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ORIGINAL ARTICLE

The effect of papaverine on tendon healing and adhesion in rats following Achilles tendon repair

Erdem Can, MD[1], Yasar Mahsut Dincel, MD[2], Derya Karabulut, MD[3], Sevil Karabag, MD[4], Yunus Ziya Arslan, MD[5]

[1]Department of Orthopedics and Traumatology, Ercis Şehit Rıdvan Çevik State Hospital, Van, Türkiye
[2]Department of Orthopedics and Traumatology, Tekirdağ Namık Kemal University Faculty of Medicine, Tekirdağ, Türkiye
[3]Department of Mechanical Engineering, Istanbul University - Cerrahpaşa Faculty of Engineering, Istanbul, Türkiye
[4]Department of Pathology, Tekirdağ Namık Kemal University Faculty of Medicine, Tekirdağ, Türkiye
[5]Department of Robotics and Intelligent Systems, Turkish - German University Institute of Graduate Studies In Science and Engineering, Istanbul, Türkiye

The Achilles tendon, recognized as the strongest tendon in the human body, plays a pivotal role in transferring the forces generated by the gastrocnemius and soleus muscles to the calcaneus. This complex mechanism is essential for fundamental activities such as walking, jumping, and running.[1,2] Notably, the incidence of acute tendon injuries tends to rise within the age range of 30 to 40 years, predominantly affecting male individuals. These injuries are frequently linked to recreational sports, such as ball games and racket sports, that involve rapid acceleration and jumping.[3,4]

Achilles tendon ruptures are characterized by prolonged recovery periods and a range of

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Correspondence: Erdem Can, MD. Erciş Şehit Rıdvan Çevik Devlet Hastanesi, Ortopedi ve Travmatoloji Kliniği, 65400 Erciş, Van, Türkiye.

E-mail: erdemcan.md@gmail.com

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ABSTRACT

Objectives: The study aimed to examine the histopathological and biomechanical effects of papaverine administered intraperitoneally and locally on Achilles tendon healing in a rat model.

Materials and methods: Forty-eight adult male Sprague-Dawley rats (range, 300 to 400 g) were used in this study conducted between October and November 2022. The rats were divided into three groups, with each group further subdivided into two for sacrifice on either the 15th (early period) or 30th (late period) day after surgery. The first (control) group received no treatment following Achilles tendon repair, while papaverine was intraperitoneally administered every other day for 10 days in the second group and locally in the third group after surgery. On the 15th and 30th days, the rats were sacrificed, and their Achilles tendons were subjected to biomechanical testing and histopathological evaluation.

Results: Histopathologically, there were no significant differences among the groups on the 15th day. However, on the 30th day, the locally applied papaverine group exhibited superior histopathological outcomes compared to the control group ($p<0.05$). Concerning the highest tensile strength values before rupture, the biomechanical assessment showed that the group receiving local papaverine treatment in the early period and both the group with systemic papaverine treatment and the one with local papaverine treatment in the late period displayed a statistically significant advantage compared to the control group ($p<0.05$).

Conclusion: Locally administered papaverine has positive biomechanical effects in the early period and exhibits a positive correlation both histopathologically and biomechanically in the late period. Novel therapeutic options may be provided for patients through these findings.

Keywords: Achilles tendon, healing, papaverine, rat, tendon repair.

variable clinical outcomes, often accompanied by complications. A potential contributing factor to both tears and impaired regenerative capacity is reduced blood perfusion within the Achilles tendon. The limited blood circulation during the recovery phase, particularly in the context of immobilized limbs, such as those involving the Achilles tendon, may partly explain these occurrences.[5] Reconstructive surgeons commonly resort to pharmacological vasodilator administration, with nearly 94% of microvascular surgeons employing some form of topical agent for this purpose.[6] Papaverine, the most commonly used vasodilator, stands out for its substantial potency, leading to an approximate 60% increase in vessel diameter upon administration.[7]

Papaverine, an opioid analog possessing myorelaxant and vasodilator effects on vascular smooth muscles, finds application in treating ischemia caused by spasms in the heart, brain, and peripheral blood vessels.[8] While a study reported that the aerosol form of papaverine accelerates wound healing and stimulates pluripotent CD34 cells,[9] another study demonstrated its application in a cream form on seven skin flaps, resulting in increased flap survival.[10] However, no prior research has explored the influence of papaverine on tendon healing.

