Marmara Medical Journal 2018; 31: 1-10
DOI: 10.5472/marumj.342046

ORIGINAL ARTICLE / ÖZGÜN ARAŞTIRMA

The effect of topical 5-fluorouracil application on epineural scar tissue in epineurectomized rat sciatic nerve

Topikal 5-fluorourasil uygulamasının epinörektomi yapılan sıçan siyatik sinir çevresinde epinöral skar dokusu oluşumu üzerine etkisi

Melekber CAVUS OZKAN, Betul TUNCEL, Guzin Yesim OZGENEL

ABSTRACT

Objective: This study investigated the effect of topical 5-fluorouracil (5-FU) application on the epineural scar tissue, which has a negative impact on the outcome after a peripheral nerve surgery, and whether the application of two doses of 5-FU would change the outcome.

Materials and Methods: The study involved 72, 3-month-old female Sprague-Dawley rats weighing 250-300 g each. After the experimental animals were observed for a week to make certain that they were healthy, they were randomly divided into 3 main groups; Group A: skin incision + epineurotomy + isotonic solution (n=24), Group B: skin incision + epineurotomy + single dose of 5-FU (n=24) and Group C: skin incision + epineurotomy + two doses of 5-FU (n=24). Each group was divided into 2 subgroups based on the weeks in which they were examined. The two subgroups in each of the three groups were made subject to a walking test, and macroscopic and histopathological examinations at Week 4 and Week 8.

Results: An evaluation of the macroscopic results showed that there was a statistically significant difference between Group A and Group C in sciatic nerve adhesion and severability of its branches. We obtained better results in Group C, when compared with the results obtained in Group A.

Conclusion: We demonstrated in our study that 5-FU had a positive effect on the scar tissue developed around the epineurectomized nerve. This positive effect was also reflected in the nerve's functional capacity, and a second dose of 5-FU application further improved this effect.

Keywords: Epineural scar tissue, 5-fluorouracil, Nerve adhesion

ÖZ

Amaç: Çalışmamızda, topikal 5-fluorouracil (5–FU) uygulamasının periferik sinir cerrahisi sonrası sonucu olumsuz yönde etkileyen epinöral skar dokusu gelişimi üzerine etkisi ve ayrıca iki doz 5–FU uygulamanın sonucu değiştirip değiştirmeyeceği araştırılmıştır.

Gereç ve Yöntem: Çalışmada 72 adet, 250-300 gr ağırlığında, 3 aylık dişi Sprague-Dawley tipi sıçanlar kullanılmıştır. Denekler bir haftalık gözlemi takiben sağlıklı olduklarına kanaat getirildikten sonra rastgele 3 ana gruba ayrıldı. Grup A: cilt kesisi + epinörektomi + izotonik (n=24). Grup B: cilt kesisi + epinörektomi + tek doz 5-FU (n=24). Grup C: cilt kesisi + epinörektomi + iki doz 5-FU (n=24). Her bir grup kendi içinde incelenen haftalar temel alınarak ikişer alt gruba ayrılmıştır. Her üç gruptaki denekler ikiye ayrılarak 4. ve 8. haftalarda yürüme testi, makroskobik ve histopatolojik incelemeler yapılmıştır.

Bulgular: Makroskobik bulgular değerleğendirildiğinde siyatik sinir yapışıklığı ve dallarının ayrılabilirliğinde Grup A ve Grup C arasında istatiksel olarak anlamlı fark olup, Grup C'de daha iyi sonuçlar elde edilmiştir.

Sonuç: Çalışmamızda, lokal 5-FU'in epinörektomi yapılan sinir etrafında oluşan skar dokusu gelişimi üzerine olumlu etkilere sahip olduğunu, bu olumlu etkinin sinir fonksiyonel kapasitesine de yansıdığını ve ikinci doz 5-FU uygulanmasının bu olumlu etkiyi pekiştirdiğini gösterdik.

Anahtar kelimeler: Epinöral skar dokusu, 5-fluorourasil, Sinir adezyonu

Introduction

Continued complaints after a surgery of entrapment neuropathies are annoying for both the patient and the surgeon. One of the reasons for this condition is the uncontrollable development of an epineural scar tissue, which cannot be predicted before the surgery [1-5]. A postoperative epineural scar causes pain and irreversible functional loss. For example, the prevalence of compression due to epineural scar tissue after a carpal tunnel surgery has been found to range between 7 to 20% [6]. The causes of the development of an epineural scar tissue can be divided

Melekber Cavus Ozkan (✉), Betul Tuncel
Department of Plastic, Reconstructive and Aesthetic Surgery, School of Medicine, Marmara University Research and Education Hospital, Pendik, Istanbul, Turkey
melekbero@gmail.com

