Effect of Nerve Growth Factor on sciatic nerve regeneration following experimental repair

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In this study, sciatic nerve cuts were repaired using an epineural suture and the effect of subcutaneously administered Nerve Growth Factor (NGF) on sciatic nerve regeneration was studied. 10 fig NGF was used for each rat, subcutaneously. Rat sciatic nerves were examined electrophysiological^ and histopathologically 21 days following the operation. In this study we observed significant difference in sciatic nerve regeneration in the NGF given group when compaired to the control groups; Histopathologically, in the group treated with NGF and epineural repair, nerve fibers distal to the anostomosis site showed increased axonal regeneration and more myelinated regions when compared to other study groups. Electrophysiological results most close to normal M-response amplitude (AMP) and distal latency time (DLT) values were found in the epineural anastomosis plus NGF group. [T Klin J Med Res 1997; 15(1): 1-5]

Key Words: Nerve Growth Factor, Peripheric nerve regeneration, Peripheric nerve cut repair

Peripheric nerve cuts cause a wide array of psycho-social and economic problems. The aim of therapy is to obtain anatomic and physiologic healing of the nerve and to return the patient to his or her pre-injury functional state.

Studies on the pathophysiology and treatment of peripheric nerve cuts are continuing since the 18th century. According to Millesi (1), Cruikshank had shown in 1795 that regeneration of the peripheric nerve is completed by axons extending from the proximal segment to the distal segment. In our experiments, we used epineural suturing which is accepted as the ideal repair method in rat sciatic nerve (2,3).

Various methods have been tried till today for peripheric nerve repair, e.g suturing, collagen tubulisation, micropore taping, fibrin glue, laser (1,4,5). The method used should aim maximal healing and regeneration with minimal side effect. In recent years, several studies showed that NGF, a protein described in 1953, may also be effective on peripheric nerve regeneration (6-9).

Neurotrophic factors are specific proteins that support the survival and general growth capabilities of neurons (10). NGF is known to be consistently present in peripheral nerve segments where it is produced by both Schwann cells and endoneurial fibroblasts (11). NGF was the first discovered and the best-characterized of the polypeptides that exert neurotrophic effects. Beside its trophic role, other important properties of NGF are its neurotropic action both in vitro and in vivo, its ability to modulate the differentiative program of neuronal precursor cells and its contribution to the maintenance of the acquired differentiated phenotype (12). NGF is a target-derived neurotrophic factor for peripheral sympathetic and sensory and basal forebrain cholinergic neurons (11). Interestingly, the mRNA for NGF receptor is expressed at high levels in rat spinal motoneurons during development at the time of naturally occuring cell death (13). Spinal motoneurons, whose axons are in the sciatic nerve.

It has recently been shown that NGF is effective on peripheric nerve regeneration (6-10,12,14-21). Thoenen (10) has found that NGF regulates neuropeptide synthesis and neurite growth. Davies (14), Kessler and Black, in separate experiments, have found the same results. NGF synthesized by target cells is taken in to the cell by sympathetic axons of the peripheric nervous system and reaches the cell body by retrograde axonal transportation. Here it shows its effect via the co-transported receptor molecule (11,22).

The aim of this study is to evaluate the effect of the single dose of NGF, especially electrophysiologically, on sciatic nerve regeneration following epineural neurorraphy.

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MATERIAL AND METHOD

Thirty-three non-pregnant female Wistar rats with a mean weight of 150 g were used in this study. The rats were

Table 1. Mean DLT, AMP values and M-response dispertion in study groups

STUDY GROUP	DLT*	AMP*	DISPERTION
Normal nerve	1.04 +0.03	14.98 ±0.13	-
Without anastomosis	6.00 ±0.10	0.53 ±0.09	+
With anastomosis	2.91 ±0.05	1.60 ±0.13	+
Anastomosis + NGF	1.83 ±0.02	6.12 ±0.74	+

* DLT and AMP values are shown as mean ± standard deviation
When the NGF treated group was compared to other study groups, In the values of DLT, AMP, the difference was found to be statistically significant with Mann-Whitney-U tests ($p<0.001$).

