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RESEARCH ARTICLE

Electrophysiological Assessment of the Effects of Silicone Tubes and Hyaluronic Acid on Nerve Regeneration in Rats with Sciatic Neurorrhaphy [1]

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Summary

One of problems after peripheral nerve injury and surgery is scar tissue formation. Although the continuity of axons is restored, often the axonal viability and recovery is blocked by scarring. For preventing from scar formation silicone tubes are widely used. In addition, in the present study was used hyaluronic acid, which prevents adhesion. The aim of the present study is to evaluate electrophysiological results on nerve regeneration obtained after incision and reconstruction of rat sciatic nerve using only silicone tubes and silicone tubes filled with hyaluronic acid. Electrophysiological measurements were performed at 30th and 60th days after surgery. The amplitude and latency of the compound muscle action potentials and nerve conduction velocities were calculated for each rat. Although sciatic nerve conduction velocities in all operated groups not reached control values, amplitude and latency values in group with silicone tube and in group with silicone tube filled with hyaluronic acid statistically achieved control values on day 60. Silicone tubes filled with hyaluronic acid had better results compared to the silicone tubes without hyaluronic acid. Hence, the use of silicone tubes and hyaluronic acid for the reconstruction of nerve injuries seems to give good results on axonal regeneration.

Keywords: *Silicone tube, Hyaluronic acid, Sciatic nerve, Regeneration, Rat*

Rat Siyatik Sinir Nörorafisinde Silikon Tüp ve Hyaluronik Asit Uygulamasının Sinir Rejenerasyonu Üzerine Etkilerinin Elektrofizyolojik Olarak Değerlendirilmesi

Özet

Periferik sinir hasarı ve cerrahisi sonrası önemli sorunlardan biri de skar dokusu oluşumudur. Aksonların devamlılığı sağlansa da, aksonun canlılığı ve iyileşmesi genellikle skar dokusu tarafından engellenmektedir. Skar dokusunun oluşumunun önlenmesi için silikon tüpler yaygın olarak kullanılmaktadır. Silikon tüpe ek olarak, sunduğumuz çalışmada adezyonları önleyebilen hyaluronik asit kullanılmıştır. Bu çalışmanın amacı, ratlarda siyatik sinir kesisi ve onarımında silikon tüplerin ve hyaluronik asit ile doldurulmuş silikon tüplerin sinir rejenerasyonu üzerine etkilerinin elektrofizyolojik yöntemlerle araştırılmasıdır. Elektrofizyolojik ölçümler cerrahi sonrası 30. ve 60. günlerde yapılmıştır. Her rat için bileşik kas aksiyon potansiyelleri amplitüdleri ve latansları ile sinir iletim hızları hesaplanmıştır. Tüm opere olmuş gruplarda siyatik sinir iletim hızlarının kontrol değerlere ulaşamamasına rağmen, sadece silikon tüp ve silikon tüp ile hyaluronik asit gruplarında amplitüd ve latans değerleri 60. günde istatiksel olarak kontrol değerlere ulaşmıştır. Hyaluronik asit kullanılmış grupta sonuçlar hyaluronik asit kullanılmayan gruba göre daha iyiydi. Böylece, sinir onarımı sırasında silikon tüplerin ve hyaluronik asidin kullanımı sinir rejenerasyonu açısından iyi sonuçlar sergilemektedir.

Anahtar sözcükler: *Silikon tüp, Hyaluronik asit, Siyatik sinir, Rejenerasyon, Rat*

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INTRODUCTION

One of the significant problems after peripheral nerve injury and surgery is scar formation among surrounding tissue and nerve. Peripheral nerve scarring usually results in loss of sensory and motor functions, persistent pain and significant morbidity. So, management of peripheral nerve adhesion, which prevents axonal regeneration, has been an important problem for surgeons. In the past few years, scientists have begun to use nerve conduits for prevent adhesion and enhancement nerve regenerations after peripheral nerve injury and surgery [1-8]. Nerve conduits are surgical methods to assist regeneration of nerve axons within an interneural stump gap through a tube of biological or artificial material [1,2]. Silicone was one of the first synthetic conduits which were used for tubulation a nerve repair site. Researchers described the reinnervation patterns and significant functional recovery after silicone tubulation, even if there is a defect in nerve [3,4]. Because silicone tubes are available in a diameter large enough to repair major nerves, use of them in human practice has been reported [5]. In addition, the mechanical properties of the silicone tube may guarantee that it will not collapse during the movements and will be sufficiently flexible.

