EXPERIMENTAL STUDY

Effects of subepineural hyaluronic acid injection on nerve recovery in a rat sciatic nerve defect model

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ABSTRACT

BACKGROUND: Maintenance of epineural integrity is very important for nerve healing. Reports on the use of substances considered to have positive effects on nerve healing in experimental nerve defect models are increasing. The present study assessed the effects of sub-epineural hyaluronic acid injection in a rat sciatic nerve defect model that was created while maintaining epineural integrity.

METHODS: The study included 40 Sprague Dawley rats. The rats were randomly divided into a control group and three experimental groups (10 rats in each group). In the control group, the sciatic nerve was dissected and no additional surgery was performed. In experimental group 1, the sciatic nerve was transected in the middle, and then, primary repair was performed. In experimental group 2, a 1-cm defect was created while preserving the epineurium, and then, the defect was repaired with end-to-end suturing of the preserved epineurium. In experimental group 3, the surgical procedure for experimental group 2 was performed, and then, sub-epineural hyaluronic acid injection was carried out. Functional and histological evaluations were performed.

RESULTS: On functional evaluation, there was no statistically significant difference among the groups during the 12-week follow-up period. On histological evaluation, nerve recovery was poorer in experimental group 2 than in experimental groups 1 and 3 ($p<0.05$).

CONCLUSION: Although the functional analysis did not reveal any significant results, the histological findings suggest that hyaluronic acid increases the regeneration capacity of axons through its anti-fibrotic and anti-inflammatory effects.

Keywords: Hyaluronic acid; nerve defect; nerve recovery; peripheral nerve injury.

INTRODUCTION

Peripheral nerve disorders have a wide spectrum of causes ranging from damage associated with long-lasting compression to acute traumatic injury. When the peripheral nerve is damaged due to trauma, primary repair without tension is the gold standard approach. If primary repair cannot be performed for peripheral nerve injury with a defect several tissues and materials, such as nerve, muscle, fat, amnion, arterial grafts, venous grafts, polyglactin meshes, and silicon tubes, can be used for repair.[1–4] The repair of small nerve defects with the epineurium was first described by Snyder et al.,[5] In 2007, Ignatiadis et al.[6] used an epineural flap to bridge a short nerve defect. In this technique, the epineurium is prepared as a flap and used for repair, resulting in a biological conduit that guides recovering axons.

Hyaluronic acid, which is one of the main components of the extracellular matrix, has an important role in wound recovery. It has been shown to decrease the risk of developing

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scar tissue and adhesions and to promote healing.[7–11] Studies have reported that hyaluronic acid administration decreased adhesion rates during interventions, such as tendon surgery, laparoscopic abdominal surgery, and spinal cord surgery.[12–16] The present study aimed to investigate the effects of sub-epineural hyaluronic acid injection in a rat sciatic nerve defect model that was created while maintaining epineural integrity.

MATERIALS AND METHODS

Animals

All procedures for the care and use of laboratory animals were conducted in accordance with the guidelines outlined by the Experimental Animal Ethics Committee of Istanbul University. The study included 40 adult Sprague Dawley rats weighing 250–300 g. Standard rat laboratory food and water were supplied *ad libitum*. All rats were kept in an animal room for 2 weeks at a constant temperature and humidity to allow them to adjust to the laboratory environment. The rats were then randomly divided into a control group and three experimental groups (10 rats in each group). All rats were healthy throughout the study.

Operative Technique

The rats were anesthetized with an intraperitoneal injection of 40 mg/kg ketamine hydrochloride and 10 mg/kg xylasine. After shaving the hair and disinfecting the skin with povidone-iodine solution, all right sciatic nerves were carefully dissected through a gluteal muscle-splitting approach. In the control group, after sciatic nerve dissection, the wound was closed without any intervention. In experimental group 1, the sciatic nerve was transected sharply, and then, the epineurium was immediately repaired using 9–0 monofilament nylon sutures. In experimental group 2, a 1-cm-long segment was marked at the middle of the sciatic nerve. The proximal and distal sides of this segment were then marked with 9–0 polypropylene sutures, and the sciatic nerve was transected in the middle of the marked 1-cm-long segment. Following transection, the epineurium layers in the proximal and distal segments were peeled off from the perineurium (5 mm in length). The fascicular nerve tissue was cut at the level of the peeled epineural sheath. The peeled epineural sheath was then repositioned and sutured end-to-end with 9–0 monofilament polypropylene sutures. Thus, a 1-cm-long nerve defect was created under an repaired epineural sheath