Guzin Yesim Ozgenel
Department of Plastic, Reconstructive and Aesthetic Surgery, School of Medicine, Uludag University, Bursa, Turkey

Submitted / Gönderilme: 25.05.2017 **Accepted/Kabul:** 05.08.2017

into two as personal and environmental depending on their etiologies. The personal factors include diabetes mellitus, hypothyroidis, obesity and, history of hypertrophic scar, and the surgical reasons include excessive tissue damage, postoperative bleeding, iatrogenic nerve incision, and infections [7, 8]. The epineural scar tissue exerts mechanical pressure on nerve fibers leading to ischemia. With extended pressure, edema occurs in endoneurium and microneural blood circulation decreases. Thickening is observed in perineurium and endoneurium. This pressure has been reported to cause decreases in the axone diameter (by up to 60%), in the number axones (by up to 40%) and in the myelin sheath thickness (by up to 90%) [9-11]. The longitudinal sliding motion, the basis of nerve physiology, is inhibited. Therefore, preventing formation of an epineural scar tissue will increase the success of a peripheral nerve surgery.

The success of 5-fluorourasil (5-FU) in preventing formation of a scar tissue has been known since 1980s and it has been widely used by ophthalmologists. 5-FU is topically applicable, non-toxic, easy to obtain and apply, and able to stop fibroblast growth with a relatively small effect on the surrounding tissues. It has been found experimentally to reduce the epidural scar tissue in rats after a laminectomy [12]. It has also been shown to reduce the development of peritendinous adhesion after a flexor tendon repair in chicken [13]. As an analog of pyrimidine, 5-FU inhibits the thymidylate synthetase, which is necessary for converting ribonucleotides to deoxyribonucleotides in replicating cells, and impairs both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) synthesis. Replicating cells are more sensitive to 5-FU than cells in resting phase. It has been shown both in vivo [14, 15] and in vitro [16, 17] that the fibroblast growth stops after one dose of 5-FU application for up to 36 days depending on the drug concentration. It has been discovered that a low dose of 5-FU stops fibroblast cell growth but fibroblasts continue their functions such as secretion and migration and the effect is reversible; however, the effect is reported to be irreversible in high doses [18].

Materials and Methods

The study was initiated upon the approval of the Uludag University Ethics Committee numbered 2009-15 and dated 10.03.2009. Seventy two 3-month-old female Sprague-Dawley rats weighing 250-300 g each were used in the study. They were monitored in a 12-hour light and 12-hour dark environment at 65±5% humidity and 21±3ºC temperature and fed with standard rat feed. After the experimental animals were observed for a week to make certain that they were healthy, they were divided randomly into 3 main groups:

Group A: skin incision + epineurotomy + isotonic solution (n=24)

Group B: skin incision + epineurotomy + single dose of 5-FU (Biosyn, Orna Pharmaceuticals, Germany) (n=24)

Group C: skin incision + epineurotomy + two doses of 5-FU (Biosyn, Orna Pharmaceuticals, Germany) (n=24).

Each group was divided into 2 subgroups based on the weeks in which they were examined. The study design is summarized in Figure 1. The rats were sacrificed at Week 4 and Week 8 to perform macroscopic and histopathologic examinations (Figure 1).

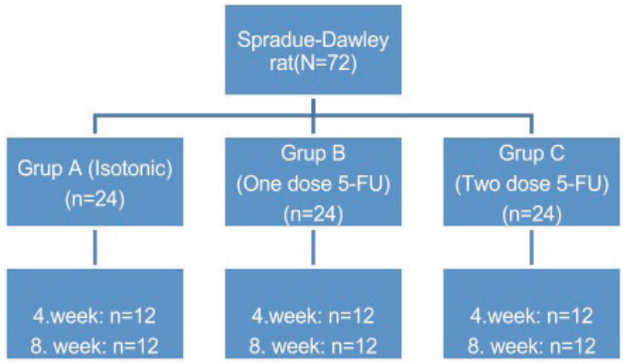

Figure 1. Experimental groups shown in a diagram according to their examination times

1: those sacrificed at Week 4 for histopathologic examination
2: those sacrificed at Week 8 for histopathologic examination

For anesthesia, a 40 mg/kg dose of ketamine hydrochloride (Ketalar, Pfizer, United Kingdom) and a 5 mg/kg dose of xylazine (Rompun, Bayer, USA) were mixed and applied intramuscularly.