Additionally, some other strategies (for example, tubes filled different biomaterials) were used for prevent scar tissue formation and for nerve tissue regeneration [6,7]. One of these biomaterials is hyaluronic acid (HA). HA, known as hyaluronan, is one of extracellular matrix (i.e. decellularized nerve tissue) component of the mammalian nerve system that has been commonly used to repair nerve injuries. HA is easy to use because it is biocompatible (non-immunogenic), has got commercially available sterilized solution and the ease with various properties can be modified [8,9]. HA- based materials have been tested in the experimental models of the peripheral nerve injury and spinal cord injury [10,11].

Often, surgical repair of peripheral nerve injuries does not result in complete functional recovery. Although the continuity of sensory and motor axons is restored, often the axonal viability and recovery is blocked by scarring, which serves as mechanical barrier to regenerating axons. For this reason, determination of the functional state of nerve is very important. One of methods which determined functional assessments of repaired nerves are electrophysiological measurement (electroneuromyography-ENMG). For the functional investigation of nerves *in vivo* and *in vitro*, the ENMG has widespread application in basic research and in clinics. In particular, for motor recovery assessment after different peripheral nerve injury the electrodiagnostic evaluation of sciatic nerve in animal models has been used widely [12]. The aim of the present study is to evaluate electrophysiological results obtained after incision and end-to-end reconstruction of rat sciatic nerve using only silicone tubes and silicone tubes filled with hyaluronic acid. The present study hypothesized that silicone nerve tube filled with HA will reduce scar formation and benefit the functional recovery of sciatic nerve after repair.

MATERIAL and METHODS

The Ethic Committee on Research Animal Care at Veterinary Faculty of Kafkas University of Kars, Turkey approved all procedures in this study (No 2012/30).

Forty-two male, adult Wistar Albino rats, weighting 200-225 g, were used in the present study. Thirty-six rats were randomly divided into equal 3 groups and six rats were allocated as controls.

Group I (n=12) - only sciatic nerve transection and repairing were applied.

Group II (n=12) - sciatic nerve transection, repairing and silicone tube were applied.

Group III (n=12) - sciatic nerve transection, repairing and silicone tube filled hyaluronic acid were applied.

Control group (n=6) no operation was carried out.

All 36 rats in 3 study groups were anesthetized with intraperitoneal combination of 10 mg/kg xylazin HCl (Rompun® 2% 50 ml Bayer), 80 mg/kg ketamin HCl (Ketasol 10% inj, 10 ml vial Richter Pharma). The medial surface of the right and left thigh was routinely prepared by shaving and the operation field was prepared with 10% polyvinylpyrrolidone iodine. The sciatic nerve was exposed by an anteromedial longitudinal straight incision between the greater trochanter and the lateral condyle of the femur. The both sciatic nerves were detached from the environment soft tissues at the level of its middle third. During every surgical step a surgical microscope set for 10 x magnification was used. In all three groups the both sciatic nerve was cut transversally and immediately repaired end-to-end using 10-0 polyglycolic acid sutures (Ethicon; Johnson & Johnson, Somerville, NJ, USA).

In group II and III 1.0-cm-long silicone tubes (internal diameter, 1.0 mm; external diameter, 2.0 mm) were used for recovery of sciatic nerve repair place. Before sciatic nerve recovery, silicone tube was placed so as to surround nerve and fixed to the environment.

In group III silicone tube was filled with HA (approximately 0.3 mcg), (Hylartin® V, Pfizer).

