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Evaluation of the Effect of Gabapentin on Tendon Healing in an Experimental Rat Model

Seyran Kılınç[1], Hatice Reyhan Eğilmez[2], Özhan Pazarcı[1], Muhammed Yasir Altunışık[1]

[1]Department of Orthopedics and Traumatology, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Turkey
[2]Department of Pathology, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Turkey

ORCID iDs of the authors: S.K. 0000-0003-0144-0916; H.R.E. 0000-0001-9666-0246; Ö.P. 0000-0002-2345-0827; M.Y.A. 0000-0002-3080-8946.

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BACKGROUND/AIMS
To investigate the effect of gabapentin used in postoperative pain prophylaxis on tendon healing.

MATERIAL and METHODS
A total of 32 Wistar albino rats were randomly divided into 4 groups. Groups A and C were administered gabapentin by oral gavage, while Groups B and D were defined as the control groups. In all the rats, a transverse cut was made on the left Achilles tendon, approximately 0.5 cm proximal to the attachment point of the bone, then it was sutured using the Kessler method. Rats in Groups A and B were sacrificed on day 15 and those in Groups C and D on day 45. Differences between the groups were evaluated biomechanically using the tensile test, and immunohistochemically by examinations of collagen type I (COLIA), Proliferating Cell Nuclear Antigen (PCNA), and Transforming Growth Factor β1 (TGF-β1).

RESULTS
In the biomechanical evaluations, no significant difference was found between the study and control groups on days 15 and 45 in terms of the tensile test results (day 15, p=0.908; day 45, p=0.798). In the semi-quantitative comparisons of positive cell involvement in the immunohistochemical data evaluations, no statistically significant difference was also found. [TGF-β1, p(15)=0.328, p(45)=0.195; PCNA p(15)=0.645]. PCNA-positive cells were seen at a high rate in the first 15 days in both groups and the involvement of these cells was found to be similar on day 45.

CONCLUSION
In the immunohistochemical and biomechanical evaluations, gabapentin was not found to have any negative effect on tendon healing. It can be concluded that gabapentin can be used in cases with appropriate indications after tendon surgery. Nevertheless, there is a need for further studies in this area to investigate the mechanism of gabapentin's effect on the tendon.

Keywords: Gabapentin, tendon healing, experimental study

INTRODUCTION

Tendon health and function is very important for orthopedic surgeons. Tendon injuries can occur in some trauma patients, and for trauma surgeons, not only the tendon repair surgery is important, but also the functional treatment outcomes. Good functional treatment outcomes are obtained with early physical therapy (1). Early movement after the repair depends on the method used and the tendon healing (2). Postoperatively or during the physical therapy, several medications are administered to the patient as pain prophylaxis. The effects of conventional analgesics on tendon healing have been frequently discussed in the literature (3, 4). The use of gabapentin for pain prophylaxis after orthopedic surgeries is increasing. In the literature, it has been shown that gabapentin leads to a reduction in postoperative pain severity and total opioid consumption (5). It is often recommended for pain prophylaxis after surgeries such as rotator cuff repair, total knee arthroplasty, and total hip arthroplasty (5-9).

It has also been found to be effective in treating patients with chronic pain syndrome and diseases such as fibromyalgia, neuropathic pain, complex regional pain syndrome, trigeminal neuralgia, post-herpetic neuralgia, and neuropathic arthropathies (10).

Corresponding Author: Seyran Kılınç
E-mail: seyrankilinc@hotmail.com

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The reported positive effects of gabapentin in postoperative pain prophylaxis have led to the increased use of this drug. There are studies in literature regarding the effects of gabapentin on fracture and wound healing (11, 12). Gabapentin [1-(aminomethyl)cyclohexane acetic acid] is an anti-epileptic agent (13). Despite the analog structure of gabapentin γ-aminobutyric acid, its function is mediated through pre-synaptic P/Q type voltage-gated calcium channels (14).

Although gabapentin use against musculoskeletal pain is gradually increasing, its effect on tendon healing is unknown. To the best of our knowledge, there is no study in the literature that has evalauted the effect of gabapentin on tendons. Thus, this study is the first in the literature to evaluate the effects of gabapentin on tendon healing. The main purpose of this study was to assess the histological and mechanical effects of gabapentin on tendon healing in a rat model of Achilles tendon transection.