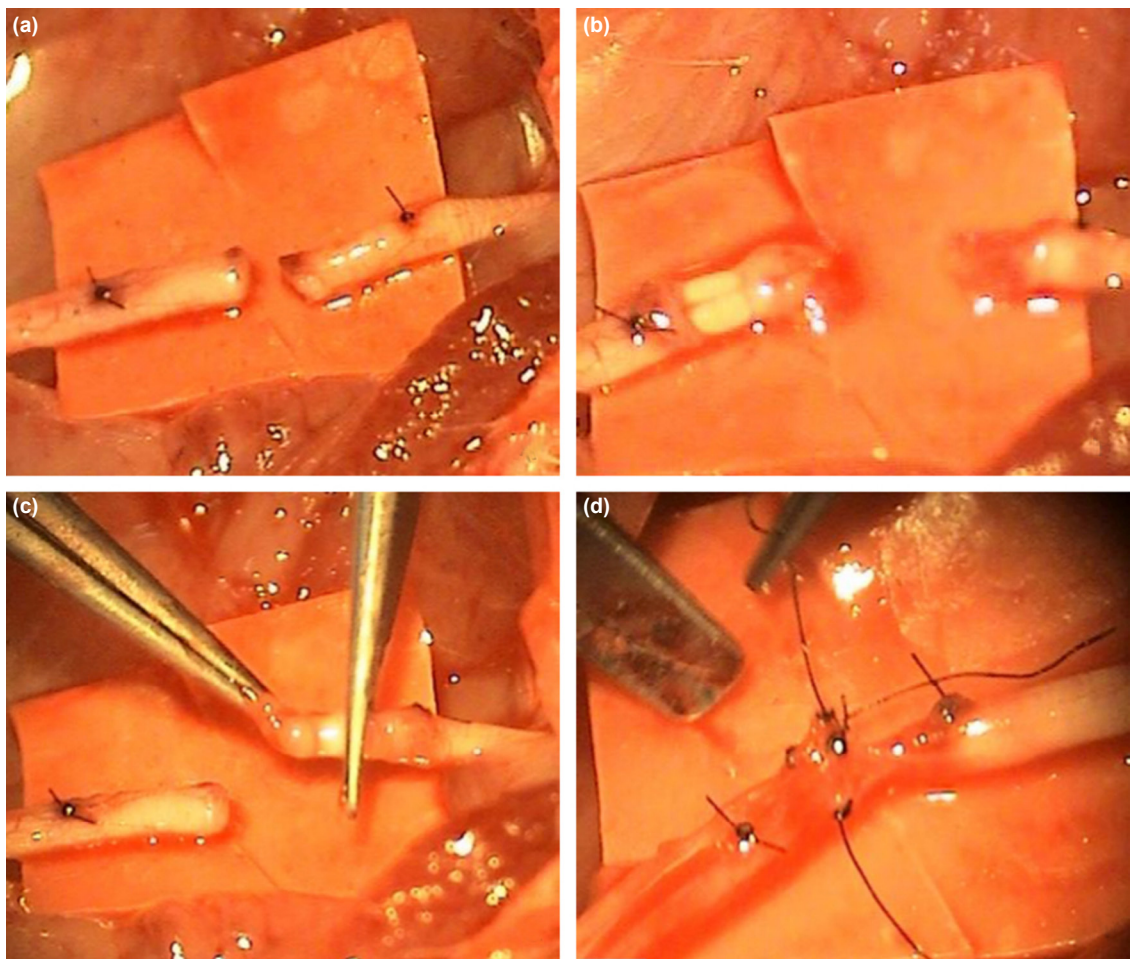

Figure 1. (a) 1 cm long segment was marked at the middle of the sciatic nerve. Note that proximal and distal sides of the 1 cm long segment were marked with suture. **(b)** Peeling of the epineurium from the perineurium. **(c)** Cutting of the fascicles while protecting the epineurium. **(d)** Repair of the epineural sheath (experimental group 2).