Abbreviations: DLT: Distal latency time
AMP: M-response amplitude
NGF: Nerve growth factor

anesthetized with intraperitoneal Ketamin (Ketalar, Parke-Davis, USA) 50 mg/kg, and fixed in prone position. Following preparation of the operation site, the left sciatic nerve was exposed. The nerve was cut with a sterile rasor at a level proximal to posterior tibial and peroneal branches, under the operation microscope (Zeiss OPMI 66, Germany). In the first group there were 11 rats. In this group the cut ends of the sciatic nerve were brought together and nothing else was done. The second group also consisted of 11 rats. In this group cut ends of the sciatic nerve were repaired epineurally with 4 to 6 sutures (Ethilon 10/0, Ethicon, Edinburgh, Scotland). In the third group, in addition to epineural repair, 10 ug NGF (NGF-7S-from mouse submaxillary glands, product no: N-0513, SIGMA, Chemical Company, St Louis, MO.) was diluted with saline (23,24). All NGF-treated rats were injected subcutaneously with a single dose of NGF.

All rats were followed for three weeks and at the end of third week sciatic nerves from all rats were examined electroneuromyographically (Nihon-Kohden -Neuropack 2). For electroneuromyographic evaluation, the platinium monopolar recording electrode was placed in the peroneal muscle while the nerve was stimulated with a superficial stimulating electrode. In all rats, the recording electrode was placed 1.5 cm distal to the anastomosis site in the peroneal muscle, and the stimulating electrode was placed 1 cm proximal to the anastomosis site on the sciatic nerve trajectory. The intensity, threshold, frequency , time and mode of stimulation were kept constant in all study groups. Analysis time was chosen as 20 and 50 msec.

In electroneuromyographic (ENMG) evaluation, DLT, AMP and M-response dispertion were chosen as study criteria.

ENMG data was analysed with Mann-Whitney-U tests in order to evaluate the difference between DLT and AMP values.

After ENMG evaluation, sciatic nerves were cut proximal and distal to the anastomosis site and were excised. The specimens were examined histopathologically for the following criteria; Anatomical continuity, anastomosis condition, fibrosis at the anastomosis site, regeneration in the distal segment and regions rich of myelin.

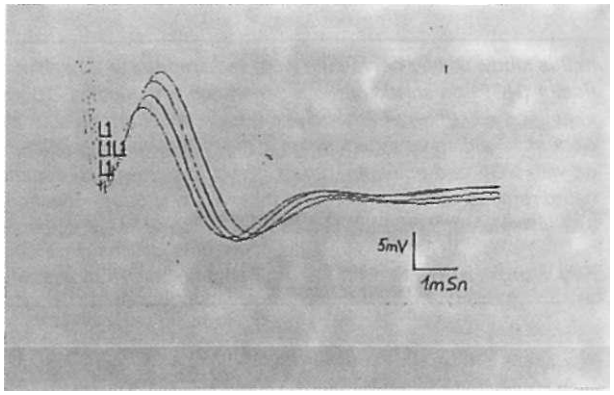

Figure 1. ENMG of a normal sciatic nerve.

FINDINGS

The rats were examined electroneuromyographically and histopathologically three weeks after the anastomosis.

A- Electrophysiological findings:

Results most close to normal AMP and DLT values were found in the epineural anastomosis plus NGF group. Mean values of DLT, AMP and M-response dispertion for study groups are shown in Table 1. When the NGF treated group was compared to other study groups, the difference was found to be statistically significant ($p<0.001$).

Sample ENMG results are shown in Figures 1 to 3.

B- Histopathological findings:

Histopathologically, in the group treated with NGF and epineural repair, nerve fibers distal to the anostomosis site showed increased axonal regeneration and more myelinated regions were noticed when compared to other study groups (Figures 4 and 5).