MATERIAL and METHODS

This study was approved by the Animal Research Local Ethics Committee (decision no:56, dated:15.08.2017). It was conducted according to the Guide for the Care and Use of Laboratory Animals. A total of 32 female Wistar albino rats, aged 10±1.2 weeks, each weighing 200–220 gr, were used in the study. The animals were obtained from the Animal Research laboratory and all through the experiment, all the care of the animals was undertaken in the same center. To ensure the adaptation to the new environment, all the animals were kept and fed in new cages in the laboratory, one week before starting the experiment. A total of 8 rats were housed in each cage in a manner that did not prevent movement. Fresh food and water were given daily.

Preparation of the Groups

The rats were randomly divided into 4 groups of 8. Groups A and C were administered gabapentin while Groups B and D as the control groups were not administered no drugs. The drugs were administered by the oral gavage route starting from 4 hours postoperatively. The daily gabapentin dose for Groups A and C was calculated based on the body surface area and was determined to be equal to the human dose of 1200 mg/day. To standardize the stress factors for the control group rats, 1% methylcellulose was administered by oral gavage at the same time as the treatment to the study group rats. The Groups A and B rats were administered ketamine anesthesia and sacrificed by cervical dislocation on postoperative day 15, and the same procedure was applied to the Groups C and D rats on postoperative day 45.

Main Points:

- To the best of our knowledge, no study in the literature has examined the effect of gabapentin on tendons.
- The fact that no difference was observed between the groups biomechanically demonstrates that gabapentin could be used in pain prophylaxis after tendon surgery, when there are suitable indications
- The immunohistochemical and biomechanical evaluations of this study showed that gabapentin does not have a negative effect on tendon healing.

Surgical Technique

Anesthesia consisting of a xylazine and ketamine HCl mixture was injected intramuscularly to all the rats by the same surgeon. A single dose of antibiotic (cefazolin sodium 5mg) prophylaxis was given. The left lower extremity was prepared with povidone-iodine solution and a sterile drape. A longitudinal 3-cm skin and subcutaneous incision was made from the midline along the course of the Achilles tendon. The Achilles and plantaris tendons were stripped from the surrounding fascia. A full-layer transverse cut was made, approximately 0.5 cm proximal from the attachment site of the Achilles tendon to the calcaneus. The plantaris tendon was also included in the cut. Primary repair of the sectioned tendons was performed with the modified Kessler method using 6-0 Ethilon monofilament nylon sutures (Ethicon, USA) (Figure 1). The skin and subcutaneous layers were sutured with 3-0 Ethilon monofilament nylon sutures. No fixation was used postoperatively. All through the experiment, standard care was applied to all the animals, and they were permitted to move freely within their cages.

Biomechanical Evaluation

The biomechanical differences between the study groups in terms of the breaking strengths of the tendons were investigated in the Biomechanics Laboratory of the Dentistry Faculty of our university. From each group, 8 calcaneus-Achilles complex samples were placed on a Lloyd LF Plus model device (Ametek Inc, Lloyd Instruments, Leicester, UK) using a specially designed holder (Figure 2). The normal tendons were evaluated using the non-operated right-side Achilles tendon complex. Tensile force was applied at 5N/sec. Before starting the test, calibration was performed for the loading and extension amounts of all the Achilles tendon com-