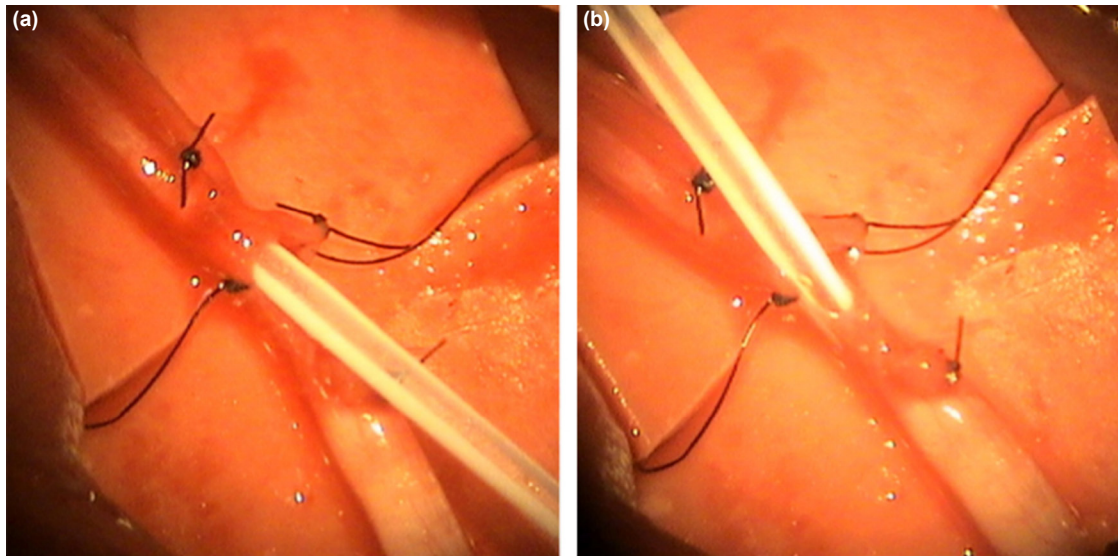

Figure 2. Hyaluronic acid injection on both sides of the epineural flap (experimental group 3).

(Fig. 1). In experimental group 3, the same procedure described for experimental group 2 was performed. Then, a 26-G intravenous cannula was inserted and 0.2 mL of hyaluronic acid was injected under the epineural sheath (Fig. 2). After completion of nerve repair, the incision site was closed in all groups.

Functional Evaluation

Functional recovery was evaluated with walking track analysis at 0, 2, 4, 8, and 12 weeks after the operation. The walking track was created using a device with an 8.2×42–cm corridor open at one end to a darkened room. The hind feet of the rats were dipped in ink, and the rats were allowed to walk along the corridor that was lined with white paper. The print length, toe spread, and intermediary toe spread were measured by hand on the experimental and control sides. Sciatic functional indices (SFIs) were estimated according to the Bain-MacKinnon-Hunter formula.[17]

Histological Evaluation

Histological evaluation was performed after harvesting the sciatic nerves at 12th week. Nerve blocks were prepared and semi-thin sections (thickness of 1 µm) were obtained from the blocks with an ultra-microtome. The semi-thin sections were stained with toluidine blue and examined under a research microscope (Olympus BX61; Olympus, Tokyo, Japan). The myelinated nerve fibers were counted in five different visual fields (each of 1000 µm^2) under ×1000 magnification. Images of the fields were obtained with a digital camera (Olympus DP72; Olympus) compatible with the microscope.