DISCUSSION

Heumann (25) has shown that, when adult rat sciatic nerves are cut, NGF synthesis increases in Schwann cells lying adjacent to the axon and distal to the lesion,

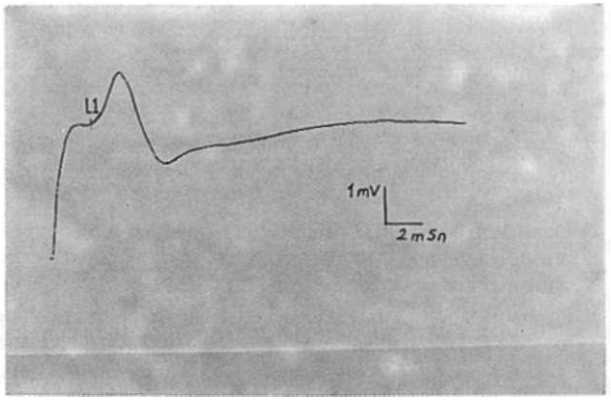

Figure 2. ENMG of a cut sciatic nerve following epineural anastomosis. DLT is shorter and AMP is lower when compared to the group without anastomosis.

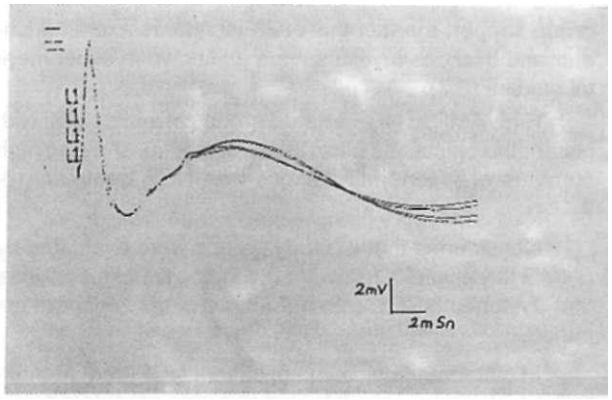

Figure 3. ENMG of a sciatic nerve treated with NGF after epineural repair. DLT and AMP values are close to normal sciatic nerve values.

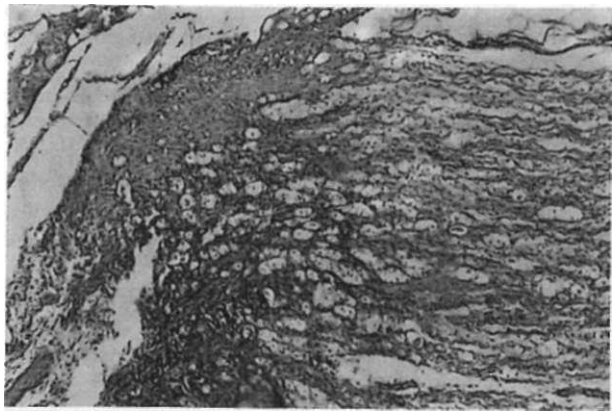

Figure 4. Increase in axonal regeneration in the group treated with NGF (Masson TrichromexlOO).

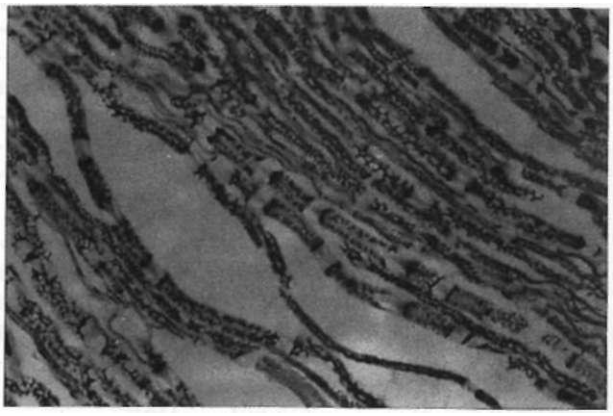

Figure 5. Increase in myelinated regions indicated with blue color in the group treated with NGF after epineural repair (Woelckex400).

similar to culture conditions. According to Davies (14), Lindholm and Bandtlow et al have found similar results and also have shown that NGF synthesis is aggrevated by stimulation of interleukin-1 originating from macrophages migrating to the injured nerve . In addition, it has been shown that following sciatic nerve injury, in the repair phase, NGF-receptor mRNA levels are elevated in spinal motor neurons (13).