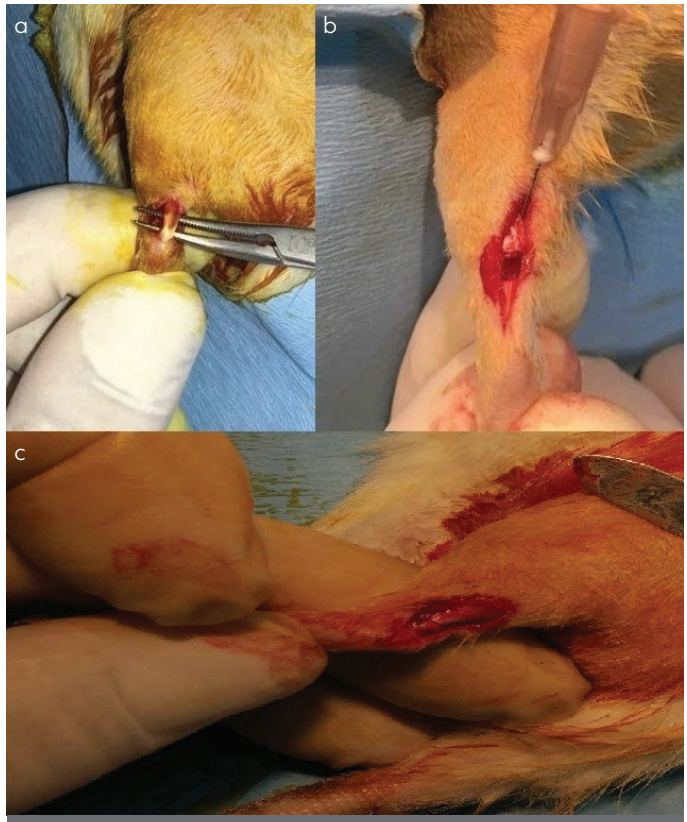

FIGURE 1. a-c. Photograph of the Achilles tendon a) before transection, b) after transection, and c) after surgical repair

FIGURE 2. Specimen during the mechanical test

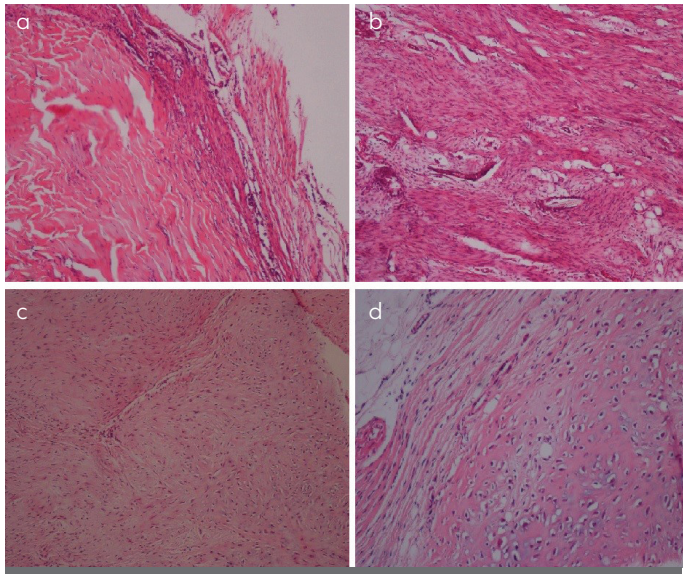

FIGURE 3. a-d. a) Group A collagenized areas, b) Group B, c) Group C, d) Group D chondrocytes-like cell (H & E, ×20)

plexes. The extension amount of the tendons was measured via a computer attached to the device. All the loading and deformation tests were applied and recorded using the Lloyd Instruments Data Analysis Package system. For pre-tensile equalization, a 2N pre-loading was applied to all the tendons.

Histological Evaluation

The samples obtained from the experimental animals were examined in the Pathology Department laboratory. Tissue samples were fixed in 10% formaldehyde for 24 hours and then subjected to routine tissue processing.

As per routine histological procedures, the tissues were embedded in paraffin blocks. Slices of 2.5 micron thickness were obtained with a Leica microtome. The slices to undergo immunohistochemical staining were placed on positive loaded slides and those to undergo hematoxylin-eosin (HE) staining on normal slides.

As per the routine processes, the HE and immunohistochemical staining processes were completed [COLIA (Santa Cruz Biotechnology, Concentrate,1/100 dilution, monoclonal, CLone COL-I,LOT NO :E0918), TGFβI (Biogenex, Santa Cruz Biotechnology, Concentrate 1/100 dilution, monoclonal, CLone 3CII,LOT NO: E0918), and PCNA (Scytek, Concentrate 1/100 dilution, monoclonal, Clone PCI0,LOT NO: 47438)]. The stained preparates were examined microscopically. These processes were performed in the same manner on day 15 and 45 of the study and control groups.