Statistical Analysis

In data analysis, mean and standard deviation values were used as descriptive statistical parameters. The Kruskal–Wallis test was used to compare quantitative continuous data between more than two independent groups. Following the Kruskal–Wallis test, the Mann–Whitney U test was used as a complementary analysis to assess differences. The Wilcoxon test was used to determine differences between repeated measurements. The obtained findings were evaluated within the 95% confidence interval. All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows v22.0 software package (IBM Corp., Armonk, NY, USA). P<0.05 was considered to indicate statistical significance.

RESULTS

Functional Evaluation

The sciatic nerve index values were obtained separately as mean and standard error for each group at each interval (Table 1). There were no significant differences in the SFI values among the four study groups preoperatively. After the surgical procedures, a sharp decline occurred in the track parameters at 2 weeks in all three experimental groups (Fig. 3). The SFI values were significantly lower in the experimental groups than in the control group during the 12–week follow-up period (p<0.05). At the end of the 12th week, the mean SFI

Table 1. Mean sciatic functional index values obtained at 0, 2, 4, 8 and 12 weeks

Weeks	Groups[a]			
	Control	I	II	III
0	-6.2±3.1	-5.9±4.8	-9.5±6.4	-5.9±5.0
2	-8.7±6.2	-92.2±2.8	-92.5±1.7	-92.2±2.4
4	-5.9±3.5	-80.0±18.4	-86.2±19.8	-84.4±16.8
8	-8.8±7.2	-76.1±23.7	-81.7±20.8	-78.3±27.8
12	-8.0±9.4	-52.5±45.9	-68.3±34.4	-58.5±37.7

[a]All values are expressed as mean and standard deviation.

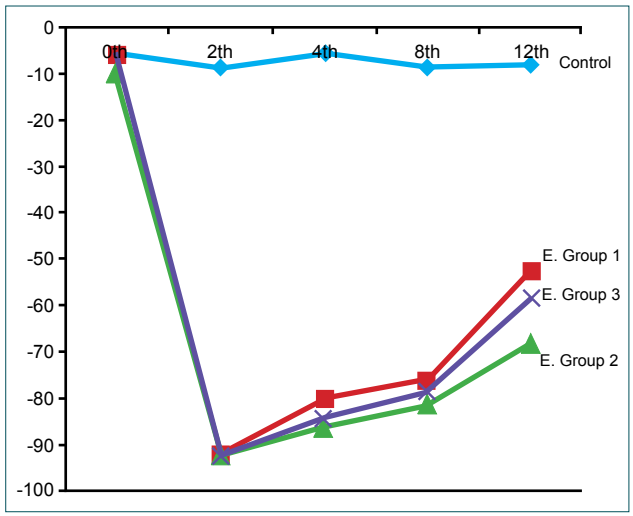

Figure 3. Mean sciatic functional index (SFI) values during the 12-week follow-up period.

values recovered significantly in all three experimental groups ($p<0.05$) (Fig. 4) and there were no significant differences in the SFI values among the three experimental groups.

Histological Evaluation

The histological examination revealed that the mean number of regenerating myelinated nerve fibers was significantly higher in the three experimental groups than in the control group ($p<0.05$). In addition, the mean number of regenerating myelinated nerve fibers was significantly higher in experimental groups 1 and 3 than in experimental group 2 ($p<0.05$). There was no significant difference in the mean number of regenerating myelinated nerve fibers between experimental groups 1 and 3 (Table 2). Extensive fibrosis in the spaces between nerve fibers was noted in experimental group 2. However, mild fibrosis was noted in experimental groups 1 and 3 (Fig. 5).

DISCUSSION

Nerve recovery is a rather complicated and multifactorial process. Following nerve injury, the best functional outcome might be achieved with primary repair. If primary repair cannot be performed, autologous tissues and synthetic materials can be used for the repair of nerve defects. However, considering restricted availability of autologous tissues, morbidity problems in the donor area, and foreign body reactions caused by synthetic materials, further research is being performed to identify other repair methods. Several different techniques related to the use of epineural flaps for the repair of small nerve defects have been described in the literature.[6,18,19] In these techniques, intact epineural tissue is used for defect repair. This prevents the loss of neurotrophic factors, which have a positive effect on nerve recovery, and establishes an optimal environment, in which regenerating axons are not affected by external factors.[20,21]

Table 2. Mean number of myelinated nerve fibres

	Groups[a]			
	Control	**I**	**II**	**III**
Mean number of myelinated nerve fibres	110.8±12.2	217.8±19.0	144.2±12.3	214.3±9.9

[a]All values are expressed as mean and standard deviation.

