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RESEARCH ARTICLE

The Effect of Silicone Tube and Silicone Tube + Hyaluronic Acid Application on Adhesion Formation in Experimental Peri- and Epi-neurorrhaphy in A Rat Model [1] [2]

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Summary

Following neurorrhaphy in Wistar albino rats with experimental sciatic nerve cut, the effectiveness of solely silicone tube (ST) and ST plus hyaluronic acid application on preventing fibrosis was clinically and histopathologically examined. After a total nerve cut is created in sciatic nerve, interfascicular and epineural anastomosis was used. While only anastomosis was applied for the first group (Control Group), for the second group (ST Group) anastomosis + silicone tube and for the third group (ST + HA Group) anastomosis + silicone tube + hyaluronic acid (HA) were applied. Animals in each group were divided into 2 sub-groups and macroscopic and histopathological examinations were conducted on the 30th and 60th postoperative day. On day 30 of the study all the animals had problematic walks. On the 60th day while animals in groups ST and ST + HA were walking normally, the problem was still going on for the control group. In the postmortem macroscopic examinations performed in the control group on the 30th and 60th days an irregular morphology and adhesion to surrounding in nerve tissue were seen. Whereas in group ST, in the anastomosis line within the tube, scar tissue which was clearer on the 60th day was observed, in group ST + HA it was seen that nerve anastomosis line was smooth on the 30th and 60th days. As a consequence, the reduction in myelin thickness and the increase in degenerated myelin for groups ST and ST + HA in 30 day show that HA does not create a positive effect on axon regeneration in the short run, on the other hand, the reduction in myelin degenerated along with the increase of axon myelin thickness and axon cross section areas in groups ST and ST + HA in day 60 has shown that silicone tube and HA application creates a positive impact on myelination in the long run.

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

Deneysel Peri ve Epinöral Nörorafi Uygulanmış Rat Modellerinde Silikon Tüp ve Silikon Tüp + Hyaluronik Asit Uygulamasının Adezyon Formasyonuna Etkisi

Özet

Bu çalışmada deneysel siyatik sinir kesisi oluşturulan Wistar albino ratlarda nörorafiyi izleyerek, ilgili alana sadece silikon tüp (ST) ve ST ile birlikte hyaluronik asit (HA) uygulamasının fibrozisi engellemedeki etkinliği klinik ve histopatolojik olarak incelendi. Siyatik sinirde total kesi oluşturulduktan sonra interfasiküler ve epinöral anastomoz uygulandı. Birinci grupta (Kontrol Grubu) sadece anostomoz yapılırken, ikinci grubta (ST Grubu) anastomoz + silikon tüp, üçüncü grubta ise (ST + HA Grubu) anastomoz + silikon tüp + Hyaluronik asit (HA) uygulandı. Her bir gruptaki hayvanlar 2 alt gruba ayrılarak postoperatif 30 ve 60. günlerde makroskopik ve histopatolojik incelemeler yapıldı. Çalışmanın 30. gününde tüm hayvanların yürüyüşleri problemliydi. 60. günde ST ve ST + HA grubundaki hayvanlarda yürüyüş normal iken kontrol grubunda problem devam etmekteydi. Prostmortem makroskopik bakıda 30 ve 60. günlerde kontrol grubunda sinir dokuda düzensiz bir morfoloji ve çevreye yapışıklık mevcuttu. ST Grubunda 60. günde daha belirgin olmak üzere tüp içerisinde anastomoz hattında skar doku ile karşılaşılırken ST + HA grubunda 30 ve 60. günlerde sinir anastomoz hattının pürüzsüz bir görünümde olduğu saptandı. Sonuç olarak, 30 günde ST ve ST + HA gruplarında miyelin kalınlığının azalması ve miyelin dejenerasyonun artması HA'in kısa dönemde akson rejenerasyonu için pozitif bir etki oluşturmadığını, buna karşın 60. günde ST ve ST + HA gruplarında aksonların miyelin kalınlığı ve akson alanlarının arttırmasıyla birlikte miyelin dejenerasyonun azalması, silikon tüp ve HA'in uzun dönemde miyelinizasyon üzerinde pozitif bir etki oluşturduğunu göstermiştir.

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

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INTRODUCTION

Peripheral nerve injury is a very common problem that medical doctors and veterinary physicians come across. These lesions develop as simple nerve rupture, ruptures that form with tissue loss and nerve laceration [1]. Common causes of nerve traumas can be listed as traffic accidents, falling down from great heights, compression, injuries by firearms, sharp object injuries, bite injuries, damages caused by fractured bone fragments, muscle tendon ruptures which appear depending upon overextension in extremities and simultaneous neurological disorders, iatrogenic reasons (false injections, intraoperative mistakes etc.) etc. [1,2].

Success rate in peripheral nerve regeneration depends on better understanding of factors that have an impact on recovery and elimination of negative factors. Regeneration rate for motor nerves is 80% and for mixed nerves 50-60% under suitable conditions. Age, diameter and length of nerve and material and technique practiced effect healing rate [3].