Statistical Analysis

Statistical analysis of the data obtained in the study were performed using SPSS vs 22.0 software (IBM SPSS Corp.; Armonk, NY, USA). Since the number of data in the groups was <30, non-parametric tests were applied. Differences between the study and control groups were evaluated using the Mann Whitney U-test. The Wilcoxon Rank test was applied to determine differences within the same group at different time points. Results were examined at a 95% confidence interval. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Histological Results

Subjective microscopic evaluations of the groups were performed with HE staining. A similarity was found in terms of neovascularization, fibroblasts, and the amount of connective tissue in the endotendineum (Figure 3). Type IA collagen (COLIA), proliferative cell nuclear antigen (PCNA), and transforming growth factor βI (TGFβI) were evaluated semi-quantitatively with immune histochemical staining. The samples were numbered and percentage slices were scored for PCNA, TGFβI, and COL-IA, according to the immunohistochemical involvement of the cells under a microscope at ×10 magnification by a pathologist blinded to the groups (Table I). Positive cell involvement was recorded. In the statistical evaluations of the positive cells, similar results were obtained in the study and control groups at 15 and 45 days (TGFβI, p(15)=0.328, p(45)=0.195. PCNA, p(15)=0.645).

In terms of PCNA, the absence of cell involvement in the samples obtained on day 45 was similar to the samples obtained from the healthy tissues, and this was interpreted as the completion of the healing process. COL-IA in all the groups showed similar properties, and no significant differences were determined (Figure 4).

Biomechanical Results

During the biomechanical examination, the greatest loading forces (Newton) were found on the calcaneus-Achilles complex. While breakage in Groups A and B was close to the area of the tendon injury, it occurred with stripping away in the form of avulsion from the calcaneus attachment point in Groups C and D.

TABLE I. Immunohistochemical results and statistics

		Minimum	Maximum	Mean	Standard Deviation	P value*	P value**
TGF-βI	Group A	25,00	75,00	40,63	22,90	0,261	0,328
	Group B	25,00	100,00	56,25	32,04		
	Group C	25,00	100,00	65,63	26,52		0,195
	Group D	25,00	75,00	46,88	20,86		
PNCA	Group A	75,00	100,00	84,38	12,94	<0,001	0,645
	Group B	50,00	100,00	78,13	20,86		
	Group C	0,00	0,00	0,00	0,00		
	Group D	0,00	0,00	0,00	0,00		
COL-IA	Group A	80,00	100,00	92,50	8,90	0,731	
	Group B	90,00	100,00	95,00	5,30		
	Group C	80,00	100,00	95,00	7,66		
	Group D	80,00	100,00	91,30	8,30		

*Significance level p≤0.05
**Comparison between the groups on days 15 and 45

TABLE 2. Biomechanical test data and statistical results (N)

		Minimum	Maximum	Mean	Standard Deviation	P value
Day 15	Group A	34,68	51,07	41,69	6,05	0,908
	Group B	23,37	53,18	41,19	10,64	
Day 45	Group C	23,54	53,28	35,83	9,60	0,798
	Group D	20,42	57,32	36,98	11,49	

Significance level p≤0.05

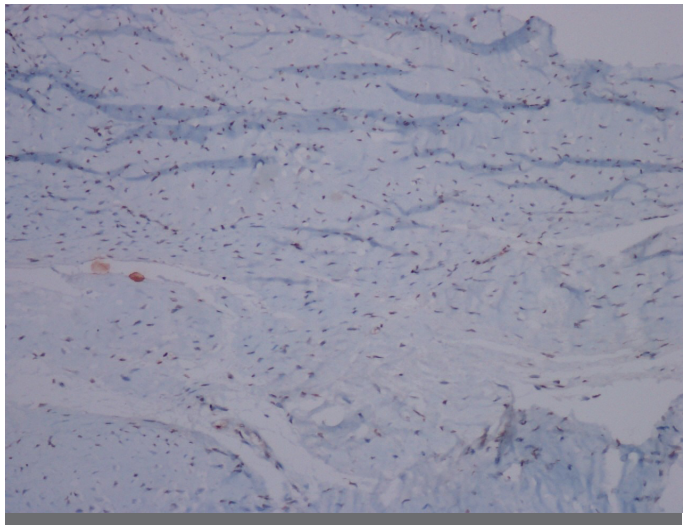

FIGURE 4. 80% staining of the tendon nuclei (COLIA, ×20)