I. Surgical technique

Following the general anesthesia, the rats were laid in prone position giving them a slight right decubitus position. The left sciatic nerve was used in all rats to make it easy for the surgeon to work on. After cutting the hair, the left gluteal area was sterilized with 10% povidone – iodine. Following a longitudinal skin incision, an entry was made with a blunt dissection between the gluteus maximus and biceps femoris

muscles to reach the sciatic nerve. After the dissection of the segment between the sciatic arch and bifurcation, epineurotomy was performed on a segment measuring approximately 1.5 cm in size. In Group A rats, gauze soaked in isotonic solution was applied to the area of epineurotomy for 5 minutes and the skin incision was closed with 4/0 prolene. In Group B and C rats, gauze soaked in 5-FU at a concentration of 25 mg/ml was applied for 5 minutes. The Group C rats were operated once more after a week using the same surgical technique to reach the sciatic nerve and gauze soaked in a 2nd dose of 5-FU at the same concentration was applied around it again for 5 minutes.

All surgical procedures were performed by the same surgeon using a loop with X4.5 amplitude and other microsurgery instruments.

II. Preparation of 5-FU

The 5-FU was obtained by diluting Biosyn 250 mg/ml ampoule, a product of Orna Pharmaceuticals, with 10 ml of physiological saline solution. The drug was applied to each animal with gauze soaked in it at a concentration of 25 mg/ml.

III. Experimental assessments

III A. Macroscopic assessment

After sacrificing the rats, their old incision areas were explored once more uncovering the nerve segment that had undergone epineurotomy. The integrity of the skin and muscle fascia, the adhesion of the nerve to the surrounding muscle tissue and its severability from the surrounding tissue were assessed according to the staging system described by Peterson [19] (Table I).

At Weeks 4 and Week 8 after the surgery, 12 sciatic nerves from each group were prepared for an examination of perineural scar tissue development.

Table I. Peterson's numeric staging table

Tissue	Stage
Closure of skin and muscle fascias	1 skin and muscle fascias fully closed 2 skin and muscle fascias partly open 3 skin and muscle fascias fully open
Nerve adhesion and severability	1 no need for a dissection or a slight blunt dissection is needed 2 a severer blunt dissection is needed 3 a sharp dissection is needed

III B. Histopathological assessment

Thirty six rats were sacrificed at Week 4 and the remaining 36 at Week 8. The sciatic nerve was reached by making another incision from the old incision site. The nerve segment from the sciatic arch to the popliteal fossa was removed as a block without so much dissecting it from the surrounding tissue. After the fixation of the removed tissues in a 10% formalin, they were dehydrated by alcohol solutions and embedded in paraffin. A series of longitudinal sections in 5 micron thickness were obtained and they were stained with hematoxylin eosin. The formations of epineural scar tissue were assessed by the same pathologist with X40 and X100 amplitude light microscope without knowing to what group the nerves belonged. The average thicknesses of the epineural scar tissue and the nerve tissue were calculated from the measurements taken with an ocular micrometer (Periplan 6.3*M; Ernst Lietz GmbH, Wetzlar, Germany) from 6 different points for each specimen. The mean scar tissue thickness was divided by the mean nerve tissue thickness to obtain an epineural scar tissue index.

III C. Functional assessment

The walking test, one of the parameters to assess sciatic nerve regeneration and function, was first described by Medinaceli et al., in 1982 [20]. This test was developed by way of observing that the interdigital space in the rat extremity with a sciatic nerve injury lessens and the front-back distance of the footprint lengthens (Figure 2) [21].

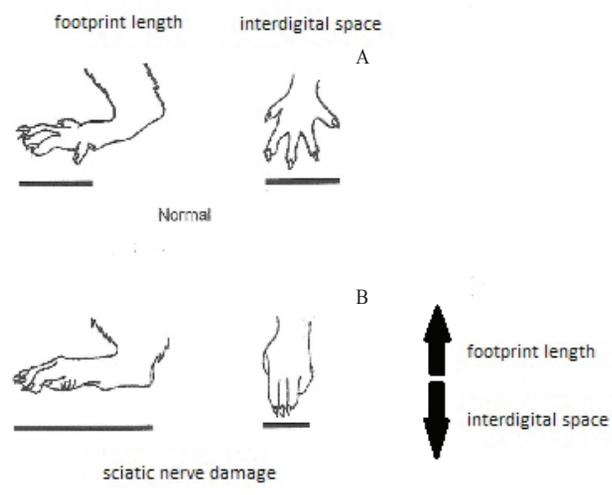

Figure 2. This is a graphical illustration of a typical walking template for normal rats (A) and after a sciatic nerve injury (B). Lengthened footprint from a side view and shortened distance between the 1st and 5th fingers from a front view after a nerve injury [21].