In ruptures where there is no neural tissue loss, by following trimmed of ruptured ends, epineural repair which is applied with solely epineural suture or interfascicular anastomosis is commonly used [1,4]. However, there are significant problems which appear during the recovery period such as intraneural fibrosis, perineural adhesion and neuroma formation that effect nerve healing in a negative way [1-5]. Extra and intraneural fibrosis forms compression on nerves along with vascular formations. Fibrosis and adhesion assume a role which induces new neurological disorders [1,3,6].

In order to make the desired recovery possible materials such as various carbohydrate polymer gels, antioxidant agents, antineoplastic preparations and corticosteroids were studied to determine their effectiveness in preventing adhesion. In addition, various organic and synthetic membranes or tube practices have been used so as to prevent adhesion to surrounding tissues and eliminate mechanical pressures and extensions on nerves [1-4,6-11].

HA is a non-sulfate glycosaminoglycan. Thanks to its viscoelastic characteristics, it has been used for many years primarily for the repair of muscle-tendon-ligament ruptures and in fields such as ophthalmology and oto-rhino-laryngology in order to prevent adhesion by eliminating fibrosis [6,12-14]. HA shows its viscoelastic characteristic acting as a barrier by forming a layer similar to film between tissues repaired and veining. Therefore this impact enables the repairing of main tissue cells directly without experiencing the inflammatory phase in which operated tissue is repaired by fibroblasts [14]. HA shows a long term impact on the tissue it is practiced by preserving its integrity for 4-5 weeks [12-14]. HA was used in combination with some gels [5] and in another study [2] by being injected into perineural area in the field of neurochirurgia.

The purpose of this study is to clinically, macroscopically and histopathologically examine the effects of only silicone tube and silicone tube plus hyaluronic acid-which is a non-sulfate glycosaminoglycan- (Na-Hyaluronate, HA) application on fibrosis following perineural and epineural neurorrhaphy in rats with experimental sciatic nerve cut.

MATERIAL and METHODS

Animals and Experimental Groups

This research was conducted after the approval of Kafkas University Animal Testing Local Ethics Council (Approval Number: KAÜ-HADYEK-2010/19-40).

Animal material of the study included 48 male adult Wistar albino rats, each weighing 250-300 g. Rats were kept under standard laboratory conditions, 12 h in darkness and 12 h in daylight, at average 20-22°C constant temperature. Animals were fed with standard rat feed and water was given ad libitum.

Rats were allocated into 3 groups containing 16 rats each.

Group I (Control Group): Only nerve cut and suture was applied.

Group II (Silicon Tube Group) (Group ST): Nerve cut, suture and silicone tube application which surrounded neurorrhaphy area were practiced.

Group III (Silicone Tube + Hyaluronic Acid Group) (Group ST + HA): Upon nerve cut and suture and intraneural silicone tube application which surrounded neurorrhaphy area approximately 0.3 ml perineural HA was applied.

Anesthesia and Experimental Procedure

For neurorrhaphy, 10/0 atraumatic polygylcolic acid suture, operation microscope, silicone tube, hyaluronic acid, routine soft tissue set; for histopathological cut and examinations routine equipments and consumables; for postoperative pain and infection control, suitable analgesic and antibiotics were used.

Upon following necessary preparations for surgical operations in all three groups, 5 minutes after intra-peritoneal injection of 5 mg/kg Xylazine HCl (Rompun® 2%, 50 ml, Bayer) and 40 mg/kg Ketamine HCl (Ketasol 10% enj, 10 ml, Richter Pharma) through anesthesia workstation (Veterinary Anesthesia WorkStation, Hallowell® EMC USA) and a suitable sized laryngeal mask with equivalent of 1 MAC isoflurane (1.4%, inspiration concentration) general anesthesia was started and went on by the end of operations. Sciatic nerve was exposed by routine surgical procedure and upon a total cut is made with bistoury

interfascicular and epineural anastomosis was practiced with 10/0 polygylcolic acid (PGA) (Ethicon; Johnson & Johnson, Somerville, NJ, USA).

In group I, surgical opening was bridged routinely and no process was applied other than this. In group II, after each nerve ending was suitably freed upon nerve cut, following neurorhapphy, silicone tube which was passed through one of the endings was placed by suture in a way that centers anastomosis line. In group III, with the same method, firstly silicone tube was passed through one of the nerve endings, secondly after neurorhapphy it was placed into the nerve tissue in stitching area and following neurorhapphy placed in a way to surround anastomosis line and then some (approximately 0.3 ml) HA (Hylartin® V, Pfizer) that could fill the tube was applied. In these groups, once again routine methods were used to close surgical opening.

Necessary processes were carried out for post operative pain and infection control and suitable care-nutrition conditions were provided for animals.

Animals in all groups were divided into 2 equal sub-groups (n: 8). Clinical assessments on postoperative 30th and 60th days were carried out and animals were decapitated under general anesthesia. Postmortem macroscopic and histomorphological examinations were conducted.