The mean tensile resistance values were determined as 41.69±6.05 N for Group A, 41.19±10.64 N for Group B, 35.83±9.60 N for Group C, and 36.98±11.49 N for Group D. The statistical analyses of the biomechanical tests applied on days 15 and 45 showed no statistically significant difference between the study and control groups in terms of tensile resistance (Table 2). [p(15)=0,908 and p(45)=0,798].

DISCUSSION

This results of this study showed that gabapentin does not have a negative effect on tendon healing. To the best of our knowledge, our study is the first study in the literature to examine the effect of gabapentin on tendon healing. Tendon health and function is very important for orthopedic surgeons. Several local and systemic factors affect the tendon healing process (1). Therefore, the orthopedic surgeons must know whether the drugs used for treatment affect the tendon healing process.

This study aimed to determine the effect of gabapentin, which is used in postoperative pain prophylaxis after orthopedic surgeries, on tendon biomechanics and the tendon healing process. Tendon healing during acute injuries is a lengthy process because of the properties specific to its connective tissue. Weakness of the vascular structure and the presence of cells at a low metabolic rate provide limited contributions to tendon healing and the regeneration potential. The healing process occurs with the simultaneous appropriate development of regenerative and scar tissues (15). The fibrous scar tissue formed causes mechanical and functional weakening of the tendon structure. Acceleration of the healing process with the actual tendon regeneration tissue is one of the desired aims (2). A soft tissue healing process is observed following all orthopedic surgical approaches, not only in acute tendon injuries. For example, total knee and hip arthroplasty surgeries aim to provide very good tendon, ligament, and soft tissue balance. The use of gabapentin is recommended after these surgeries (8). In this context, the current study ex-

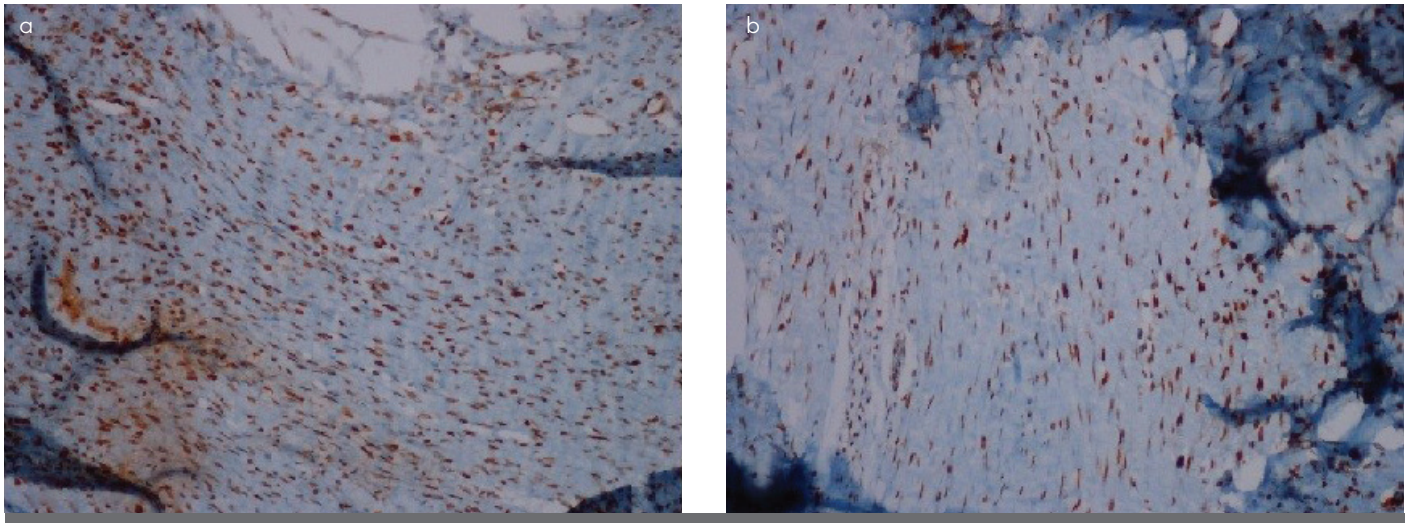

FIGURE 5. a, b. a) Group A nuclear 100% positive, b) Group B nuclear 80% positive (PCNA, X20)

amining the effect of gabapentin on tendon healing provides a contribution to the scarce literature in this field.

