EXPERIMENTAL STUDY

Histological examination of the effects of epidermal growth factor on regeneration of acute peripheral nerve injuries on rabbit model

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ABSTRACT

BACKGROUND: Peripheral nerve injuries are one of the most common and costly injuries especially in the young population. In this study, it is aimed to determine the histological role of epidermal growth factor (EGF) in nerve regeneration with an acute damage made on sciatic nerve in the rabbit model.

METHODS: We used 18 New Zealand rabbits (nine in control group and nine in experimental group). Each group was divided into two groups consisting of five rabbits planned for diameter measurement and four rabbits planned for spatial measurement. The sciatic nerve exploration in the right flank of each animal, full-thickness nerve damage, and then epineural repair was made by a single researcher. 10 µg/kg EGF was given to the repair area of the experimental group and five more EGF injections were given to the experimental group every other day postoperatively. In the control group, we used saline solution. Rabbits were observed for 8 weeks. During follow-up, two rabbits died. At the end of 8 weeks, the nerve tissue of each animal was evaluated histologically and morphologically.

RESULTS: In the experimental group consisting of five rabbits, the mean thickness of connective tissue (epineurium+ mesoneurium) was 156,867 µm; while, in the control group, the thickness was 25,170 µm. In the other groups, the numerical increase in epineurium and mesoneurium areas was detected in the EGF (+) group as a result of the comparative spatial measurements. Epineurium and mesoneurium enlargement was observed in the EGF-given group. Adipocyte and capillary increase was observed in connective tissue.

CONCLUSION: EGF increases epineurium and mesoneurium diameters in peripheral connective tissue in acute peripheral nerve injury regeneration. However, further studies are needed to understand this effect clinically and physiologically.

Keywords: Epidermal growth factor; nerve regeneration; peripheral nerves; sciatic nerve.

INTRODUCTION

Traumatic peripheral nerve damage is one of the leading causes for disability and impairment across the world.[1] Nerve injuries result in approximately $150 billion spent annually in the United States.[2] Around 2–3% of patients admitted to Level I trauma centers have peripheral nerve injuries. When plexus and root injuries are also included, the rate rises up to 5%.[1] The most commonly injured nerves in the upper extremity are radial, ulnar, and median nerves, respectively. In the lower extremity, sciatic nerve injuries are the most common followed by peroneal nerve and rarely occurring tibial and femoral nerve injuries.[3]

Cite this article as: Ayık G, Huri G, Hashemihesar R, Yürüker S, Doral MN. Histological examination of the effects of epidermal growth factor on regeneration of acute peripheral nerve injuries on rabbit model. Ulus Travma Acil Cerrahi Derg 2023;29:30-39.

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Ulus Travma Acil Cerrahi Derg 2023;29(1):30-39 DOI: 10.14744/tjtes.2022.99201 Submitted: 16.10.2021 Revised: 28.02.2022 Accepted: 10.05.2022

Among main etiological factors of such injuries are motor vehicle accidents, penetrating traumas such as stabbing, firearm injuries, industrial work accidents, stretching, and crush injuries which may occur during falling or similar situations. Among these, the most common one is motor vehicle accidents.[3]

According to the study of peripheral nerve injury of 456 cases by Kouyoumdjian,[4] males were more exposed to injuries. The mean age of patients was 32.4 years. The most common injury site was the upper extremity with 73.5%, followed by the lower extremity and the face. Especially young people and military personnel were affected more.[2]

Epidermal growth factor (EGF) was first discovered in 1962 by Doctor Stanley Cohen while studying on nerve growth factors (NGF).[5] The receptor to which it binds is the EGF receptor (EGFR, ErbB1), which shows tyrosine kinase activity and is a member of the EGFR/ErbB super family.[6] Through this receptor, it affects many physiological processes in the cell such as proliferation, differentiation, apoptosis and organ development, growth, regeneration, and ion transport.[7]

The main surgical methods used for peripheral nerve injuries consist of epineural repair, fascicular repair and repair with nerve graft.[8] However, it is limited to assisting the very own regeneration potential of the nerve which we can intervene by means of main surgical procedures. What we can do through the selected surgical method is to bring the nerve ends together by trying to achieve fascicle alignment. We cannot yet control the cellular and molecular responses in this area. In this context, many analyses have been ongoing on growth factors and molecules together with their impact over nerve damage and its regeneration. The aim of our study is to explore the histological effect of EGF particularly on surrounding connective tissue during the regeneration process of acute peripheral nerve injuries.

MATERIALS AND METHODS

Ethics committee approval was received for our study from the Çukurova University Animal Experiments Local Ethics Committee.

Study Plan and Experimental Groups

Eighteen white New Zealand rabbits aged 9–12 months with a body weight varying from 3000 to 3500 g and an average body weight of 3320 g were included in the study. Right sciatic nerves of the rabbits were acutely damaged and repaired epineurally on day 0. No invasive procedure was applied to the left sciatic nerves of the rabbits.

The rabbits were divided into two equal groups. In a randomized and controlled manner, laboratory animals were randomized and grouped by the use of sealed envelope method. Groups are named EGF (+) and EGF (–). Each group was then randomly divided into another two groups: The first group consisting of five rabbits for diameter measurement and the second group of four rabbits for spatial measurement. The first group consisting of five rabbits for diameter measurement was called as EGF (+) -1 and EGF (–) -1, and the second group with four rabbits for spatial measurement was named EGF (+) -2 and EGF (–) -2. Tissues were examined histologically with *Masson's Trichrome* and *Hematoxylin-Eosin* staining, especially for the purpose of evaluating the regeneration of micro-surroundings and connective tissues.