Histological Analysis

- Tissue Processing

On the 30th and 60th postoperative day, under general anesthesia, the distal blocks of sciatic nerves were removed from the nerves after electrophysiological assessment of the nerve. The nerves were stretched to *in situ* lengthwise by pinning them onto a card and then fixing them with 2.5% glutaraldehyde in 0.1M phosphate buffer (pH 7.4) for 4-6 h in 4°C. Following fixation, the tissues were rinsed in phosphate buffer (pH 7.4) two times. Specismens were postfixed in 1% osmium tetroxide for 2 h, dehydreted in ascending alcohol series and took into propylene oxside for 16 min. These prosedurs were completed by embeding the tissue 48 h in Epon embeding kit (Fluka Gmbh, Swithzerland). For embedding, we used a silicon embedding mold that has 21 consecutively numbered, bullet-shaped cavities with a depth of 5 mm each. Semi-thin sections of 1 µm thickness were cut by an ultra microtome (Super Nova Reichert- Yung, Austria) and stained with 1% toluidine blue.

- Stereological Analysis

Stereological analyses of sciatic nerves were conducted according to principles described previously [15,16]. A stereological workstation composed of a digital camera (mbf/Bioscience, Qimaging), otomatic controlled specimen stage, a light microscope (Leica, DM400B) and a software program (mbf/Bioscience, Stereo investigator, version 9) was used to count axons. To obtain an estimation of total myelinated axon number in an unbiased manner, the axon profiles in the nerve cross-section are sampled with equal probability regardless of shape, size, orientation and location that means each sampled item was selected with a systematic random manner [17]. For this aim, we used an area fraction approach. In the application, area of unbiased counting frame was 900 µm^2. Meander sampling of each sectioned nerve profiles was done in 70 µm x 70 µm step size in a systematic-random manner. This ensures that all locations within a nerve cross-section were equally represented and that all axon profiles were sampled with an equal probability regardless of shape, size, orientation and location [17-19].

The same stereological workstation was also used for stereological analyses of myelin thickness and axon cross-sectional area. A two-dimensional isotropic uniform random nucleator [20,21] was used for estimation of cross-sectional axon area and the thickness of myelin sheet using an oil objective (100x, NA 1.40). Meander sampling of each sectioned nerve profiles for axon cross-section area and myelin sheet thickness was done over successive, systemic-random steps of 70 µm-70 µm. Two dimensional nucleator at isotropic uniform random positions was used for estimation of axonal areas and the thickness of myelin sheet using an oil objective (100x, NA 1.40).

Statistical Analysis

Six rats were used for each experimental group. "n" which is used for statistical analysis is the number of animals. Non-parametric tests were used in statistical analysis. For the comparison of groups of two, Mann-Whitney-U test was used while Kruskal Wallis test was preferred for the comparison of groups of three. Statistical difference is important on $P<0.05$ level.

RESULTS

Clinical Findings

Animals' individual locomotion on a suitable platform before experiment ~~was~~ terminated was monitored in each group, findings were as follows: on 30th day, locomotion in group ST and ST + HA were problematic while there was no problem in groups ST and ST + HA on 60th day. The locomotion problem still existed in the control group.

Postmortem Macroscopic Findings

During postmortem macroscopic examinations on the 30th and 60th days, an irregular morphology and adhesion to surrounding were observed in the control group. When tissue growths similar to fibrosis mass in the anastomosis line within the tube, which was clearer on the 60th day in

groups ST, in group ST + HA it was observed that on the 30th day, HA in the tube preserved its transparent structure and it was still present on the 60th day. In this group it was observed that nerve anastomosis line was smooth and bright on the 30th and 60th days.

Histomorphological Findings

Upon histomorphological examination of nerve tissues which belonged to a 30 day period, reduction in myelin thickness though more apparent in groups ST and ST + HA and increase in myelin degenerated was observed. When data from 60 day subgroups for groups ST and ST + HA were compared to 30 day data, it was seen that there was an increase in axons myelin thickness and axon cross section areas and a decrease in myelin degenerated *(Fig. 1, 2, 3, 4* and *5)*.

DISCUSSION

Different types of biological and artificial grafts have been studied in comparison with autologous nerve grafts with regards to nerve regeneration and functional healing lately and important developments have been made. The tubulization is a good alternative for peripheral nerve reconstruction [22].

Three different tubulization methods which are biological, synthetic and combined are used for peripheral neurosurgery. In cases when nerve spaces are shorter than 30 mm, effectiveness of vein grafts was confirmed by experimental and clinical studies [18,23]. When nerve spaces are longer than 30 mm, in order for a successful nerve regeneration vein tubes and other tissues like nerve parts and skeletal muscle suture are used together in some studies and resulted in a better functional feedback [18,24,25]. In addition, use of silicone tubes for nerve spaces shorter than 5 mm has been reported to lead to a successful nerve regeneration [18,26]. In this study solely ST and ST plus HA which is a viscoelastic material were used with the purpose of a functional healing.