The walking test was performed at post-operational Week 4 and Week 8. Each rat was made to walk on a 10-cm wide and 100-cm long wooden corridor with one edge ending in a dark box. A white absorbing paper was placed on the floor of the corridor separately for each rat. The rear feet of the rats were dipped into methylene blue and they were made to walk on the paper floor [22-26]. Their footprints at the middle point were assessed, because they were not willing to walk at the beginning and their footprints appeared falsely longer when they tended to beg towards the end [27]. Some standard measurements were made from the footprints on the absorbing paper (Figure 3) [20].

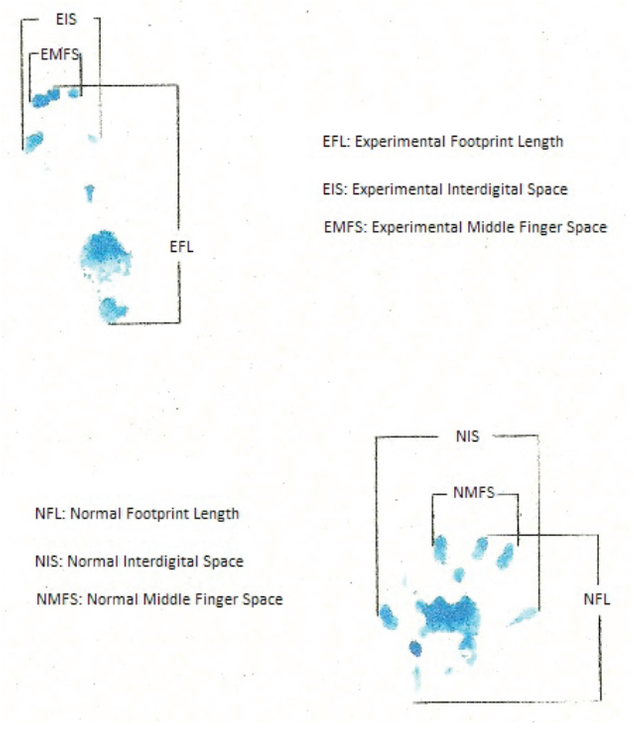

Figure 3. Standard measurements from the normal side and the side with sciatic nerve lesion [20].

- Footprint length (FL): Length from the heel to the 3rd finger
- Interdigital space (IS): Distance between the 1st and 5th fingers
- Middle finger space (MFS): Distance between the 2nd and 4th fingers

All these measurements were made from both the experimental side that underwent epineurotomy FL(EFL), epineurotomy IS (EIS) and epineurotomy MFS (EMFS) and the control side normal FL (NFL), normal IS (NIS) and normal MFS (NMFS). From these measurements, the footprint length factor (FLF), interdigital space factor (ISF) and middle finger space factor (MFSF) were calculated as shown below. These 3 factors were obtained by dividing the difference between the experimental and control side measurements by the control side values.

- Footprint Length Factor (FLF): (EFL – NFL) / NFL
- Interdigital Space Factor (ISF): (EIS – NIS) / NIS
- Middle Finger Space Factor (MFSF): (EMFS – NMFS) / NMFS

These factors were used to calculate the 'Sciatic Function Index' according to the formula described by Brown-Mackinnon-Evans et al. [28] in 1989.

Sciatic function index (SFI): – 38.3*FLF + 109.5*ISF + 13.3*MFSF – 8.8

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) (Chicago, IL) was used for statistical analysis. The changes in the epineural scar tissue index and the sciatic function index values were analyzed for each group using the Mann-Whitney U test and Kruskal-Wallis test. The $P<0.05$ value was considered statistically significant.

Results

I. Macroscopic Results

The sciatic nerves were uncovered by entering from the old incision line at Weeks 4 and 8 following the removal of skin sutures. The rats were assessed for wound site infections and splits, and suture reactions. Apart from a local wound infection in one rat in the group that was administered isotonic solution, there were no wound site splits or suture reactions in any of the rats. Then, the integrity of the skin and muscle fascias, the presence and appearance of a perineural scar tissue, and the severability of the sciatic nerve from the surrounding tissues were assessed macroscopically in line with the Peterson staging system (Table I) and noted. The macroscopic results are summarized in Table II.

I.A. Skin Closure

Week 4: The median Peterson staging value was found to be 1 (1-1) for the group that was administered isotonic solution(Group A), 1 (1-1) for the group that was administered a single dose of 5-FU (Group B) and 1 (1-1) for the group that was administered two doses of 5-FU (Group C). There was no statistically significant difference between the groups.

Week 8: The median Peterson staging value was found to be 1 (1-2) for the group that was administered isotonic solution (Group A), 1 (1-1) for the group that was administered a single dose of 5-FU (Group B) and 1 (1-1) for the group that was administered two doses of 5-FU (Group C). There was no statistically significant difference between the groups.