Surgical Technique

All surgical procedures were performed by a single researcher. The same suture material (8.0 Prolene, Ethicon, USA-for nerve repair) was used in all animals for nerve repair and repair of skin and subcutaneous tissues.

Before the surgical procedures, the rabbits with no abnormalities detected that may hinder surgery in terms of general view, behavior, in-cage movements, clinical findings, food, and water intake were, respectively taken from the room where their cages were located and they were monitored, in another room where anesthesia would be performed. All rabbits were intramuscularly injected with 20 mg/kg Cefazolin Sodium (Cefamezin, Sanofi, France) antibiotic prophylaxis and anesthetized by 35 mg/kg Ketamine HCL (Ketalar, Pfizer, USA) and 5 mg/kg Xylazine (Xylazin Bio, Bioveta, Czech Rebuplic). The adequate depth of anesthesia was decided by observing absence of cornea reflexes. Following this process, the right femurs of the rabbits were carefully shaved from the pelvis to the knee. The right lower extremity was then washed with a liquid soap solution, 7.5% Polyvinylprolidone-Iodine complex (Batticon®, Adeka, Istanbul) and 0.9% isotonic NaCl solution (Polifleks, Polifarma, Istanbul). After the cleaning procedure, the rabbits were taken to the surgical room. The surgical site in the right femurs of the rabbits was stained with an antiseptic solution, 10% Polyvinylprolidone-Iodine complex. The rabbits were placed in a lateral decubitus position. They were then covered with sterile covers. Following surgical site sterilization, a slightly oblique skin incision of approximately 5 cm was made about 4 cm distal to the iliac wing which was palpated to the right side. A sharp dissection was used to pass through the subcutaneous tissues. The muscle was split longitudinally parallel to the direction of the muscle fibers. The sciatic nerve was reached and the nerve was explored for approximately 4 cm (Fig. 1a). Following the sciatic nerve exploration, the nerve was damaged in neurotmesis type with a guillotine-sharp scalpel about 6 cm distal to the palpated iliac wing while the lower extremity was in neutral position (Fig. 1b). After the sciatic nerve was damaged, it was repaired by the same researcher with non-absorbable sutures (8.0 Prolene, Ethicon, USA) using the epineural technique with the help of the surgical loupe (Fig. 1c and d). The same procedure was repeated for the right sciatic nerves of all groups.

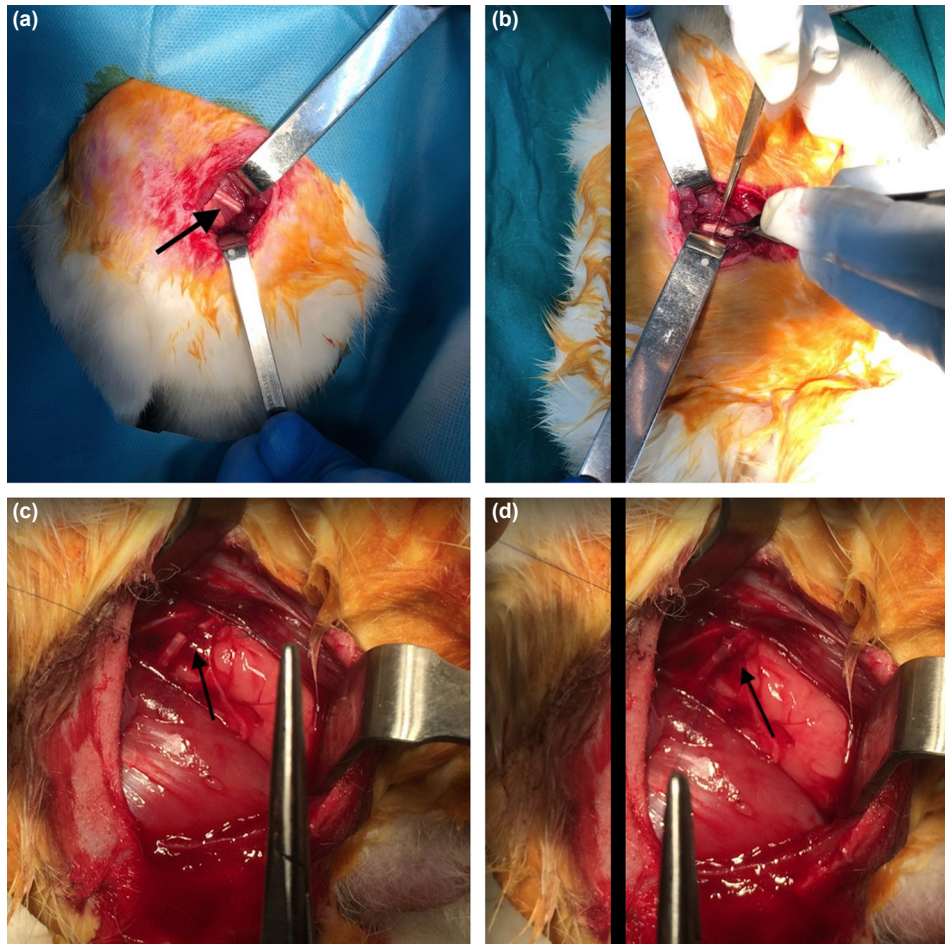

Figure 1. (a) The Sciatic nerve is seen with the black arrow, **(b)** Sciatic nerve damage, and **(c** and **d)** before repair sciatic nerve damage is seen with black arrow. The second black arrow indicates the sciatic nerve during repair.

The depth of the repaired area from the skin was measured through using a ruler for subsequent injections and noted for each rabbit. A dose of 10 µg/kg EGF (Heberprot - P®, Heber Biotech, S.A., La Habana, Cuba) was administered to the EGF (+) experimental group by use of an injector during the repair, without closing the skin and other tissues. The repaired areas of EGF (–) control group were injected with the same amount of physiological saline solution. After the repair stability was checked, the muscle and skin were closed with the same suture materials for all groups. A marking suture was used on the repair area with non-absorbable suture material in a manner that was visible on the skin.