With silicone nerve guidance conduits (NGCs) which are used for nerve repair, silicone tube is filled with serum within the first couple of hours as blood vessels are cut. Inflammatory cells such as serum macrophages contain some cytokine, neuropathic factors and fibrin. Fibrin creates axon regeneration by forming two nerve ends on a longitudinal direction as a fibrin cable bridge. Fibrin bridge structure is a critical step towards healing [27]. Besides, Schwann cells and macrophages excrete mitogens which have an impact on neuropathic (growth) factors starting remyelination with axonal regeneration. Both cells express

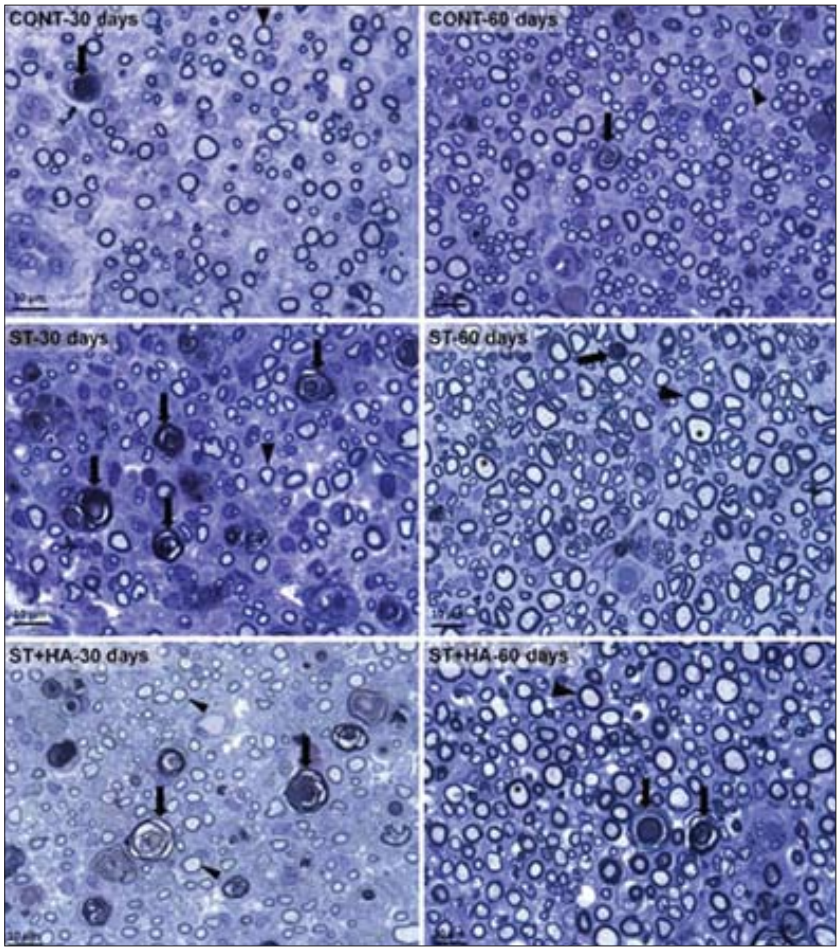


Fig 1. Light microscopic image of myelin degenerated, myelin thickness and axon cross section areas in Control group, ST and ST+HA groups (myelin degenerated in 30 day groups is more intense in groups ST and ST+HA compared to control group *(arrows)*. Myelin thickness is less in groups ST+HA than control group in the same period *(arrowheads)*. Myelin thickness and axon cross section areas are more in groups ST and ST+HA of 60 days than of 30 days (asterisk) and the number of myelin degenerated axons are less compared to 30 day animals (Scale bar: 10 µm)

Şekil 1. Kontrol, ST ve ST+HA gruplarında myelin dejenerasyonu, myelik kalınlıkları ve akson alanlarının ışık mikroskobik görünümü

(30 günlük gruplarda miyelin dejenerasyonu ST ve ST+HA gruplarında kontrol grubuna göre daha yoğundur *(Oklar)*. Aynı dönemde kontrol grubuna göre ST+HA grubunda miyelin kalınlıkları daha incedir *(Ok başları)*. 30 günlüklere göre 60 günlük hayvanların ST ve ST+HA gruplarında miyelin kalınlıkları ve akson alanları daha fazladır *(asteriks)* ve dejenere olmuş miyelinli aksonlar daha azdır) (Skala bar: 10 µm)