The mechanism of the effect of gabapentin has not yet been fully understood. However, reducing glutamate release by preventing calcium flow in the nociceptive pathways reduces pain transmission and sensitivity (14). Part of the effect is seen in voltage-gated calcium channels (16), which are located in tendons (17). There is a need for further studies in this area to examine the mechanism of the effect of gabapentin on tendons. In this respect, the current study can be considered as being of value in providing a viewpoint on this.

Although gabapentin is used in several clinical indications, it is used especially for postoperative and chronic pain control and to effectively reduce opioid consumption (18). There are studies in the literature related to gabapentin and pain prophylaxis, wound healing, and fracture healing, but its contribution to tendon healing has not yet been evaluated previously. The results of the current study showed that there was no significant difference biomechanically between the groups administered with gabapentin and the control groups (Table 2). The fact that no difference was observed between the groups biomechanically demonstrates that gabapentin could be used in pain prophylaxis with suitable indications after tendon surgery. Similar to the current study, there are studies in the literature that have recommended gabapentin in pain prophylaxis following tendon repair such as the rotator cuff repair surgery (7).

In the samples obtained on day 15 in the current study, PCNA-positive cells were observed to be dense around the epitenon; in other words, to have been intensified in the tendon periphery (Figure 5). The absence of PCNA-positive cells in all the groups on day 45 was interpreted as that the cellular regeneration had been completed (Figure 6). This was proven by the similar effect seen in the control samples obtained from the intact extremity. PCNA plays an important role in nucleic acid metabolism during the replication and repair process. PCNA interacts with the proteins necessary for the controlled cell cycle (19). In the early stages of the tendon repair process, it has been shown that the number of PCNA-positive cells increases. It is thought that these cells are undifferentiated mesenchymal stem cells that migrate from the paratenon toward the tendon healing area approximately 1 week after injury (20).

TGF-β1 plays a part in the tendon healing process associated with many different cytokines. It is known that TGF-β1 plays a role in collagen production and angiogenesis (21, 22). TGF-β1 expression has been shown to vary greatly at different times in tendon healing (20). This status in contrast to PCNA was seen in the immunohistochemical evaluations on day 45 of the current study. Similar results were obtained in terms of the rates of PCNA and TGF-β1-positive cells in the comparisons of the study and control groups on days 15 and 45. This was interpreted as gabapentin not having any negative effect on the tendon healing process.

This study has some limitations. Primarily, the histological evaluations were conducted semi-quantitatively. However, the microscopic examination was made by a pathologist blinded to the groups. Secondly, since the study was conducted on healthy animals, it was difficult to conclude that the same effects would be observed in humans.

In conclusion, the immunohistochemical and biomechanical evaluations of this study showed that gabapentin does not have a negative effect on tendon healing. Therefore, gabapentin can be used in cases with the appropriate indications after tendon surgery. Nevertheless, there is a need for further studies in this area to examine the mechanism of the effect of gabapentin on the tendon.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Cumhuriyet University Animal Experiments (56/15.08.2017).

Informed Consent: The study was conducted according to the Guide for the Care and Use of Laboratory Animals.

Peer-review: Externally peer-reviewed.

Author contributions: Concept - S.K.; Design - S.K., H.R.E.; Supervision - Ö.P., M.Y.A.; Resource - S.K., H.R.E.; Materials - S.K., Ö.P.; Data Collection and/or Processing - S.K., H.R.E., M.Y.A.; Analysis and/or Interpretation - Ö.P., M.Y.A.; Literature Search - S.K., H.R.E., Ö.P.; Writing - S.K., Ö.P.; Critical Reviews - H.R.E., M.Y.A.

Conflict of Interest: Authors have no conflicts of interest to declare.

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