I.B. Muscle Fascia Closure

Week 4: The median Peterson staging value was found to be 1 (1-2) for the group that was administered isotonic solution (Group A), 1 (1-1) for the group that was administered a single dose of 5-FU (Group B) and 1 (1-2) for the group that was administered two doses of 5-FU (Group C). No statistically significant difference was found between the groups.

Week 8: The median Peterson staging value was found to be 1 (1-3) for the group that was administered isotonic solution (Group A), 1 (1-2) for the group that was administered a single dose of 5-FU (Group B) and 1 (1-2) for the group that was administered two doses of 5-FU (Group C). No statistically significant difference was found between the groups.

An example of the muscle fascia closure is shown in Figure 4.

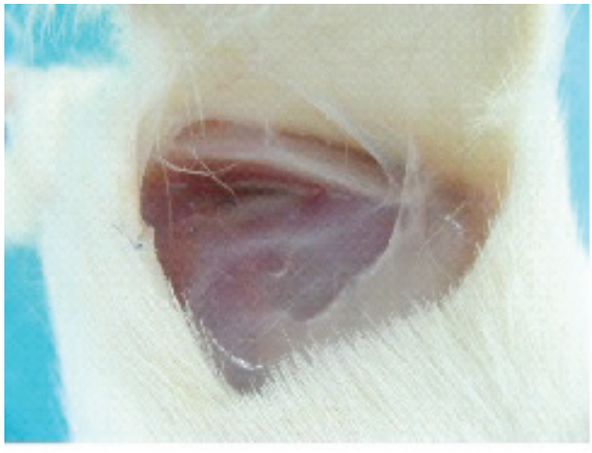

Figure 4. Fascia closure defect

I.C. Nerve Adhesion

Week 4: The median Peterson staging value was found to be 2 (1-3) for the group that was administered isotonic solution (Group A), 2 (1-2) for the group that was administered a single dose of 5-FU (Group B) and 1 (1-2) for the group that was administered two doses of 5-FU (Group C). When the groups were compared to each other, the only statistically significant difference was between the group that was administered two doses of 5-FU and the group that was administered isotonic solution (P=0.01). Although application of 5-FU in two doses produced a better result compared to its application in a single dose, the difference was not statistically significant.

Week 8: The median Peterson staging value was found to be 2 (1-3) for the group that was administered isotonic solution (Group A), 2 (1-2) for the group that was administered a single dose of 5-FU (Group B) and 1 (1-2) for the group that was administered two doses of 5-FU (Group C). When the groups were compared to each other, the only statistically significant difference was between the group that was administered two doses of 5-FU and the group that was administered isotonic solution (P=0.01). Although two doses of 5-FU application produced a better result compared to its single dose application, the difference was not statistically significant.

Examples for nerve adhesion are shown in Figure 5.

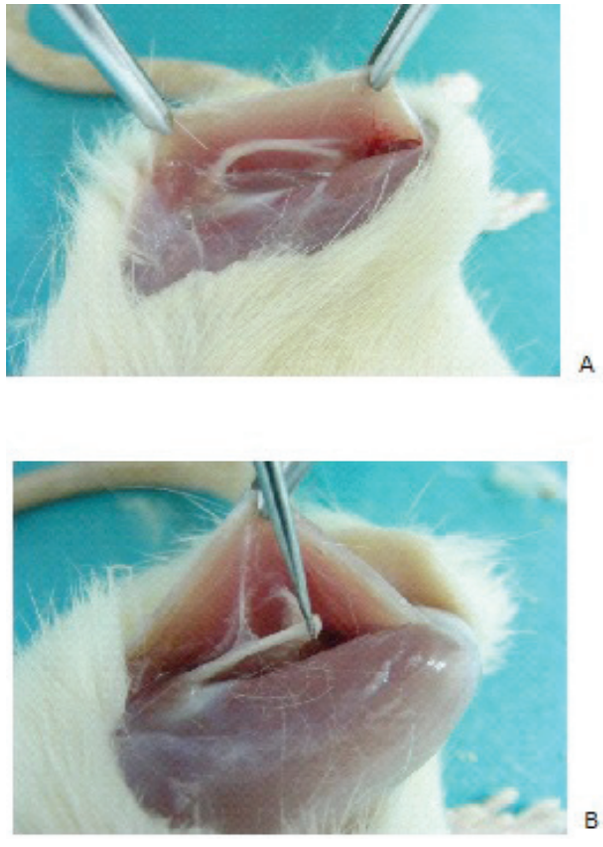

Figure 5. Nerve adhesion was done according to the Peterson staging system. Stage 1 (A) and Stage 2 (B).