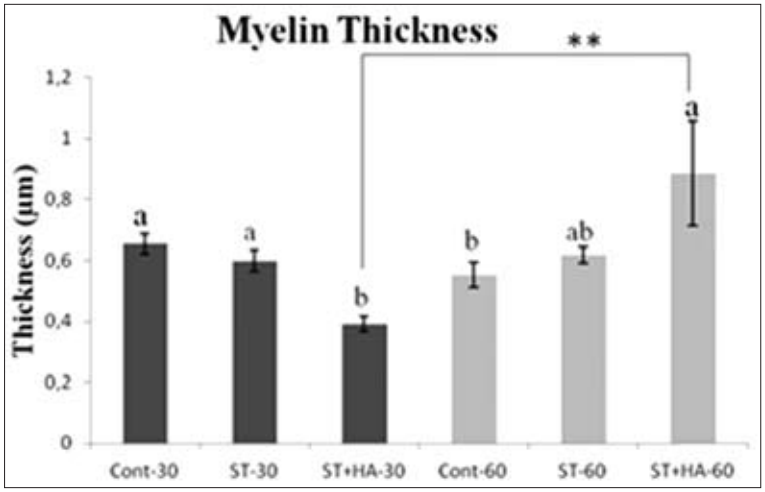


Fig 2. Myelin thickness

30 day groups (Cont-30, ST-30, ST+HA-30 ** P<0.01), 60 day groups (Cont-60, ST-60, ST+HA-60 * P<0.05), (Cont-30, Cont-60 ** P<0.01), (Average ± SEM)

Şekil 2. Miyelin kalınlığı

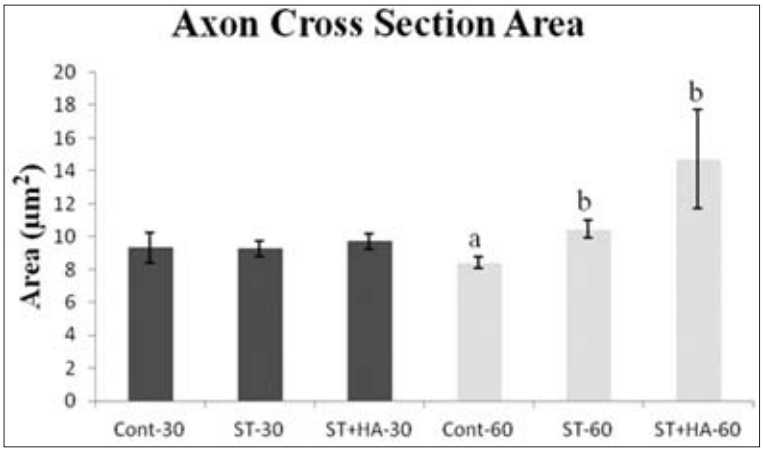
30 günlük gruplarda (Cont-30, ST-30, ST+HA-30 ** P<0.01), 60 günlük gruplarda (Cont-60, ST-60, ST+HA-60 * P<0.05), (Cont-30, Cont-60 **P<0.01), (Ortalama ± SEM)

Fig 3. Axon cross section area

30 day groups (Cont-30, ST-30, ST+HA-30, P>0.05), 60 day groups (Cont-60, ST-60, ST+HA-60, * P<0.05), (Average ± SEM)

Şekil 3. Akson alanı

30 günlük gruplarda (Cont-30, ST-30, ST+HA-30, P>0.05), 60 günlük gruplarda (Cont-60, ST-60, ST+HA-60, * P<0.05), (Ortalama ± SEM)



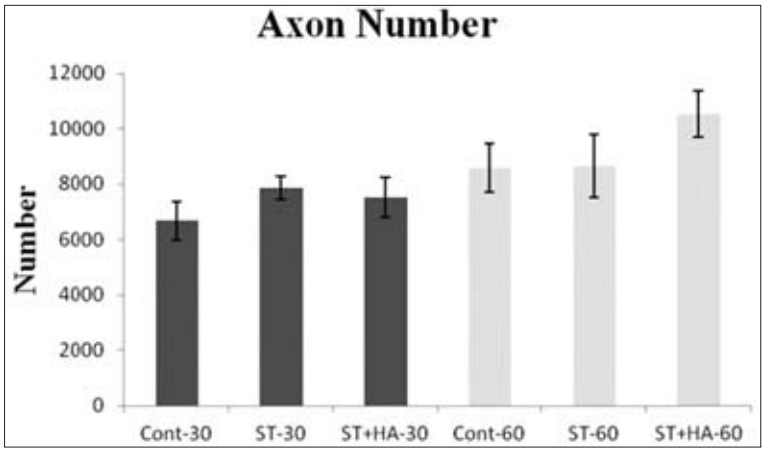


Fig 4. Axon number

30 day groups (Cont-30, ST-30, ST+HA-30, P>0.05) and 60 day groups (Cont-60, ST-60, ST+HA-60, * P<0.05) (Average ± SEM)

Şekil 4. Akson sayısı

30 günlük gruplarda (Cont-30, ST-30, ST+HA-30, P>0.05) ve 60 günlük gruplarda (Cont-60, ST-60, ST+HA-60, * P<0.05) (Ortalama ± SEM)

anti-inflammatoire cytokines such as interleukin (IL)-10 which represses the inflammatory process staring in Wallerian degeneration [28]. Schwann cells have shown to form a regeneration tube by forming long cell bands also known as Bungnerin Bands which make axon proceed along with axon growth. While Schwaan cells regenerate to form long cell bands, macrophages and monocytes migrate to degenerated nerve bruise to eliminate myelin and axon debris [28-30].