Following the surgical procedure, 15 mg/kg Paracetamol (Perfalgan, Bristol-Myers Squibb, USA) was administered intravenously for analgesic purposes through the marginal ear veins of the rabbits. Antibiotic and analgesic were continued to be administered until the 2nd post-operative day. The rabbits were then taken from the room where the surgical procedures were completed to the room locating their cages. All the rabbits were observed to be moving in their cages following their waking up after the surgery. All the rabbits who had undergone surgery were dressed on post-operative day 2 and the dressing procedure continued for 10 days at 2-days' intervals. Considering the measured distances to the skin, EGF at a dose of 10 µg/kg was given to the EGF (+) experimental group while the same amount of saline solution was administered to the EGF (-) control group by the same researcher every other day, with the help of an injector to the repair area-except the day 0–5 doses in total for 10 days.

One rabbit each died from EGF (–)-2 and EGF (+)-2 groups during the 2nd and 3rd weeks of post-operative follow-up. The study continued in this way.

The laboratory animals were euthanized at the 8th week intravenously administrating 100 mg/kg Thiopental Sodium (Pental Sodyum, I.E. Ulagay, Istanbul) through marginal ear veins. The wound site was found out to be clean in all of the rabbits who were euthanized, and no signs of infection (discharge, malodor, splitting sutures, etc.) were observed on any of them (Fig. 2a). The skin was opened using the same incision and marking sutures on the skin and the sciatic nerve was then reached. It was observed that the suture materials were not absorbed in the repair area. The sciatic nerve was excised without causing any damage, approximately 1 cm proximal

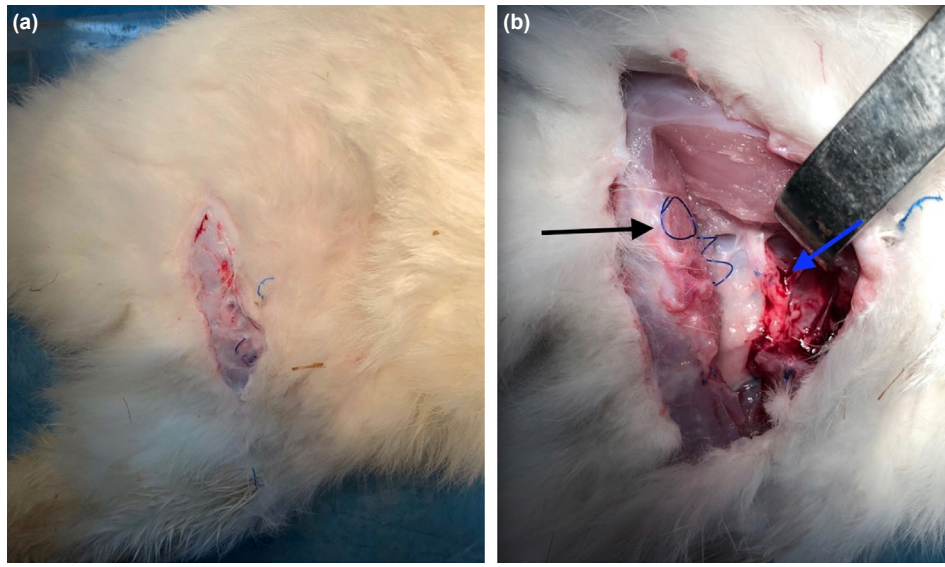

Figure 2. (a) No signs of infection and **(b)** the blue arrow shows the repair area. Marking suture is shown with the black arrow.

and 1 cm distal to the repair site (Fig. 2b). The left sciatic nerves that had not undergone surgery were also excised to the same amount from the same location. For better understanding the direction of the excised nerve tissue, one non-absorbable marking suture was placed on the most distal part. The excised sciatic nerve tissue was examined macroscopically and microscopically.

Histological Analysis

Histological examination and measurements of the tissues were performed by histologists in the Departments of Histology and Embryology of Medical Faculties in Hacettepe University and Uşak University. Tissues were arranged in similar sizes for examination in a laboratory environment.

The tissue samples were fixed in a buffered 10% neutral formaldehyde solution for 48 h, and then passed through alcohol, xylene, and paraffin in automatic tissue tracking device and embedded in paraffin blocks in a horizontal position. Serial 5 µm thick sections were taken from the tissues with a sliding microtome (Leica SM2000R). Serial sections were stained with *Masson's Trichrome* and *Hematoxylin-Eosin* stains and were examined with a research microscope.

All tissues were imaged with a Leica DFC 7000 color camera under the Leica DM6B research microscope (Leica Microsystems, Wetzlar, Germany). Tissue healing was reviewed for all groups. Connective tissue (epineurium and mesoneurium), adipocyte tissue in connective tissue, cell migration, and vascularity were examined.

For quantification, Leica DM4000B research microscope and Optronics Microfire digital camera (Optronics, Oklahoma, USA) were used.

Quantification of nerve tissue was done using stereological methods. For estimation of volume, Cavalieri estimator probe in Stereo Investigator software (MBF Bioscience, Williston, USA) was used. ×2.5 objective was used for quantification with step interval of 100 µm (Fig. 3).