I.D. Nerve severability

Week 4: The median Peterson staging value was found to be 2 (1-3) for the group that was administered isotonic solution (Group A), 2 (1-2) for the group that was administered a single dose of 5-FU (Group B) and 1 (1-2) for the group that was administered two doses of 5-FU (Group C). When the groups were compared to each other, the only statistically significant difference was due to the two doses of 5-FU application. Two doses of 5-FU application significantly changed the nerve severability compared to both isotonic solution application and single dose 5-FU application (P=0.014 and P=0.039, respectively).

Week 8: The median Peterson staging value was found to be 2.5 (2-3) for the group that was administered isotonic solution (Group A), 1.5 (1-2) for the group that was administered a single dose of 5-FU (Group B) and 1 (1-2) for the group that was administered two doses of 5-FU (Group C). When the groups were compared to each other, no statistically significant difference was found between Group B and Group C. However, significant differences were found between Group A and Group B, and between Group A and Group C, the difference being stronger with C (P=0.001 and P<0.001, respectively).

Examples for severability of nerve branches are shown in Figure 6. The macroscopic results are shown in Table II.

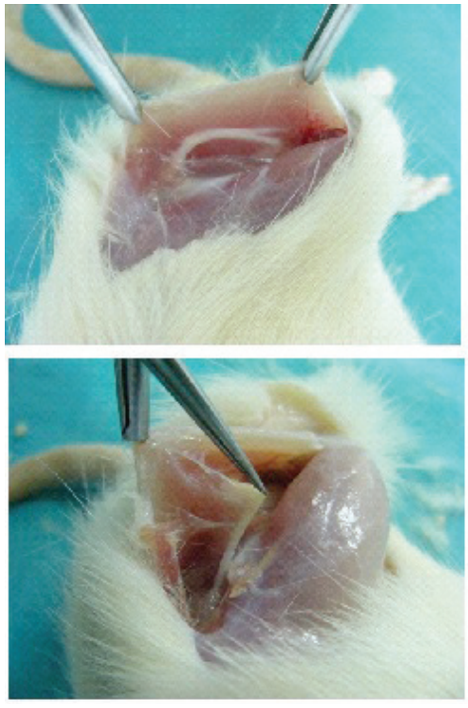

Figure 6. Severability of nerve branches was done according to the Peterson staging system. Stage 1 (A) and Stage 3 (B).

Table II. Macroscopic results

	Group A	Group B	Group C	*P*
Skin closure				
Week 4	1 (1-1)	1 (1-1)	1 (1-1)	> 0.05
Week 8	1 (1-2)	1 (1-1)	1 (1-1)	> 0.05
Muscle fascia closure				
Week 4	1 (1-2)	1 (1-1)	1 (1-2)	> 0.05
Week 8	1 (1-3)	1 (1-2)	1 (1-2)	> 0.05
Nerve adhesion				
Week 4	2 (1-3)	2 (1-2)	1 (1-2)	***0.017***
Week 8	2 (1-3)	2 (1-2)	1(1-2)	***<0.001***
Nerve severability				
Week 4	2 (1-3)	2 (1-2)	1 (1-2)	***0.008***
Week 8	2.5 (2-3)	1.5 (1-2)	1 (1-2)	***<0.001***

II. Histopathological Results

The formations of epineural scar tissue were assessed by the same pathologist with X40 and X100 amplitude light microscope without knowing to what group the nerves belonged. The average thicknesses of the epineural scar tissue and the nerve tissue were calculated from the measurements taken with an ocular micrometer (Periplan 6.3*M; Ernst Lietz GmbH, Wetzlar, Germany) from 6 different points for each specimen. The histopathological assessment showed that the collagen fibers were thicker in the group that was administered isotonic solution (Figure 7A) and the collagen fibers were thinner in the groups that were administered single and double doses of 5-FU (Figure 7B).

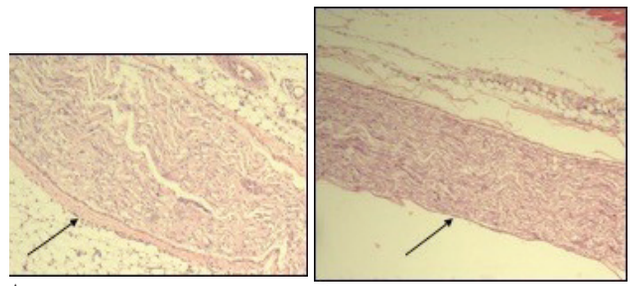

Figure 7. The scar tissue is thicker in the group that was administered isotonic solution (A) compared to the group that was administered 5-FU (B) (hematoxylene-eosine staining X4).