Substances that are not biodegradable stay where they are after nerve degeneration like foreign substances. Therefore, they cause a chronic foreign substance reaction with excessive scar tissue structure and in the end they are reported to prevent nerve function recovery [30,31]. After peripheral nerves are cut in a latitudinal way a set of molecular and cellular events named as Wallerian degeneration are triggered in the distal damaged zone [30,32].

In this study we observed that silicone tube applied after nerve damage reduced myelin thickness in the control group for 30 day animals, on the other hand, for

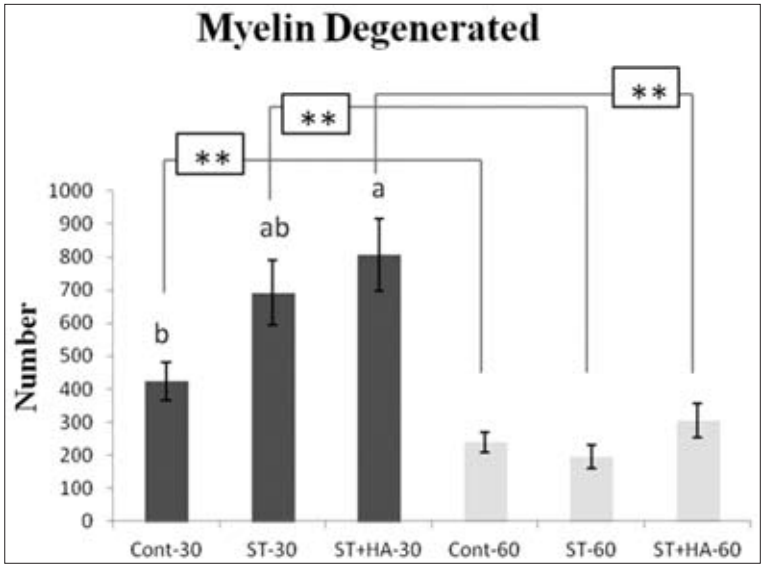


Fig 5. Myelin degenerated

30 day groups (Cont-30, ST-30, ST+HA-30 * P<0.05), 60 day groups (Cont-60, ST-60, ST+HA-60, P>0.05), (Cont-30, Cont-60, ** P<0.01), (ST-30, ST-60, ** P<0.01), (ST+HA-30, ST+HA-60, ** P<0.01). (Average ± SEM)

Şekil 5. Miyelin dejenerasyonu

30 günlük gruplarda (Cont-30, ST-30, ST+HA-30 * P<0.05), 60 günlük gruplarda (Cont-60, ST-60, ST+HA-60, P>0.05), (Cont-30, Cont-60, ** P<0.01), (ST-30, ST-60, ** P<0.01), (ST+HA-30, ST+HA-60, ** P<0.01). (Ortalama ± SEM)

60 day animals with an opposite impact, myelin thickness increased with the axon cross section area. This may suggest that negative impact of short term silicone tube application on recovery as mentioned in literature [30,31] may stem from foreign substance effect it creates on that area. High myelin degeneration in 30 day ST and ST + HA groups proves that there is Wallerian degeneration in distal zone as mentioned in literature [30,32].

Neuropathic factors and extracellular matrix molecules (ECM) are produced by Schwann cells for successful axon regeneration. With the help of these factors, it is stated that a regenerated unit surrounded by a basal lamina on the proximal part of nerves that are latitudally cut begin to sprout. Newly sprouting axons generally spread from nodes of Ranvier and become remyelinated by Schwann cells [30,33,34].

Hyaluronic acid is naturally composed of glucuronic acid and linear polysaccharides which are formed by recurring disaccharide units composed of N-acetylglucosamine and it plays an important role for tissue repair [30,35]. Superficial Hyaluronan gel application has been determined to prevent perineural scar structure and increase peripheral nerve regeneration [30,36]. Because of its characteristic that organize fibrin, HA may create axonal ingrowth and pathway for cells in the acellular fibrin matrix phase of peripheral nerve regeneration [32,37].

Certain studies [35,38] conducted by various materials combined with hyaluronic acid have shown a significant rise in axon number.

In this study, though silicone tube and ST+HA application upon nerve damage in 30 and 60 day groups did not cause a statistical difference compared to 30 day control group, the rise in numerical values showed that new axon sprouts developed as stated by certain studies [30,33,34]. The rate of increase stated by different researches [35,38] has not been detected in our study. However, our results are compatible with the results of various researches [30,32,35,36] which stated HA's positive impact on recovery.

In summary, the reduction in myelin thickness and the rise in myelin degenerated in silicone tube and hyaluronic acid groups of 30 day animals shows that hyaluronic acid does not have a positive impact on axon regeneration phases in the short run. In contrast, when silicone tube and hyaluronic acid groups of 60 day animals are compared to 30 day animals, the fact that axons increase myelin thickness and axon cross section areas and the decrease in myelin degenerated may shows that silicone tube and hyaluronic acid have a positive impact on myelination in the long run.

ST + HA application has contributed to early functional strength of extremities and created a proper walking. There has been a smooth recovery without fibrosis in the nerve tissue with macroscopic operation.