In our study, we aimed to evaluate the effect of papaverine on the healing process, hypothesizing that its vasodilatory effect can enhance microcirculation in the repair area, and intended to validate this hypothesis by conducting histopathological and biomechanical evaluations.

MATERIALS AND METHODS

A total of 48 adult male Sprague-Dawley rats (range, 300 to 400 g) were used in this study conducted between October and November 2022. To investigate the effects of papaverine on Achilles tendon repair, the rats were initially categorized into three groups based on the drug administration method. They were then further divided into subgroups for sacrifice either on the 15th or 30th day. In Group 1, the rats did not receive any medication after Achilles tendon repair. In Group 2, the rats were administered 100 mg/kg of papaverine perioperatively and 60 mg/kg/day of papaverine (Papaverin HCl; Galen İlaç, Istanbul, Türkiye) intraperitoneally every other day for the subsequent nine days. In Group 3, the rats were applied 1 mL/30 mg of papaverine locally to the surgical site after the repair. Within each group, further divisions were made for the rats to be sacrificed on either the 15th day (designated as Groups 1a, 2a, and 3a) or the 30th day (designated as Groups 1b, 2b, and 3b). From each subgroup, four Achilles tendons were separated for histopathological examination, and an additional four were reserved for biomechanical assessment.

All groups received antibiotic prophylaxis, consisting of 8 mg/kg of gentamicin (Genthaver; Osel, Istanbul, Türkiye) administered subcutaneously 30 min before the surgery. Pain management was achieved by administering 5 mg/kg of carprofen (Rimadyl; Zoetis Inc., Parsippany, NJ, USA) every 12 h for the initial 24 h following surgery. Surgical procedures and wound dressing were conducted under general anesthesia, initiated with 4% isoflurane (Isoflurane USP; Adeka İlaç, Istanbul, Türkiye) as an inhalation anesthetic for induction, followed by maintenance at a dose of 2%.

A 2-cm longitudinal incision was made along the Achilles tendon, and the cutaneous and subcutaneous layers were dissected. Upon reaching the Achilles tendon, it was carefully separated from the surrounding tissues, including the plantaris tendon, which was preserved for its internal splinting effect. The Achilles tendon was cut transversely, using a size 11 scalpel (Plasmed, Istanbul, Türkiye), approximately 0.5 cm proximal to its insertion on the calcaneus and meticulously repaired using the modified Kessler method, employing a 3/0 atraumatic polypropylene suture (Nevolene; Betatech, Istanbul, Türkiye). Subsequently, the incision in the skin was properly closed with a 3/0 sharp propylene suture (Nevolene; Betatech, Istanbul, Türkiye) and dressed with povidone-iodine. While the control group did not receive papaverine, Groups 3a and 3b received a local application of 30 mg (1 mL) of papaverine solution before the closure of the skin.[11] Groups 2a and 2b received an initial dose of 100 mg/kg of papaverine intraperitoneally following skin closure, followed by doses of 60 mg/kg given every other day for the ensuing nine days.[12,13]

No immobilization was imposed on the rats after surgery. For the first 24 h, they were individually housed in single cages and closely monitored. Afterward, the groups were regrouped into their original cages, each containing eight rats. The general health and condition of the rats, as well as the condition of their surgical wounds, were monitored daily.