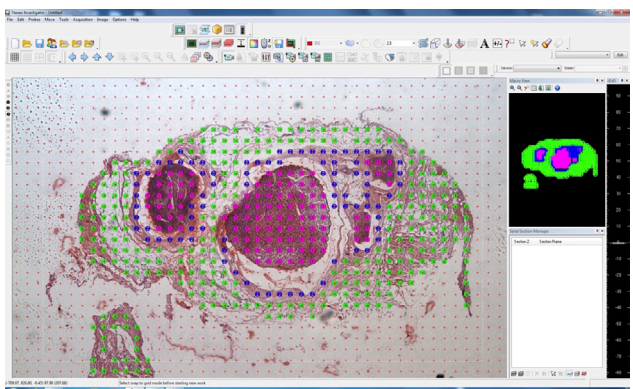

Figure 3. Stereo Investigator (MBF Bioscience, Williston, USA) Software, measurement with cavalieri probe (http://www.mbfbioscience.com/stereo-investigator).

Statistical Analysis

Statistical analysis was conducted with IBM SPSS 23rd version (IBM Corp., Armonk, NY, USA) software. Whether continuous variables were normally distributed or not was analyzed through the Shapiro–Wilk test. According to the results of this analysis, independent sample t-test or Mann–Whitney U test was used for comparison of paired groups, while one-way analysis of variance test or Kruskal–Wallis test was used to compare more than two groups. Intergroup differences were evaluated by *post hoc* analysis for a comparison of more than two groups. Descriptive statistics were presented as mean ± standard deviation (minimum-maximum) for continuous variables, and as numbers and percentages. $P<0.05$ was considered statistically significant.

RESULTS

Nerve continuity was observed in the repaired area of all subjects. During the macroscopic observation, the repair area in the experimental group was found out to have relatively more volume than the control group (Fig. 4).

Both nervous and connective tissues were examined through Masson's trichrome and HE staining. An increase in adipose tissues, which is a component of connective tissue (epineurium and mesoneurium), was observed in the EGF (+)-1 experimental group (Fig. 5).

An increase in vascularity was observed in the connective tissue (epineurium and mesoneurium) in the EGF (+)-1 group (Fig. 6). In EGF (+)-1 group, in addition to epineurium and increased vascularity, cells with a morphological resemblance to mesenchymal stem cells were observed between connective tissue and nervous tissue (Fig. 7).

Connective tissue (epineurium and mesoneurium) diameters of 5 randomly determined rabbits from EGF (+)-1 and EGF(–)-1 groups were examined and compared. The measurements were conducted on the thickest area (Table 1).

An increase in the diameter of the connective tissue (epineurium + mesoneurium) in the right sciatic nerves which are repaired was observed in EGF (+)-1 group. The difference between the three groups was analyzed using the Kruskal–Wallis test, and their *post hoc* analysis was conducted with the Mann–Whitney U test. The p value is 0.003 according to Kruskal–Wallis test results. The mean diameter of EGF (+)-1 group was found to be 156,867 (SD 78,306) µm, the mean diameter of EGF (-)-1 was 25,170 (SS 3,721) µm, and the mean diameter of the unprocessed healthy left side was 20,421 (SD 5,405) µm. According to the subgroup analysis, a statistically significant difference was detected between EGF (+)-1 and EGF (–)-1 as well as between EGF (+)-1 and the healthy side (p=0.008 and <0.001, respectively), whereas no statistical difference was found between EGF (–)-1 and the healthy side (Table 2).

The connective tissue (epineural and mesoneural) areas were compared in EGF (+)-2 (rabbits numbered 11, 13, 14) and

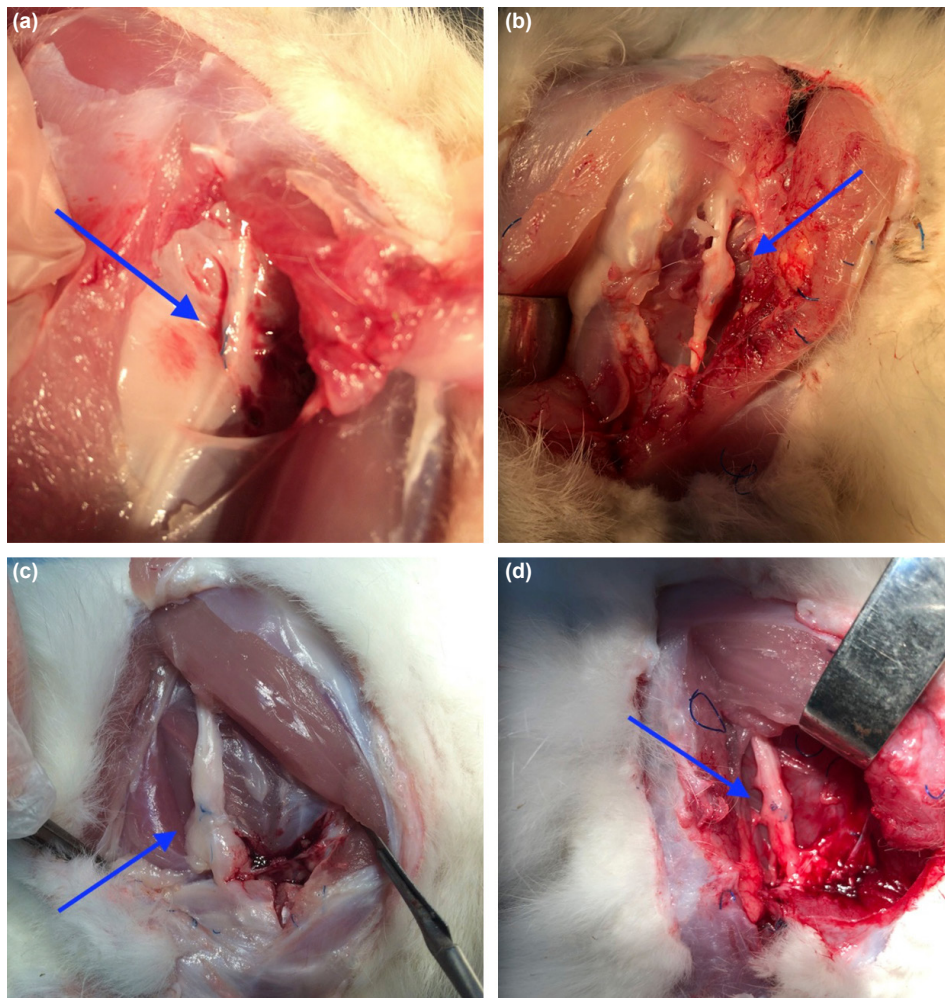

Figure 4. (a and **b)** Samples from EGF (–) group. The blue arrows show the repair area. **(c** and **d)** samples from EGF (+) group. The blue arrows show the repair area.