We used a repair technique different from the described techniques involving an epineural flap. In our study, 5–mm parts of the epineurium were preserved in the proximal and distal nerve stumps. Later, the perineural tissue under these flaps was resected and 1–cm-long nerve defect was repaired with the prepared epineural flaps. In previously described epineural flap techniques, flaps were prepared only with one-sided pedicles or were used as epineural grafts. In these cases, the vascularity of the epineural tissue might not be appropriate. In our study, we prepared two separate flaps that were perfused from the proximal and distal stumps and attempted to overcome this problem with well vascularized two flaps.

We found that hyaluronic acid decreased perineural adhesion and fibrosis and thus supported nerve recovery.[7,10,11] This might be associated with its anti-inflammatory effect. In some studies, hyaluronic acid was topically applied to the area of primary repair.[22,23] In the studies by Seckel et al.[24] and Wang et al.,[7] nerve defects were repaired with conduits filled with hyaluronic acid and the effects of hyaluronic acid on neural recov-

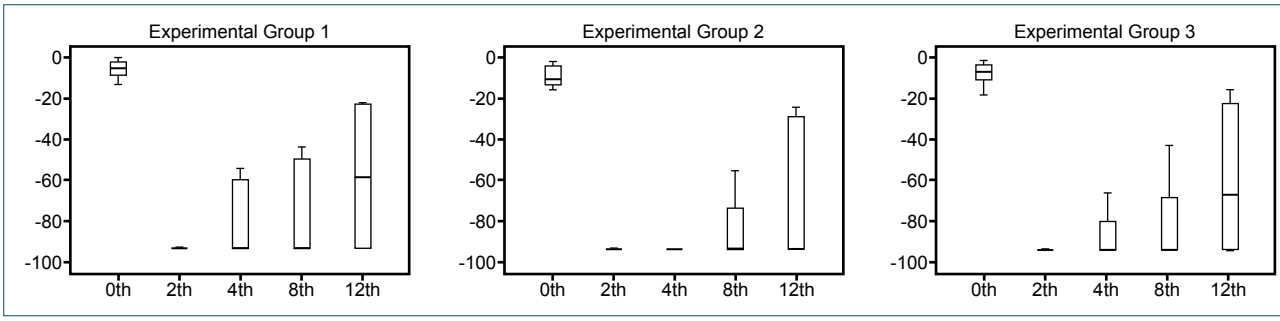

Figure 4. Recovery of the sciatic functional index (SFI) values in the experimental groups.