Week 4: The median epineural scar tissue index was 0.238 (0.11-0.38) for Group A (isotonic solution), 0.077 (0-0.17) for Group B (single dose 5-FU) and 0 (0-0.26) for Group C (two doses of 5-FU). There was no significant difference between the applications of two doses and a single dose of 5-FU. However, there was a strong statistically significant difference between the two groups that were administered 5-FU and the group that was administered isotonic solution (P<0.001).

Week 8: The median epineural scar tissue index was 0.203 (0-0.5) for Group A (isotonic solution), 0.093 (0-0.25) for Group B (single dose 5-FU) and 0 (0-0.23) for Group C (two doses of 5-FU). When the groups were compared to each other, the only statistically significant difference was seen to originate from the application of two doses of 5-FU. The two doses of 5-FU application was changing the histopathological results significantly in comparison to both isotonic solution application and single dose 5-FU application and the difference was statistically stronger with the isotonic solution group (P=0.003 and P=0.039) (Figure 8). The histopathological results are shown in Table III.

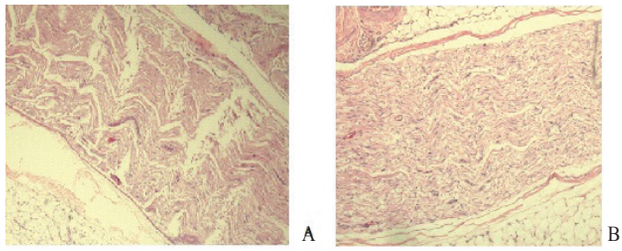

Figure 8. The thickness of the epineural scar tissue in the group that was administered double doses of 5-FU (A) is seen to diminish more compared to the group that was administered isotonic solution (B) (hematoxylene-eosine staining X4).

Table III. Summary of histopathological results

	Group A	Group B	Group C	*P*
Scar Tissue Index Week 4	0.0238 (0.11 – 0.38)	0.077 (0-0.17)	0.00 (0-0.26)	***<0.001***
Scar Tissue Index Week 8	0.20 (0-0.5)	0.0925 (0-0.25)	0.00 (0-0.23)	***0.005***

III. Functional Results

Week 4: The sciatic function index was found to be median – 25,5 ([-42,3] – [-10,7]) for the group that was administered isotonic solution (Group A), median – 9,1 ([-13,3] – [-3]) for the group that was administered a single dose of 5-FU (Group B) and median – 7,3 ([-13,4] – [-2,3]) for the group that was administered two doses of 5-FU (Group C). Although two doses of 5-FU application seemed to increase the capacity more compared to a single dose of 5-FU application, the difference was not statistically significant. However, there was a strong statistically significant difference between the two groups that were administered 5-FU and the group that was administered isotonic solution (P<0.001).

Week 8: The sciatic function index was found to be median – 20,2 ([-35,6] – [-10,6]) for the group that was administered isotonic solution (Group A), median – 7,9 ([-15,4] – [-4]) for the group that was administered a single dose of 5-FU (Group B) and median – 5,6 ([-13,6] – [-1,1]) for the group that was administered two doses of 5-FU (Group C). Although the two doses of 5-FU application seemed to increase the capacity more compared to a single dose of 5-FU application, the difference was not statistically significant. However, there was a strong statistically significant difference between the two groups that were administered 5-FU and the group that was administered isotonic solution (P<0.001).

Our sciatic nerve functional assessment results are summarized in Table IV.

Table IV. Sciatic nerve functional assessment results

	Group A	Group B	Group C	*P*
Sciatic function index Week 4	25.5 ([-42.3] – [-10.7])	-7.3 ([-13.4] – [-2.3])	-9.1 ([-13,3] – [-3])	***<0.001***
Sciatic function index Week 8	20.2 ([-35.6] – [-10.6])	-7.9 ([-15.4] – [-4])	-5.6 ([-13,6] – [-1.1])	***<0.001***

Discussion

As in many studies carried out on peripheral nerve surgery, we also preferred rat sciatic nerve in our study as it is agreed to be an appropriate model to create an entrapped neuropathy [7, 29-31]. The reasons include: 1) It resembles human nerve structure, 2) Ease of dissection due to its anatomic localization, 3) Ease of obtaining experimental rats as their life cycle is short and their fertility rate is high, 4) The size of the animals are big enough to recognize the tissue and small enough to manipulate, and 5) It follows a repair process similar to that of humans after an intervention. As a generally accepted surgical method to enhance the adhesion of the sciatic nerve to the surrounding tissues, a circular epineurotomy was used in our study in the 15-mm segment between the sciatic arch and sciatic bifurcation [18, 32].