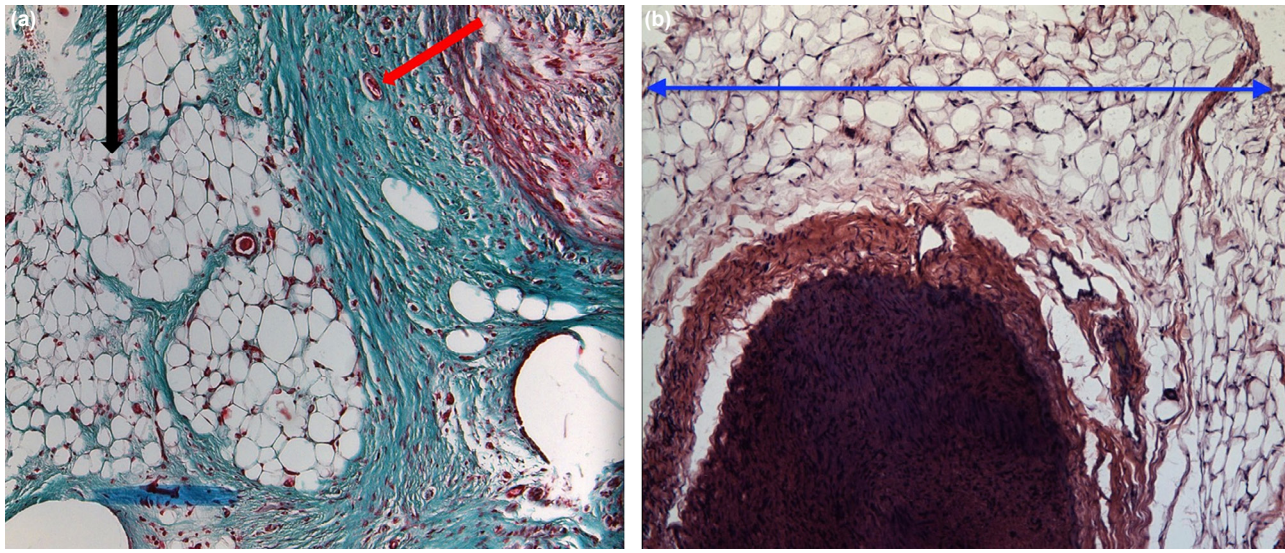

Figure 5. (a) ×10 objective. An increase in adiposes (black arrow) and capilleries (red arrow) is seen in connective tissues (epi and mesoneurium) of EGF (+)-1 group. *Masson's trichrome* **(b)** ×10 objective. Transverse section from EGF (+)-1 group repair zone. Adipous tissue amount is remarkable. Hematoxylin Eosin.

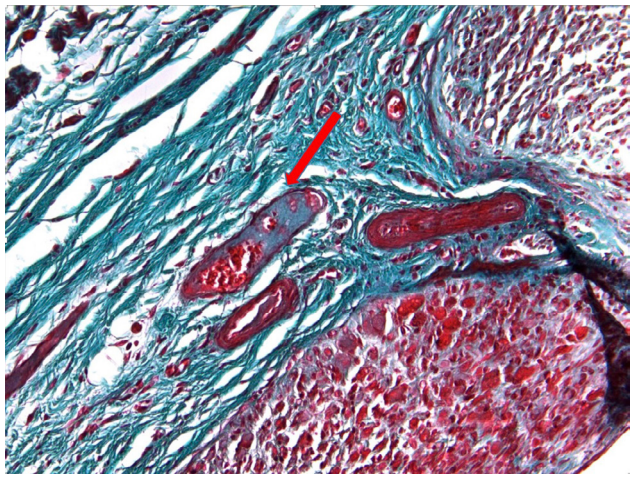

Figure 6. ×20 objective. Capillary increase is observed in connective tissues (red arrow) in EGF (+)-1 group. *Masson's trichrome.*

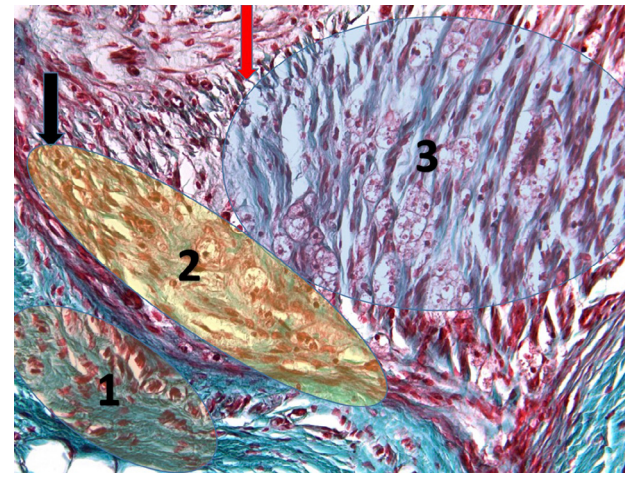

Figure 7. ×20 objective. A vascular site surrounding the nerve fibers as well as a site like a recovery area in between is observed in EGF (+)-1 group. Cells with a morphological resemblance to mesenchymal stem cells were observed in zone 2. Epineurium is highlighted as 1, buffer zone as 2 and peripheral nerve as 3. *Masson's. trichrome.*