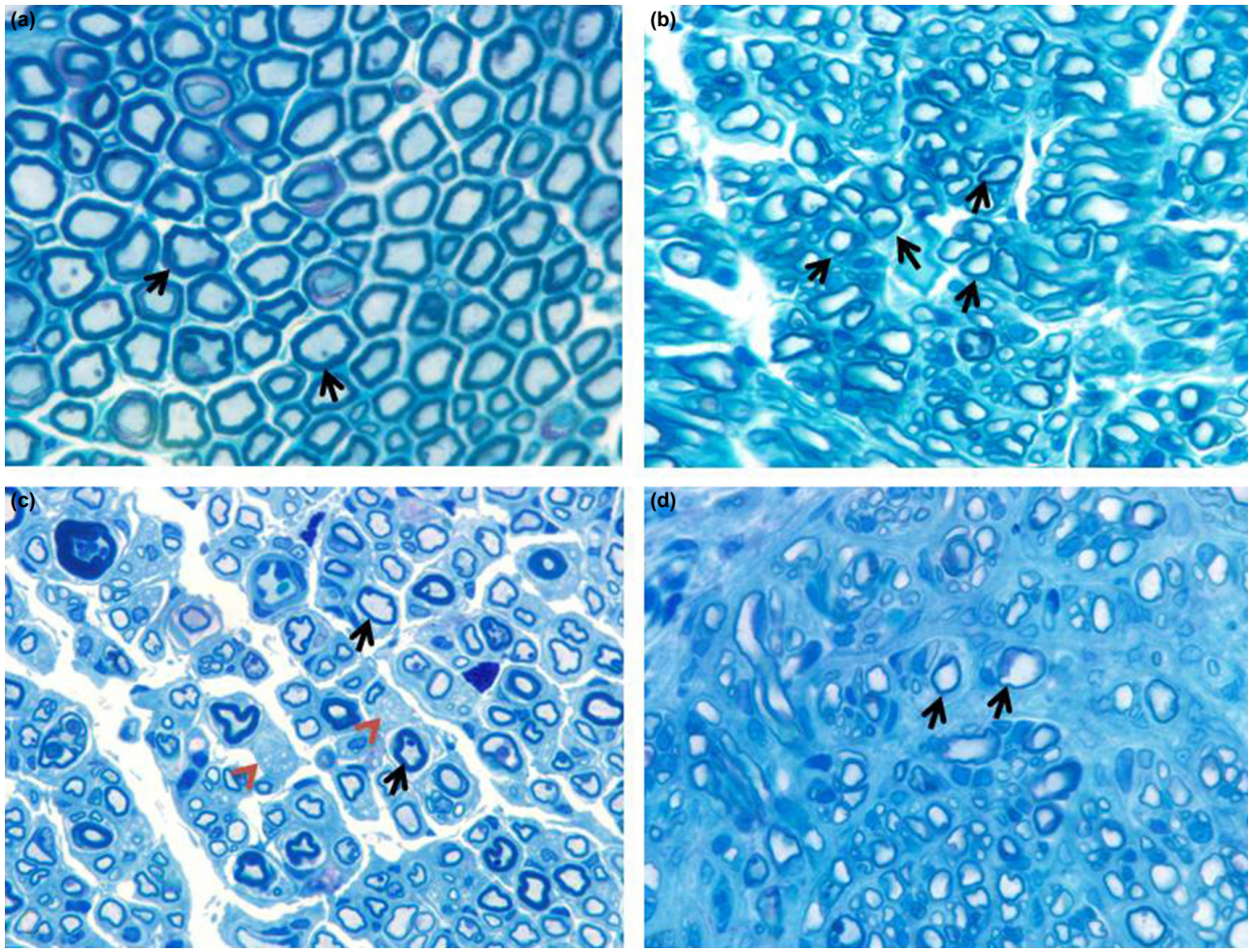

Figure 5. (a) Control group: Myelinated nerve fibers (black arrow). **(b)** Experimental group 1: Multiple regenerating myelinated nerve fibers with small and large diameters (black arrow). **(c)** Experimental group 2: Regenarating myelinated nerve fibers with different diameters. Degenerative areas along with nerve fibers (red arrow) and increased connective tissue are seen. **(d)** Experimental group 3: Multiple regenerating myelinated nerve fibers with small and large diameters.

ery were evaluated. We did not find any study focused on the effects of hyaluronic acid after repair involving epineural flaps.

We observed neural recovery in all experimental groups. The changes in the mean SFI values in the experimental groups during the 12-week follow-up period provide evidence for nerve recovery. Although the SFI values in experimental groups 1 and 3 were similar throughout the 12-week follow-up period, the SFI values in experimental group 2 remained subdued starting from the 2nd week. These findings indicated that the recovery rate was comparable in experimental groups 1 and 3 and was subdued in experimental group 2. However, there were no statistically significant differences among the groups throughout the 12-week follow-up period. The absence of a difference might be explained by the mixed structure of the sciatic nerve and the proximal location of the defect.