Then, the operation field was closed with traditional methods. All rats were fed at 22-24°C and light-controlled conditions (12-12-h light- dark cycle) with free access to food and water.

Rats in all three groups were randomly divided into equal groups of six rats in each. Electrophysiological measurements were performed at 30 (I-30, II-30 and III-30 groups) and 60

(I-60, II-60 and III-60 groups) days after surgery to all six surgery groups containing six rats in each. In addition, electrophysiological evaluation of the sciatic nerve was used in six rats of the control non-surgery group.

The animals were anesthetized using intraperitoneal 10 mg/kg xylazin HCl and 80 mg/kg ketamin HCl and positioned prone with maximally extended hindlimbs. The rat's body temperature was maintained by keeping the animals on an electric pad that was switched off during recording. Electrophysiological measurements were taken using Neuropack M1 MEB-9200 (Nihon-Cohden Corp, Tokyo, Japan) device. The sciatic nerve was stimulated percutaneously from bipolar needle electrodes which were placed proximally and distally to the operated site at the level of hip joint and popliteal fossa, respectively. Recording electrodes were placed in medial gastrocnemius muscle belly. Exact placement of the recording electrodes on the gastrocnemius muscle is very important, because the volume conduction from neighboring muscles could result in false positive signals, mostly from the biceps femoris muscle [12,13]. The common reference (ground electrode) was placed on the tail *(Fig. 1)*. Electrical stimulation was square pulse with an frequency of 1 Hz and duration of 0.2 ms. The intensity was gradually increased up to supramaximal intensity (current intensity 30% above the value to evoke the maximal compound muscle action potentials amplitude - CMAP). The stimulation was repeated 5 times for all measurements and the average values were used.

The peak amplitude of the CMAP, CMAP latency of onset and motor nerve conduction velocity (MNCV) were calculated for each rat. The CMAP recorded from indicator muscle reflects the three values: the size of the motor unit innervated by the axons, the size of motor nerve fibers responding to the stimulus and the synchronization of their response. So, the CMAP amplitude represents an indirect measure of the number of motor nerve fiber [14]. The CMAP amplitudes were measured from peak-to-peak and latencies were measured from stimulus to negative deflection onset. Evaluation of the latency is an indicator the quality and the number of viable and functional axons and thus the functional status of examined motor nerves. The latency difference between the proximal and distal responses was divided into the distance between stimulation sites to generate a conduction velocity. The MNCV reflects the degree of myelination of the fastest axons [12] *(Fig. 2)*.

The electrodiagnostic results were analyzed using one - way analysis of variance (ANOVA). The following variables were considered: MNCV, amplitude and latency. Values were considered statistically significant when $P<0.05$.

RESULTS

In this study all rats survived, with no wound infection. Some rats have got bedsore, usually on the knee joints and took adequate treatment. Complete flaccid paralysis of the operative extremities was observed after operations.

All results presented in *Table 1*.

Latencies

The latencies of the control group were 1.21±0.40 ms. The latencies of the group I-30, II-30 and III-30 were 2.69±0.578 ms, 1.95±0.8 ms and 2.22±0.78 ms respectively. On day 60 latencies in group I, II and III were 2.13±0.57 ms, 1.19±0.3 ms and 1.42±0.38 ms respectively.

The one-way ANOVA test found overall statistically significant differences in the latencies between control group

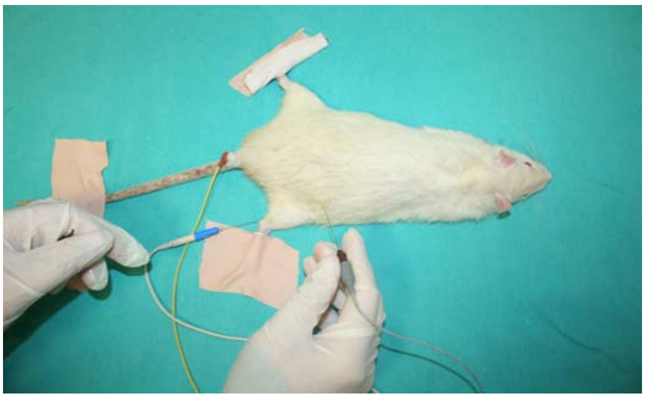

Fig 1. Sciatic nerve electrophysiological study: the ground electrode was placed on tail; recording electrode was placed in medial gastrocnemius muscle belly. The sciatic nerve was stimulated percutaneously from bipolar needle electrodes which were placed proximally and distally to the operated site