The epineural scar tissue, which has a negative effect on the peripheral nerve surgery, is an undesirable situation that cannot be predicted before the surgery. Many surgical methods, pharmacologic agents and chemical substances have been tried to prevent formation of an epineural scar tissue, but although promising, the clinical outcomes have not been satisfactory. In their study, Özgenel et al., wrapped human amniotic membrane around the epineurectomized

rat sciatic nerves and injected hyaluronic acid [33]. They reported that sciatic nerve adhesion and perineural scar tissue development decreased noticeably compared to the control group. Dam-Hieu et al., have reported that the use of topical hyologlide gel reduced the development of postoperative perineural adhesion in rabbit sciatic nerves [34].

The pharmacological agents or chemical substances used for preventing perineural scar tissue formation are also desired to not delay wound recovery. Another pharmacologic agent that meets these criteria is 5-FU. Being an antimetabolite taking part in DNA synthesis, 5-FU inhibits proliferation of cells in their replication phase. Occleston et al., have found that use of a single dose of 5-FU or mitomycin-C (MMC) stops the proliferation of fibroblasts but does not affect their other functions involved in wound healing such as growth factors, expression of extracellular matrix molecules, and migration capacities [35]. Besides cancer treatment, 5-FU has been shown to decrease formation of scar tissue in peritendineous area and tendon capsule preventing adhesion [13-18]. Moreover, it has been found in the previous studies made on tendons in dog [36] and chicken [37] models that 5-FU reduces the peritendineous scar tissue by preventing type 1 and type 2 collagen formation and by suppressing TGF-B1. In their study on a rabbit laminectomy model, Yildiz et al., used topical MMC, 5-FU, and cyclosporine A to minimize development of spinal epidural fibrosis and found as a result that development of epidural and arachnoidal fibrosis diminished to a large extent [12]. Moran et al., have investigated in their study published in 2000 the effect of a single dose of 5-FU applied in various concentrations on the formation of a scar tissue and have shown that the 25 mg/kg concentration of 5-FU is the most effective dose for reducing the adhesion of flexor tendons [13]. Although many methods have been tried so far to prevent a scar tissue around a nerve, no other study where 5-FU was used has been published, which made us use the 25 mg/kg concentration of 5-FU in our study based on the study of Moran et al. [13]. Looking at the literature, we see that according to the in vitro study of Khaw et al. [16] and the in vivo study of Lanigan et al. [38], an application of 5-FU for 5 minutes stops the proliferation of fibroblasts up to 36 days. Another experimental study has shown in a cell culture medium that a 25 mg/kg concentration of 5-FU shortens fibroblast migration by as long as 48 hours [27]. The mean plasma half-life of 5-FU is between 10 and 20 minutes depending on the dose. For this reason, we also applied the gauze soaked in 5-FU for 5 minutes in our study. However, it was not possible to measure the 5-FU concentration remained in the epineurotomy region after removing the gauze.

The macroscopic, histopathological and functional assessments were made in Week 4 and Week 8 for each group. The macroscopic assessments made in both of these weeks showed that the connective tissue in the epineurotomy region was thinner and more transparent in the rats that were administered either a single or double dose of 5-FU compared to the rats that were administered isotonic solution. Furthermore, the adhesion of the sciatic nerve to the surrounding tissue and its severability (as per the Peterson staging) showed improvements with the use of 5-FU. In our study, better histopathological outcomes were obtained in Group B and C rats that were administered 5-FU compared to Group A rats that were administered isotonic solution. The perineural scar tissue index improved with the application of 5-FU. The application of 5-FU in two doses further increased the effectiveness.

We observed in our study that the positive effect of the 5-FU application on the macroscopic and histopathological recoveries continued further in the functional recoveries of the rats. Although the rats that were administered 5-FU showed significantly better functional improvement compared to the rats that were administered isotonic solution, the application of two doses of 5-FU did not produce an additional contribution. The reflection of the macroscopic and histopathological improvements in the functional improvements is an expected outcome, but the failure of the second dose of 5-FU application to produce an additional benefit in statistical terms is rather unexpected. Considering that the recovery of a nerve tissue requires a long process, a study investigating the results of applying a second dose of 5-FU later instead of after a week would clarify this issue. Although the results obtained in our study are promising, its being the first study assessing the effect of 5-FU on epineural scar tissue made us think that our results should be supported by further studies and the optimal application time of a second dose of 5-FU should be determined.

In conclusion, we have demonstrated that a topical application of 5-FU has positive effects on the development of a scar tissue around an epineurectomized nerve, and these positive effects are also reflected in the nerve's functional capacity, and a second dose of 5-FU application further improved this effect.

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