EGF (–)-2 (rabbits numbered 16, 17, 18) groups consisting of three subjects each (Table 3). The comparison was made between the nerves undergoing invasive procedure in EGF (+)-2 and EGF (–)-2 groups. Serial sections were taken starting from the distal segments of the nerve; and the 1st, 100th, 200th, 300th, and 400th sections were examined in terms of the area. Repaired zone falls within the compared areas. In the statistical analysis performed, the P value of the 100th section was found to be 0.023. In addition, the p value was found to be 0.058 in the 200th section area. Apart from these, the p values of the 1st, 300th, and 400th sections of the were determined to be, respectively, 0.248, 0.213, and 0.207.

In general, an expansion was observed in the connective tissue (epineurium and mesoneurium) of the EGF (+) group. Adipocyte and capillary increase was observed in the connective tissue. Around the nerve fibers was detected an area which is rich in terms of vascularity and consisted of morphologically stem cell-like cells. In the EGF (+) group, there was an increase in the diameter of the peripheral nerve.

DISCUSSION

Peripheral nerve injuries are a common clinical problem. Approximately 2–3% of patients admitted to Level I trauma centers have peripheral nerve injuries. These types of injuries are mostly seen in the upper extremity, resulting in loss of functions and a subsequent suboptimal functional recovery. There has not been much surgical innovation in the past 50 years. Direct nerve repairing and nerve autografts when defects in-between are in question are the current gold stan-

Table 1. Connective tissue (epineurium + mesoneurium) diameters of EGF (+)-1 and EGF (-)-1 groups (values written in μm)

	Rabbit number	Right side where surgery was performed	Unprocessed healthy left side
EGF (+)-1	1	53.336	16.776
EGF (+)-1	2	269.650	27.100
EGF (+)-1	3	175.112	19.039
EGF (+)-1	4	129.378	22.469
EGF (+)-1	5	156.859	28.395
EGF (-)-1	6	30.766	25.083
EGF (-)-1	7	23.860	20.929
EGF (-)-1	8	20.888	11.558
EGF (-)-1	9	26.584	17.648
EGF (-)-1	10	23.752	15.213

Table 2. Comparison of the diameters

Statistics between groups	p-value
Between EGF (+)-1 and EGF (-)-1	0.008
Between EGF (+)-1 and healthy left side	<0.001
Between EGF (-)-1 and healthy sol side	0.165

EGF: Epidermal growth factor.

dard.[2] What we can do with today's repairing techniques is to bring the nerve endings together by trying to align the fascicles. Therefore, even if a perfect repair is made, we can only achieve good and perfect results at 50%.[9,10] However, we cannot yet fully control the cellular and molecular responses in this area.

The fact that repairing results remain at 50% success rate even with modern microsurgery techniques has triggered researches on regeneration mechanisms.[8] The target mechanisms of current studies on nerve healing can be grouped under two main titles. First is to accelerate axon regeneration and second is to ensure adaptation of the surrounding tissue.[2] Our study is especially important in determining change of the regeneration micro-surrounding with EGF.

When we consider the substances involved in regeneration process in general, NGF (neurotrophins-*Nerve Growth Factor*) are the molecules released naturally during nerve regeneration. They are released from nerve endings following a nerve damage and play important roles in nerve growth, differentiation, and survival.[11] These growth factors are at low levels in healthy nerves. Their release increases after an injury, especially from the distal nerve stump. Today, many growth factors regarding nerve regeneration have been identified, which are glial growth factor, fibroblast growth factor (FGF), glial cell derived neurotrophic factor (GDNF), neurotrophin 3, ciliary neurotrophic factor, and leupeptin.[11] The current studies try to improve the repair results, especially in injuries with defects, by revealing, in addition to these known substances, unknown molecules which would affect regeneration and integrating them into the nerve tubes. However, both unknown side effect potentials of these factors and their synergistic impacts with other factors constitute a clinically important problem. Horowitz states that gangliosides have positive effects on rat sciatic nerve regeneration.[12] Klein demonstrates that forskolin increases axon growth.[13] Azathioprine and hydrocortisone are thought to exert the protective effect of gangliosides by reducing autoantibodies.[8] Cyclophosphamide has been shown to increase motor function recovery in rat sciatic nerve.[14] Recently, N-acetyl cysteine and acetyl-L-carnitine have been found experimentally to be two pharmacological agents having neuroprotective effects.[15,16]

Schwann cell (SC) has a very important role in peripheral nervous system both during the development process and after injury. Allogeneic transplantation of these cells has experimentally been shown to increase regeneration.[17] Embryonic stem cells, neural stem cells, induced pluripotent stem cells,

Table 3. Comparison of connective tissue areas (epineural and mesoneural) (Values are given in μm²)

Group	Number	Epineural + mesoneural area (average) 1st section	Epineural + mesoneural area (average) 100th section	Epineural + mesoneural area (average) 200th section	Epineural + mesoneural area (average) 300th section	Epineural + mesoneural area (average) 400th section
EGF(+)-2	3	2450000	3120000	3540000	3400000	3600000
Rabbit Nr: 11,13,14		(Min: 1360000	(Min: 2560000	(Min: 2010000	(Min: 1960000	(Min: 1820000
		Max: 3660000)	Max: 3830000	Max: 4300000)	Max: 5220000)	Max: 4500000)
EGF(-)-2	3	1340000	1490000	1270000	1800000	1950000
Rabbit Nr: 16,17,18		(Min: 620000	(Min: 1040000	(Min: 510000	(Min: 880000	(Min: 700000
		Max: 2260000)	Max: 1940000)	Max: 1840000)	Max: 2510000)	Max: 2800000)