HA still preserved its presence in silicone tube in both groups (30 day and 60 day SA + HA groups), which was interpreted as a sign that as viscoelastic material it had very strong physical characteristics.

The decrease in myelin thickness and increase in myelin degenerated in ST and ST + HA groups of 30 day animals has shown that HA does not have a positive impact on axon regeneration in the short run, the rise in myelin thickness and axon cross section areas and the decrease in myelin degenerated in 60 day animals has shown that ST and HA have a positive impact on myelination in the long run.

However, it can be said that multi dimensional experimental studies which provide the following are required: a- formation of a model that makes it possible for HA to hold on to the area in larger volumes and that enables to repeat HA injection periodically, b- thus, examination of

clinical and microscopic table in future phases of recovery by keeping time of experiment longer, c- determination of the most suitable application method by detecting HA's effect of inhibiting scar formation in scored measurements, d- recovery table is pointed out by functional measurement (biomechanical and electrophysiological tests).

REFERENCES

1. Radkey WG: Peripheral nevre surgery. **In,** Slatter D (Ed): Textbook of Small Animal Surgery. Vol. 1, Second ed., 1135-1141, WB Saunders Co. Phyladelphia, 1993.

2. Smit X, von Neck JW, Afoke A, Hovius SER: Reduction of neural adhesion by biodegradable autocrosslinked hyaluronic acid gel after injury of peripheral nerves: an experimental study. *J Neurosurg,* 101 (4): 648-652, 2004.

3. Özaydın İ, Ünsaldı E, Durmuş AS, Cihan M, Karabulut E: Tavşanlarda hyaluronik asitin periferal sinir rejenerasyonu ve postoperatif adezyon formasyonuna etkisi: Klinik ve elektrofizyolojik bulgular. *XI. Ulusal Veteriner Cerrahi Kongresi,* 26-29 Haziran, Kuşadası, Aydın, Türkiye, s.46-47, 2008.

4. Ayan I, Esenkaya I, Karakaplan M, Germen B, Milcan A, Zrlu Demir S, Ozcan C: The effect of human placental suspention on rat sciatic nevre healing. *Acta Orthop Traumatol Turc,* 41 (2): 140-146, 2007.

5. Adanali G, Verdi M, Tuncel A, Erdogan B, Kargi E: Effects of hyaluronic acid-carboxymethylcellulose membrane on extraneural adhesion formation and peripheral nerve regeneration. *J Reconstr Microsurg,* 19 (1): 29-36, 2003.

6. Hüseyinoğlu N, Özaydın İ, Yayla S, Yıldırım CH, Aksoy Ö, Kaya M, Şengöz A, Taşdemiroğlu E: Electrophysiological assessment of the effects of silicone tubes and hyaluronic acid on nerve regeneration in rats with sciatic neurorrhaphy. *Kafkas Univ Vet Fak Derg,* 18 (6): 917-922, 2012.

7. Kotil K, Kirali M, Eras M, Bilge T, Uzun H: Neurprotective effects of avetyl-L-carnithine in experimental chronic compression neuropathy. A prospective, randomized and plasebo-control trials. *Turk Neurosurg,* 17 (2): 67-77, 2007.

8. Senoglu M, Nacitarhan V, Kurutas EB, Senoglu N, Altun I, Atli Y, Ozbag D: Intraperitoneal alpha-lipoic acid to prevent neural damage after crush injury to the rat sciatin nevre. *J Brachial Plex Peripher Nerve Inj,* 4, 22, 2009.

9. Albayrak BS, Ismailoglu O, Ilbay K, Yaka U, Tanriover G, Gorgulu A, Demir N: Doxorubicin for prevention of epineurial fibrosis in a rat sciatic nerve model: outcome based on gross postsurgical, histopathological, and ultrastructural findings. *J Neurosurg Spine,* 12 (4): 441, 2010.

10. Pei-Ran HO, Coan GM, Cheng ET, Nielle C, Tarn DM, Zhou H, Sierra D, Terris DJ: Repair with collagen tubules linked with brain-derived neurtrophic factor and ciliary neurotrophic factor in a rat sciatic nevre injury model. *Arch Otolaringol Head Neck Surg,* 24, 761-766, 2010.

11. Yamamoto M, Endo N, Ito M, Okui N, Koh S, Kaneko H, Hirata H: Novel polysaccharide-derived hydrogel prevents perineural adhesions in a rat model of sciatic nerve adhesion. *J Orthop Res,* 28 (3): 284-288, 2010.

12. Çolak A, Özaydın İ, Okumuş Z, Özcan K, Oral H: Koyunlarda deneysel meme başı lezyonlarının viskoşirurjikal sağaltımı. *Kafkas Univ Vet Fak Derg,* 1 (1-2): 36-41, 1995.

13. Özaydın İ, Özba B, Okumuş Z, Maraşlı Ş, Cihan M, Utlu N: Atlarda tendinitis ve tenosinovitislerin sağaltımında hyaluronik asit kullanımı. *Kafkas Univ Vet Fak Derg,* 2 (2): 211-217, 1996.

