied, the regeneration was accompained with myelination whereas in control group although regenerated axones can be seen but they are unmyelinated or not myelinated yet. We reported that another factor other than influence of distal segment of a transected nerve is essential for successful regeneration. Also Lundborge (1982) reported that with in the silicone chamber, the growth of myelinated fibers, compared with unmyelinated fibers, were significantly reduced by the greater gap of distal stump. These myelinated axons were with decreased number, density and diameter. We found that removal of a part of sciatic nerve will cause unmyelination. It is now clear that well known functions of ensheathment and myelination are specifically regulated by contact with axons, that the schwann cell is centrally involved in extracellular matrix production in the peripheral nerve trunk, and that the schwann cell plays a critical role in promoting axonal regeneration in the peripheral nervous system (9). Additional schwann cell functions are to induce provision of a versatile source of trophic factors, the capacity to remyelinate central nervous system axons and the restoration of electrophysiological conduction (10). NGF may provide additional or different

elements to myelination. Studies in which schwann cell proliferation was inhibited, suggest that schwann cell products may provide chemotactic influences to regenerating axons in vivo (11). Indeed NGF as an outgrowth of schwann cells plays a role in axon myelination. Fluid accumulated in silicone chamber in proximal-distal-10 mm arrangement contained significant neuronotrophic activity for sensory, sympathetic and motor neuron cultures (12,13,2). Attentive to our results, this amount does not seem enough for myelination of nerve in which there is a gap between two ends of transected nerve. If such factors do promote axonal myelination in the present chamber system, they may be over diluted diffusion-limited with increasing chamber length or increasing the excised length. In adult life schwann cell proliferation can be activated under pathological conditions which plays a crucial role in nerve regeneration (9). The contribution of nonneuronal cells to the supply of neurotrophic factors such as NGF is negligible (14). These neurons are trophically supported by factors produced in peripheral target tissue. However, after nerve lesion, there is a massive increase in the synthesis of NGF by the sciatic nonneuronal cells. Both fibroblast and schwann cell contribute to the synthesis of NGF-mRNA. It is already reported that the expression of growth factors, except for cNTF are quite low in intact sciatic nerve (15). On the other hand schwann cells respond to injury by the massive expression of gene encoding neurotrophic factors such as NGF and BDNF which would maintain neuronal survival and promote nerve regeneration. Perhaps removing a segment of sciatic nerve causes production of NGF in peripheral target tissue that does not perform its function for successful regeneration. Indeed an elevated NGF level is required for optimal synthesis of myelin in nerve after being removed. Whether or not this activity is effective on greater distance and or whether or not NGF acts directly on myelination axons by schwann cells are questions yet to be answered. The effectiveness of acellular nerve for grafting was suggested, but did not receive much attention from other investigators. Indeed, we showed that application of NGF is as effective as schwann cells columns as conduits in the early stage regeneration. The effects of basic fibroblast growth factor on growth of regenerating axons have been demonstrated by many in vivo studies (1). Acellular nerve transplants for allogenic grafting and the effects of fibroblast growth factor on the regenerating axons are thin compared to those of normal fibers. We showed that applying NGF causes the growth of myelination axons in the rat sciatic nerve in which schwann cells had been eliminated by removal of a part of nerve. Also basal lamina is a suitable extracellular matrix for nerve regeneration that can provide a scaffold and at the same time probably trophic factors for growing axons. Our findings indicated that in the absence of basal lamina in removed nerve, axons regenerated to the distal part of nerve. In addition the effects of NGF to myelination axons, and separation into groups as bundles, should also be considered. On the other hand, lines of in vitro evidence have shown that schwann cells degrade myelin and proliferate in the absence of macrophages (16). We observed that in the absence of injected NGF some of schwann cells represented macrophage morphology of themselves. In other words schwann cells did not synthesize myelin around the axons, although they showed characterstic macrophages.

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