Şekil 1. Siyatik sinir elektrofizyolojik çalışması: toprak elektrod kuyruğa, kayıt elektrodu gastroknemius medialis kasına yerleştirilmiştir. Siyatik sinir ameliyat bölgesinin proksimal ve distalinden bipolar iğne elektrodlarla perkutan olarak stimüle edilmiştir

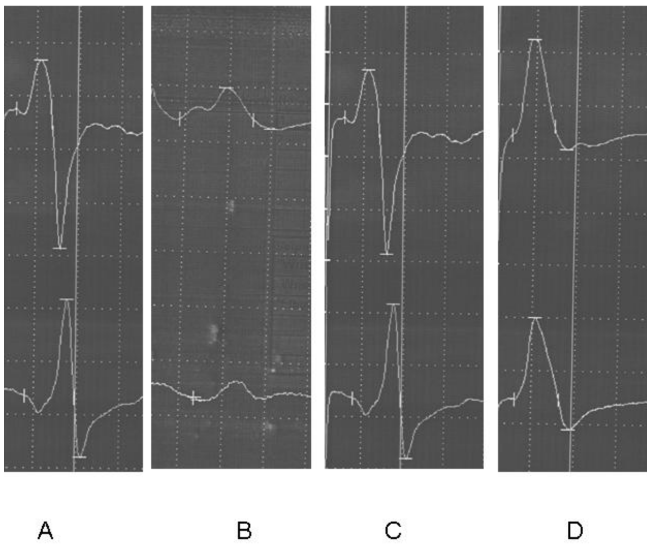

Fig 2. Representative traces of electrophysiological study (A. Control group, B. Group I-60, C. Group II-60, D. Group III-60)

Şekil 2. Elektrofizyolojik çalışmanın örnek traseleri (A. Kontrol grup, B. Grup I-60, C. Grup II-60, D. Grup III-60)

Table 1. *Electrophysiological data (latency, amplitude and NCV) in control and surgery groups on 30th and 60th days*
Tablo 1. *Kontrol ve cerrahi uygulanmış gruplarda 30 ve 60. günlerde elektrofizyolojik veriler (latans, amplitüd ve sinir iletim hızı)*

Data	Control Group	Group-I		Group-II		Group-III	
		30th day	60th day	30th day	60th day	30th day	60th day
Latency	1.21±0.39b	2.69±0.57a	2.13±0.56a	1.95±0.80ab	1.19±0.30b	2.21±0.78ac	1.41±0.38bc
Amplitude	17.82±6.80a	3.28±0.51b	6.61±2.52bc	5.15±2.47b	6.08±4.49bc	3.07±1.92b	11.78±5.75ac
NCV	59.08±5.19	16.75±3.43b	23.56±9.07bc	23.16±9.07bc	27.56±5.76bc	21.58±13.27bc	31.63±3.31c

Differences between average values are shown by different letters (a-c) on the same line (P<0.05)

and rats group I-30, I-60 and III-30. No statistical differences between control group and II-60 and III-60 groups. On day 60 the latencies in group II-60 and III-60 were significantly better than in group I-30 and I-60. No statistically significance between latencies in groups II-60 and III-60 *(Fig. 3)*.

CMAP Amplitudes

In the control group sciatic nerve CMAP amplitudes were 17.82±6.8 mV. In the group I-30, II-30 and III-30 CMAP amplitudes were 3.28±0.52 mV, 5.15±2.47 mV and 3.07±1.92 mV respectively. On the 60th day CMAP amplitudes were 6.61±2.52 mV, 6.08±4.5 mV and 11.78±5.57 mV in the groups I, II and III respectively.