EGF: Epidermal growth factor.

and adult mesenchymal stem cells are alternatively tested and there are many *in vivo* and *in vitro* studies on them. It is aimed to express myelin protein in peripheral nerve regeneration through forming SC precursors with these stem cells. Mesenchymal stem cells can be found in many adult tissues such as bone marrow, adipose tissue, liver, dental pulp, skin, and skeletal muscle and have especially active involvement in tissue repair after injury.[18] Stem cells used for nerve healing increase peripheral nerve regeneration by differentiating into SC-like cells. Adiposed-derived stem cells also form functional SC-like cells and enhance nerve regeneration.[18] Recently, studies have been carried out to increase axonal regeneration and to create a neuroprotective effect by incorporating culture cells and biological scaffolds. However, the long cultivation periods cause a tendency to stem cells instead. There are studies aiming at increasing neuronal survival by use of adipose-derived stem cells with various scaffolds.[19]

The role of the connective tissues around the nerve in the injury process is very important. In Seddon's classification, axonotmesis is defined as the stage in which connective tissues are preserved at various rates, while neurotmesis is defined as complete transection of both axon and connective tissues. In the Sunderland classification, the damage to the epineurium increases the injury from Stage IV to V. In this context, it is remarkable that the perineural connective tissues increased in the EGF group according to the results, we obtained from the study. Epineurium and mesoneurium have great importance in nerve regeneration both in vascular and micro-surrounding terms. Mesoneurium is a loose connective tissue whose importance increases particularly in nerve injuries by providing the extrinsic circulation of the nerve.[20]

In this study, we observed that there is an increase in volume of connective tissue. This increase was due to the accumulation of new vascular structures and stem cells that comes with it. Each vessel has satellite cells that surrounds it. Satellite cells which are stem cells are brought to the injury site by blood vessels. EGF facilitates the formation of new blood vessels and thus enhance the formation of both adipose cells and stem cells which will help the repair process of the injured nerve. Further study needs to be done to understand the process that starts with satellite stem cells and the stem cell that enhances the repair process of the injured nerve. The accumulation of adipose cells is due to fuel the need for energy in the repairing process. It is known that cells in the repair zone, especially Schwann cells, are activated for regeneration and the nerve tissue switches from "transmission" mode to "synthesis" mode.[21]

Despite many recent studies mentioned above, there is currently no clinically proven and applicable pharmacological treatment for nerve injuries. There are many candidate growth factors, peptides, and molecules (NGF, BDNF, and CTNF). However, the clinical practices of such molecules are highly problematic due to time and dosage, mode of administration, interactions with other in vivo growth factors, and potential side effects.[18]

There are evidences pointing out that EGF and its related receptor family are effective on mammalian nervous system injuries. EGF is a mitogenic factor that stimulates the proliferation of various cell types, including epithelial cells and fibroblasts. EGFR is thought to be expressed in the cerebral cortex, cerebellum, hippocampus, and many other areas in the central nervous system. EGF not only affects mitotic cells but also post-mitotic neurons. Various studies show that EGF increases neurite growth and survival and has neurotrophic and neuromodulatory impacts over cerebral cortical and cerebellar neurons. Although the neurotrophic effect mechanism of EGF has not been fully clarified, it is thought that it acts through the activation of mitogen-activated protein kinase through EGFR. It has an effect on glial cells as well as neurons. Especially, the studies conducted on the CNS show that it causes both proliferation and differentiation in glial cells. For mammalian nervous system injuries, the necessary steps such as proliferation and differentiation of the glial population required for axonal regeneration, activation of glial cells with the EGF/ErbB signaling system, and establishment of glial and neuronal relationships are supported by EGF.[22]

In the organ culture study performed by Wildering et al.[23] on *Lymnaea stagnalis*, a mollusk, EGF was tested on various neuron types. Again, based on the data obtained in the previous studies of the same team, EGF was used at the maximum neurotrophic effect concentration specifically for the studied neuron. As a result, it was shown that EGF in three different neuron types increases axonal regeneration in organ cultures. In addition, in the same study, it was shown that reducing the tyrosine kinase activity of this receptor using EGFR receptor inhibitors results in a decrease in axonal regeneration. The therapeutic effect of EGF on axonal regeneration was antagonized by selective EGFR tyrosine kinase inhibitors. Although the study showed axonal regeneration on *Lymnaea*, the lack of studies showing the effect of EGF and its homologues on the mammalian peripheral nervous system *in vivo* was also mentioned.

Dubuisson et al.,[24] in their study on 15 Sprague-Dawley rats, created a gap on the sciatic nerve and grafted this gap with a tube of collagen. They added approximately 75 µg EGF on the experimental side and Type I collagen on the other side. They then euthanized five rats in the 4th week and 10 rats in the 8th week. In the electrophysiological study in the 4th week, they could not detect any significant electrical signal on both sides. On the 8th week, they found an average nerve conduction velocity on both sides, which was not significant. In histological examination, they focused on the distal of the repair and looked at the myelinized nerve fiber density, width, and axon/myelin ratio, but could not find any signif-

icant difference between both sides. As a conclusion, they stated that the EGF-loaded collagen tube did not increase nerve regeneration. They presented some reasons to explain this situation. First is that EGF could be a weak neurotrophic factor. The other reason is that EGF was not used in sufficient concentration. Third, they stated that EGF might have a short half-life under in vivo conditions. However, the histological characteristics of the regenerative site in particular were not fully specified. Our study differs in terms of the absence of defects in-between, examining the impacts of EGF following an end-to-end repair for acute nerve injuries and determining histological features.