14. Cihan M: Viskoşirurji. *Kafkas Univ Vet Fak Derg,* 4 (1-2): 119-122, 1998.

15. Larsen JO: Stereology of nerve cross-sections. *J Neurosci Methods,* 85, 107-118, 1998.

16. Geuna S, Tos P, Guglielmone R, Battiston B, Giacobini-Robecchi MG: Methodological issues in size estimation of myelinated nerve fibers in peripheral nerves. *Anat Embryol (Berl.),* 204, 1-10, 2001.

17. Canan S, Aktaş A, Ulkay MB, Colakoglu S, Ragbetli MC, Geuna S, Kaplan S: Prenatally exposed to a non-steroidal anti-inflammatory drug impairs sciatic nerve morphology: A stereologıcal and histological study. *Int J Develop Neurosci*, 26 (7): 733-738, 2008.

18. Piskin A, Kaplan S, Aktas A, Ayyıldız M, Raimondo S, Alic T, Bozkurt HH, Geuna S: Platelet gel does not improve peripheral nerve regeneration: An electrophysiological, stereological, and electron microscopic study. *Microsurgery,* 29, 144-153, 2009.

19. Ulkay MB, Aktas A, Aksoy Ö, Haktanır D, Bozkurt HH: Comparison of fixation methods for peripheral nerve fiber. *Kafkas Univ Vet Fak Derg,* 19 (1): 51-56, 2013. DOI: 10.9775/kvfd.2012.7098

20. Gundersen HJ, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K, Nyengaard JR, Pakkenberg B, Sorensen FB, Vesterby A, West MJ: Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. *Apmis,* 96, 379-394, 1988.

21. Gundersen HJ, Jensen EB, Kieu K, Nielsen J: The efficiency of systematic sampling in stereology-reconsidered. *J Microsc*, 193, 199-211, 1999.

22. Mackinnon SE, Hudson AR: Clinical application of peripheral nerve transplantation. *Plast Reconstr Surg,* 90, 695, 1992.

23. Chiu DT: Autogenous venous nerve conduits. A review. *Hand Clin,* 15, 667-671, 1999.

24. Tang JB: Vein conduits with interposition of nerve tissue for peripheral nerve defects. *J Reconstr Microsurg,* 11, 21-26, 1995.

25. Battiston B, Tos P, Cushway TR, Geuna S: Nerve repair by means of vein filled with muscle grafts I. Clinical results. *Microsurgery,* 20, 32-36. 2000.

26. Dahlin LB, Lundborg G: Use of tubes in peripheral nerve repair. *Neurosurg Clin N Am,* 12, 341-352, 2001.

27. Dodla MC, Mukhatyar VJ, Bellamkondav RV: Peripheral nerve regeneration. Principles of Regenerative Medicine. Second ed., Chapter 56, pp. 1047-1063, Academic Press, New York, 2011.

28. Ngeow WC: Scar less: A review of methods of scar reduction at sites of peripheral nerve repair. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod,* 109, 357-366. 2010.

29. Stoll G, Griffin JW, Li CY, Trapp BD: Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. *J Neurocytol,* 18, 671-683, 1989.

30. Gu X, Ding F, Yang Y, Liu J: Construction of tissue engineered nerve grafts and their application in peripheral nerve regeneration. *Prog Neurobiol,* 93, 204-230, 2011.

31. Deng M, Chen G, Burkley D, Zhou J, Jamiolkowski D, Xu YM, Vetrecin R: A study on in vitro degradation behavior of a poly(glycolide-co-L-lactide) monofilament. *Acta Biomater,* 4, 1382-1391, 2008.

32. Seckel BR: Enhancement of peripheral nerve regeneration. *Muscle Nevre,* 13, 785-800, 1990.

33. Lundborg G: Nerve regeneration. **In,** Lundborg G (Ed): Nerve Injury and Repair. 149-195, Churchill Livingstone, London, UK, 1988.

34. Muir D: The potentiation of peripheral nerve sheaths in regeneration and repair. *Exp Neurol,* 223, 102-111, 2010.

35. Wang KK, Nemeth IR, Seckel BR, Chakalis-Haley DP, Swann DA, Kuo JW, Bryan DJ, Cetrulo Jr CL: Hyaluronic acid enhances peripheral nevre regeneration *in vivo. Microsurgery* 18, 270-275, 1998.

36. Ozgenel GY: Effects of hyaluronic acid on peripheral nerve scarring and regeneration in rats. *Microsurgery*, 23, 575-581. 2003.

37. Richard D. Price RD, Berry MG, Navsaria HA: Hyaluronic acid: the scientific and clinical evidence. *J Plast, Reconstr Aesthet Surg,* 60, 1110-1119, 2007.

38. Mohammad JA, Warnke PH, Pan YC, Shenaq S: Increased axonal regeneration through a biodegradable amnionic tube nerve conduit: Effect of local delivery and incorporation of nerve growth factor/hyaluronic acid media. *Ann Plast Surg,* 44, 59-64, 2000.