In all groups on day 30 and in I-60 and II-60 groups CMAP amplitudes were statistically lower than in control group. But no statistically differences in the CMAP amplitudes between control group and III-60 group were detected *(Fig. 4)*.

Sciatic Nerve Conduction Velocity (NCV)

Sciatic NCV was 59.08±5.2 m/s in the control group.

In group I-30, II-30 and III-30 NCV were 16.75±3.43 m/s, 23.17±9.07 m/s and 21.58±13.27 m/s respectively. On day 60 NCV were 23.57±3.53 m/s, 27.57±5.77 m/s and 31.63±3.31 m/s respectively.

In all groups on day 30 and 60 sciatic nerve NCV were significantly lower than in control group *(Fig. 5)*.

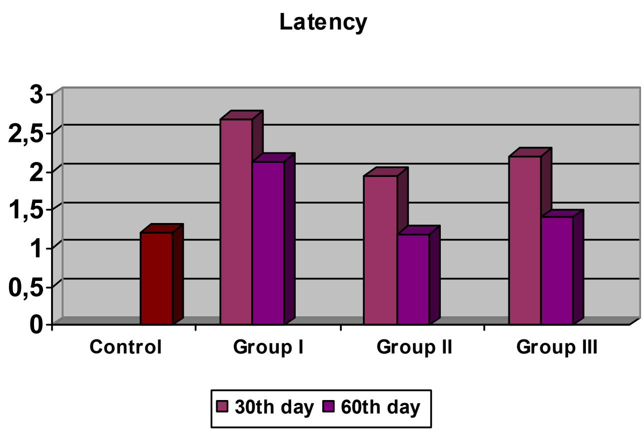

Fig 3. Latencies in control and surgery groups on days 30 and 60
Şekil 3. Operasyon ve kontrol gruplarında 30. ve 60. günlerde latanslar

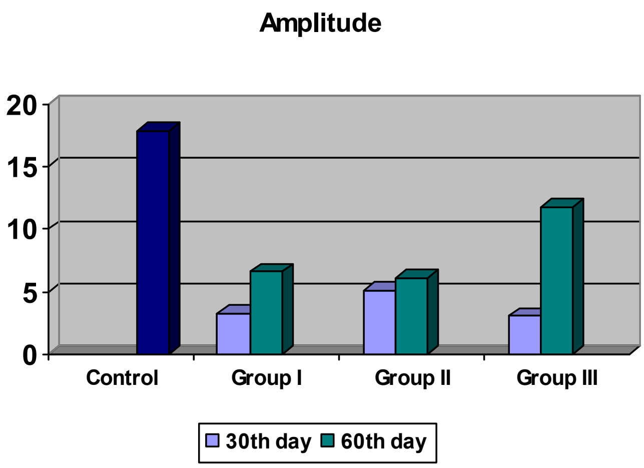

Fig 4. Amplitude values in control and surgery groups on days 30 and 60
Şekil 4. Kontrol ve operasyon uygulanan gruplarda 30. ve 60. günlerde amplitüd değerleri

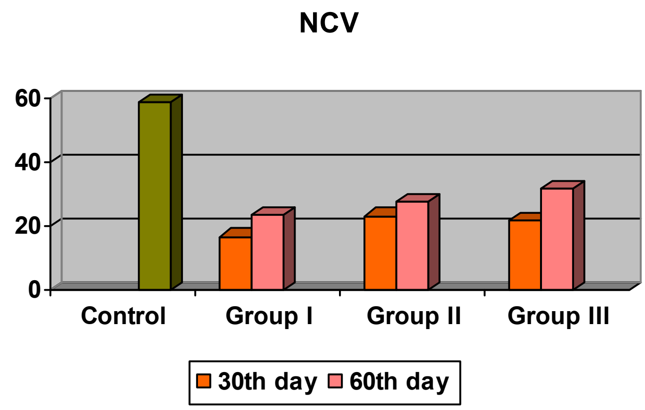

Fig 5. NCV values in control and surgery groups on days 30 and 60
Şekil 5. Kontrol ve operasyon uygulanan gruplarda 30. ve 60. günlerde siyatik sinir iletim hızları