EGF has a dose dependent effect. In our study, we used a relatively higher dose compared to similar studies in the literature. We made injections every other day to reduce the effect of possible short half-life of EGF and due to the fact that inflammatory response required for regeneration after damage appears in the 1st days. We administered 10 μg of EGF per kilogram into the site, which means an EGF administration between 180 and 210 μg per rabbit with five injections every other day. Thus, we tried to ensure that the EGF concentration remained relatively sufficient compared to the previous studies and that its effects were more clearly revealed. However, EGF dosage raises safety concerns in ulcer and wound healing applications that are currently used clinically as repetitive applications could induce hyperplasia and hypertrophy as well as increase angiogenesis and predispose the development of cancer, especially in patients with immunity problems.[7] Increased EGF levels as well as increased EGFR activity and mutations have been detected in many cancers such as glioblastoma, non-small cell lung cancer, head-and-neck, breast, colorectal, ovarian, prostate, and pancreatic cancer.[25]

The main limitation of our study is the lack of an electrophysiological and clinical evaluation. While euthanasia in the 8th week allows for histological interpretation, possibly longer time is required for regeneration from damaged site to the distal target organ, especially in a relatively large animal such as a rabbit. Therefore, the results to be obtained through electrophysiological studies for earlier periods will probably not be distinctive. However, it is for sure that electrophysiological studies are required for the clinical evaluation and definite understanding of the findings.

Conclusion

As a result of our study, it has been shown that EGF leads to an expansion in the surrounding connective tissues together with an increase in adipose tissue and vascularity during the regeneration process of acute peripheral nerve injury. It is not possible to make a clear clinical interpretation of the histological effects occurring at this point. In this respect, further studies are needed to make clinical evaluation based on the present study.

Ethics Committee Approval: This study was approved by the Çukurova University Animal Experiment Ethics Committee (Date: 28.11.2016, Decision No: 10).

Peer-review: Externally peer-reviewed.

Authorship Contributions: Concept: G.A., G.H., S.Y., M.N.D.; Design: G.A., G.H., R.H., S.Y., M.N.D.; Supervision: G.A., G.H., M.N.D.; Resource: G.H., S.Y., G.A.; Materials: G.A., G.H., R.H.; Data: G.A., G.H., R.H., S.Y.; Analysis: G.A., G.H., S.Y., M.N.D.; Literature search: G.A., S.Y., G.H., R.H.; Writing: G.A., G.H., S.Y., M.N.D.; Critical revision: G.A., G.H., S.Y., M.N.D.

Conflict of Interest: None declared.

Financial Disclosure: The authors declared that this study has received no financial support.

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DENEYSEL ÇALIŞMA - *ÖZ*

Akut periferik sinir yaralanmalarının rejenerasyonunda epidermal büyüme faktörünün etkilerinin tavşan modeli üzerinde histolojik incelenmesi

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AMAÇ: Periferik sinir yaralanmaları, özellikle genç nüfusu etkilemekte, yüksek maliyetlere sebep olmakta ve sık görülmektedir. Bu çalışmada, tavşan modelinde siyatik sinir üzerinde oluşturulan akut bir hasar ile sinir rejenerasyonunda EGF'nin histolojik rolünün belirlenmesi amaçlandı.
GEREÇ VE YÖNTEM: On sekiz adet Yeni Zelanda türü tavşan; dokuz adet kontrol grubu, dokuz adet deney grubu olacak şekilde kullanıldı. Kontrol ve deney grupları kendi içlerinde dört ve beş tavşan içeren iki gruba ayrıldı. Dört tavşan içeren gruplarda alan ölçümleri yapılırken, beş tavşan içeren gruplarda çap ölçümleri yapıldı. Her bir tavşana sağ kalçasından siyatik sinir eksplorasyonu, tam kat sinir hasarı ve ardından epinöral tamir tek bir araştırmacı tarafından uygulandı. Deney grubuna 10 µg/kg EGF bölgeye enjekte edildi. Deney grubuna ameliyat sonrası günaşırı olacak şekilde beş enjeksiyon daha yapıldı. Kontrol grubunda aynı miktarlarda serum fizyolojik kullanıldı. Tavşanlar sekiz hafta boyunca gözlendi. Takipler sırasında iki tavşan öldü. Sekiz hafta sonunda hayvanlardan alınan siyatik sinir dokuları histolojik ve morfolojik olarak değerlendirildi.
BULGULAR: Beş tavşan içeren EGF (+) grupta ortalama bağ doku (epinöryum + mezonöryum) çapı 156.867 µm; beş tavşan içeren kontrol grubunda ise 25.170 µm idi. Diğer gruplarda yapılan karşılaştırmalı alansal ölçümlerde ise EGF (+) grubunda bağ doku (epinöryum+ mezonöryum) alanlarında kontrol grubuna göre artış gözlendi. EGF verilen grupta epinöryum ve mezonöryumda genişleme izlendi. Bağ dokusunda adiposit ve kapiller artışı görüldü.
TARTIŞMA: EGF, akut periferik sinir yaralanma rejenerasyonunda çevre bağ dokuda epinöryum ve mezonöryum çaplarını arttırmaktadır. Fakat bu etkinin klinik ve fizyolojik açıdan anlamlandırılabilmesi için ileri çalışmalara ihtiyaç duyulmaktadır.
Anahtar sözcükler: Epidermal büyüme faktörü; periferal sinirler; sinir rejenerasyonu; siyatik sinir.

Ulus Travma Acil Cerrahi Derg 2023;29(1):30-39 doi: 10.14744/tjtes.2022.99201