In recent histopathologic and ultrastructural studies evaluating the effect of exogeneous NGF on cut peripheric nerves, it has been shown that neuronal death is decreased by 55% and regeneration with elevated diameter and thicker myelin sheath has been observed (6-9).

In our study, we investigated the electrophysiological aspect of the effect of NGF on peripheric nerve regeneration in the rat sciatic nerve model.

Systemic administration of the NGF both elevates synthesis of NGF at the distal segment and increases NGF-receptor expression in the target cells, thus potentiating the present effect of NGF (19).

Electroneuromyographic examination of the injured peripheric nerve gives important clues about regeneration. Nerve conduction velocity (NCV), DLT, AMP and configuration are basic criteria (26). In our study, we used DLT, because it directly reflects NCV. In addition, a positive correlation between AMP and active axon number is also known (27). For this reason short DLT, high AMP and lack of M-response dispertion is electrophysiological parameters showing a good regeneration.

In our study, DLT values were significantly shorter in the NGF group when compared to the anastomosis only group. In the NGF treated group, DLT shorthening was statistically significant and AMP was significantly high when compared to the anastomosis group. These findings show electrophysiologically that parenteral NGF has a positive effect on nerve regeneration. Axonal diameter and thickness of the myelin sheath are important parameters of NCV. Short DLT values in the NGF treated

group support the fact that NGF increases axonal diameter and thickens myelin sheath as shown in experimental studies (6,7).

High AMP in NGF treated group correlates well with histopathological and ultrastructural studies showing high numbers of regenerating axons with NGF treatment (6-9)-

Considering that all study groups were evaluated by ENMG three weeks following the injury, we can conclude that systemic NGF treatment shortens the regeneration period.

In histopathological examination, NGF treated neurofilaments were more tidy and this was considered as a sign of regeneration closer to normal when compared with the untreated group. In addition, in the group treated with NGF there was an increase in axonal regeneration distal to the peripheric nerve. This finding correlated well with prior studies on the subject (6-9).

Fibrosis at the anastomosis site was observed in all groups, being most prominent in the group with unsutured nerve endings. There are prior studies recommending the use of corticosteroids to decrease fibrosis (7,28,29). As dexamethasone has been shown to decrease NGF-receptor mRNA accumulation in an experimental study, it may be more convenient to search other agents to decrease fibrosis (30).

In prior studies, NGF was administered subcutaneously during a period of 4 to 10 days (23,24). In our study NGF was administered subcutaneously in a single dose, and this regimen was found to be effective in the regeneration of the sciatic nerve by shortening the regeneration period. It has been shown in an experimental study that long-term use of high dose NGF causes mechanical and heat hyperalgesia in adult rats (31). Therefore, we may conclude that a single dose regimen will cause less side effect.

In conclusion, NGF administration must be kept in mind in the repair of peripheric nerves. In this study we showed that NGF shortens the regeneration period following peripheric nerve repair.

Nerve Growth Faktör'ün deneysel onarımı takiben siyatik sinir rejenerasyonu üzerindeki etkisi

Bu çalışmada, siyatik sinir kesileri epinöral sutur kullanılarak onarıldı ve subkutan verilen Nerve Growth Faktör'ün siyatik sinir rejenerasyonu üzerindeki etkisi incelendi. Her rat için subkutan 10 ug NGF kullanıldı. Rat siyatik sinirleri operasyonu takiben 21 gün boyunca incelendi. Bu çalışmada, kontrol gruplarıyla karşılaştırıldığında siyatik sinir rejenerasyonunda belirgin bir fark gözlendi. Histopatolojik olarak NGF ve epinöral onarım ile muamele edilen grupta, diğer çalışma grupları ile kıyaslandığında anastomoz hattı altındaki sinir liflerinde artmış aksonal rejenerasyon ve daha fazla myelinize bölge mevcuttu. Epinöral anastomoz+NGF grubunda normal M-Response Amplitude (AMP) ve distal latans zamanına yakın elektrofizyolojik sonuçlar bulundu. [T Klin Araştırma 1997; 15(1):1-5]

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