DISCUSSION

After the nerve injuries, when the endoneural tube is disrupted, axon sprouts may grow into scar tissue and as a result a functionally inappropriate endoneural tube, such as a potentially painful neuroma, may develop. This interruption halts the axonal development and leads to the permanent nerve dysfunction. For preventing from scar tissue formation silicone tubes are widely used in human and animal practice [1,3]. Azizi et al.[15] demonstrated that silicone tubes could be a useful conduit in different nerve injuries. In this study authors described the reinnervation patterns between nerve stumps in a rodent model and up to 80% recovery of rat sciatic nerve defects.

Additionally, several substances were used to prevent adhesions and indirectly improve axonal regeneration in injured nerves. One of these substances is HA, which produced at the cell surface of fibroblasts by extrusion into the extracellular matrix [16]. HA known as an agent which enhances peripheral regeneration and reduce the extent of scar formation by inhibiting lymphocyte and macrophage migration, proliferation and chemotaxis [17]. HA- based biomaterials have been shown to be non-cytotoxic, completely biodegradable and biocompatible [18]. HA has been implicated a wide variety of medical and veterinary fields as diverse as surgery, orthopedics for scarless wound healing [19-21]. HA has also been used as an agent that prevents peripheral nerve adhesion after neurolysis in animal model [9]. Studies have shown that HA- based nerve conduit may be suitable as a guide in peripheral nerve repair [22]. In other study, the authors suggested that the application of the HA into the extracellular environment after spinal cord injury has resulted in reduced glial scar and inflammatory response [23]. It is hypothesized that HA may interact directly with astrocytes to modulate the injury environment and HA bindings to cells via the CD44 receptor down regulates the production of inhibitory proteoglycans in the extracellular matrix [23].

In addition, Özgenel reported that a single-dose topical application of HA significantly reduced extraneural and epineural scar formation, resulting in enhancement of peripheral nerve regeneration [24]. Finally, some studies have shown that HA improves the formation of fibrin matrix and this means that the acid acts as an agent, which helps axonal growth and repair [10,25,26]. However, we noted, there is insufficient data about effects of combination silicone tubes and long-term HA exposure on the peripheral nerve regeneration *in vivo,* which were confirmed by the detailed electroneuromyographic data, as in present study. Our study based on electrophysiological evaluation of rats' sciatic nerves and obtained data support the above studies [10,24-27]. Though sciatic NCV did not reach control values, we still found better results in the silicone tube group and HA + silicone tube group, when CMAP amplitudes and latencies were taken into consideration. Latencies in groups II-60 and III-60 reached the control values and it indirectly proves that the axonal regeneration of these groups is better than in the group where silicone tubes and HA were not used.

In addition, the increase in CMAP amplitudes in group III-60 and achieves them to the values of the control group suggest better axonal regeneration in group where silicone tube + HA was used.

Present study showed the slow increase of sciatic NCV in all three groups. But some studies led to the conclusion that the NCV is not a good indicator to differentiate between regenerative properties or severity of lesion [14].

Finally, in present study we choose the minimally invasive electrodiagnostic method without euthanizing the animal, because the non- invasive measurements are valuable tool to displaying axonal changes within the regenerating rat sciatic nerve and there is no significant difference between non-invasive and invasive methods. These data were confirmed by histomorphometric evaluation. In addition, electrodiagnostic assessment has demonstrated clear advantages over other functional tests, such as sciatic function index and static sciatic index [12].

In conclusion, our study demonstrated that using of silicone tubes or silicone tubes filled with HA after end-to-end repair of the cut nerve can provide a positive effect on the CMAP amplitudes and latencies and hence, on the axonal regeneration. Silicone tubes filled with HA was the most effective method for axonal regeneration after nerve repair in comparison to the silicone tube without HA. We think that further histological investigations would shed light on these findings.

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