After nerve injury, the proximal axon continues to proliferate and forms more than one terminal bud. These buds extend distally and complete the recovery process.[20] Therefore, at the end of the 12th week, the presence of more myelinated nerve fibers in the experimental groups than in the control group was considered a normal finding. The mean number of regenerating myelinated nerve fibers was higher in experimental group 1 and 3 than in experimental group 2 ($p<0.05$). In addition, in experimental group 2, there was extensive fibrosis between nerve fibers but in experimental groups 1 and 3, only mild fibrosis developed. These findings confirmed that the recovery rate was lower in experimental group 2 than in the other groups and the recovery rate of experimental group 1 and 3 were comparable. In experimental group 3; the increased number of regenerating myelinated nerve fibers, absence of fibrotic areas, and presence of recovery comparable to that in experimental group 1 despite a nerve defect, might be related to the regulatory effects of hyaluronic acid on fibrosis and inflammation. Furthermore, hyaluronic acid fills the epineurium and prevents the collapse of epineural flaps, and thus provides a suitable microenvironment for the regeneration of axons distally.

Conclusion

Although the functional analysis did not reveal any significant results, the histological findings suggest that hyaluronic acid increases the regeneration capacity of axons through its anti-fibrotic and anti-inflammatory effects and thus contributes to the recovery of nerve fibers. The use of hyaluronic acid involves a high cost; however, it has certain advantages, such as availability, low risk of hypersensitivity reactions due to its natural biochemical structure, and convenient use. Therefore, hyaluronic acid, which is clinically used in several fields, can be considered in the current microsurgical treatment of neural defects.

Ethics Committee Approval: This study was approved by the İstanbul University Animal Experiment Ethics Committee (Date: 26.05.2011, Decision No: 2011/68).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: A.A.; Design: A.A.; Supervision: O.C.; Resource: A.A.; Materials: A.A., G.C.; Data: A.A.; Analysis: A.A.; Literature search: A.A., G.C.; Writing: A.A.; Critical revision: O.C.

Conflict of Interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZ*

Sıçanlarda oluşturulan siyatik sinir defekt modelinde subepinöral hyalüronik asid enjeksiyonunun sinir iyileşmesine etkisi

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AMAÇ: Sinir iyileşmesi açısından epinöral bütünlüğün sağlanması büyük önem taşımaktadır. Deneysel olarak oluşturulan sinir defekti modellerinde iyileşmeyi olumlu yönde etkilediği düşünülen maddelerin kullanımının bildirimi giderek artmaktadır. Bu çalışmada epinöral bütünlük korunarak oluşturulan siyatik sinir defekti modelinde subepinöral hyalüronik asid enjeksiyonunun sinir iyileşmesine olan etkisi araştırıldı.

GEREÇ VE YÖNTEM: Çalışmada 40 adet Sprague Dawley cinsi sıçan kullanıldı. Her grupta 10 adet sıçan olacak şekilde 1 kontrol ve 3 deney grubu oluşturuldu. Kontrol grubunda, siyatik sinir disseksiyonu dışında işlem uygulanmadı. Deney grubu 1'de siyatik sinir tam kat kesildi ve primer onarıldı. Deney grubu 2'de epinöryum korunarak 1cm'lik sinir defekti oluşturuldu. Defekt epinöral dokuların uc uca suture edilmesi ile onarıldı. Deney grubu 3'de deney grubu 2'de uygulanan cerrahi prosedür tekrar edildi, buna ek olarak subepinöral hyaluronik asid enjeksiyonu gerçekleştirildi.

BULGULAR: Fonksiyonel değerlendirmede, 12 haftalık takip süresi boyunca gruplar arasında anlamlı istatistiksel fark gözlenmedi. Histolojik değerlendirmede sinir iyileşmesinin deney grubu 2' de, deney grubu 1 ve 3' e oranla daha düşük olduğu saptandı ($p<0.05$).

TARTIŞMA: Fonksiyonel değerlendirmede anlamlı fark saptanmamasına karşın, bu çalışmada elde edilen histolojik bulgular hyaluronik asidin antifibrotik ve antiinflamatuar etkileri ile aksonların rejenerasyon kapasitesini artırdığını göstermiştir.

Anahtar sözcükler: Hyalüronik asid; periferik sinir yaralanması; sinir defekti; sinir iyileşmesi.

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