On the 15th and 30th days, the rats were sacrificed through cervical dislocation while under general anesthesia. The right Achilles tendons of all groups

were carefully isolated from surrounding tissues, including the proximal femur attachment site and the distal calcaneus. To prevent confusion, the samples were numbered. The first four samples from each group were placed in containers filled with 10% formaldehyde for subsequent histopathological examination. Additionally, the untreated Achilles tendons of rats in Groups 1a and 1b, where no drug interventions occurred on the 15th and 30th days, were collected as the sham group and also preserved in 10% formaldehyde. All samples were promptly sent to the Department of Medical Pathology at Tekirdağ Namık Kemal University on the day of collection.

For biomechanical examination, the first four samples from each group were wrapped in gauze soaked with 0.9% saline, grouped into containers, and frozen at –20°C. These samples were then transferred to the Machinery Materials Laboratory at the Faculty of Engineering of Istanbul University-Cerrahpaşa, where the relevant analyses were conducted.

Histopathological evaluation

Tendon specimens designated for histopathological analysis were fixed in 20 mL of 10% formaldehyde for 24 h and subsequently placed into cassettes in the macroscopy unit, encompassing the tendon-muscle junction at the proximal end and the muscle-bone junction at the distal end. These samples underwent a process of dehydration within closed tissue tracking devices, progressing through alcohol, xylene, and paraffin stages, followed by paraffin blocking. Subsequently, 4-µm sections were extracted from each sample, and every section was subjected to staining with hematoxylin and eosin. All sections were closed using a fully automatic closing device. A pathologist assessed the results in a blinded manner using an Olympus CX41 microscope (Olympus, Tokyo, Japan).

A semiquantitative scoring system was employed, incorporating scores derived from the scoring systems of Svensson et al.,[14] Cook et al.,[15] and Soslowsky et al.[16] This system encompassed four distinct parameters: *(i)* fiber structure, *(ii)* cellularity, *(iii)* vascularity, and *(iv)* cartilage formation. The samples were scored for the presence of any significant abnormal appearance, with each parameter receiving a score on a 4-point scale ranging from 0 (normal) to 3 (markedly abnormal; Table I).[17] The total score was calculated as the sum of individual parameter values, yielding a range from 0 (normal tendon) to 12 (most severe abnormality). Adhesion on the 30th day was histopathologically evaluated following the criteria established by Tang et al.,[18] which categorized adhesion into six levels: 0 for no adhesion, 1-2 for slight adhesion, 3-4 for moderate adhesion, and 5-6 for severe adhesion.

Biomechanical evaluation

The biomechanical assessment involved attaching the specimens on sandpaper and interposing it between the probes of a tensile device (Instron 5892; Instron, Norwood, MA, USA), securing the Achilles origin proximally and the calcaneus distally. Tendons were then subjected to tension at a rate of 6 mm/sec along their longitudinal axis until rupture occurred (Figure 1). The maximum tensile strength reached by the tendons before rupture was computed in MPa for each group and subjected to comparison. The elasticity modulus was calculated by measuring tendon lengths using precision calipers ($E=\sigma/\varepsilon$).

Statistical analyses

Statistical analyses were conducted using IBM SPSS version 25.0 software (IBM Corp., Armonk, NY, USA) for histopathological assessment. The normal distribution of all data was evaluated with the Shapiro-Wilk test. Histopathological healing scores across groups were compared utilizing

TABLE I
Modified Soslowsky, Svensson and Cook scoring system

Parameter	Grade 0	Grade 1	Grade 2	Grade 3
Fiber structure (Soslowsky and Svensson)	Normally parallel collagen fibers	Slight changes (less than 25% individual fibers)	Moderate changes (25-50% separated and distorted fibers)	Marked changes (>50% disorganized and hyalinized fibers)
Cellularity (Cook)	Elongated nuclei and absence of cytosol	Oval nuclei and absence of cytosol	Round nuclei and small cytosol	Round nucleus and abundant cytosol
Vascularity (Svensson)	Few veins, parallel to fibers	Slight increase in veins	Moderate increase in veins	Significant increase in veins
Cartilage formation	No cartilage	Isolated cartilage nodules	Moderate cartilage formation (25-50%)	Extensive cartilage formation (>50%)

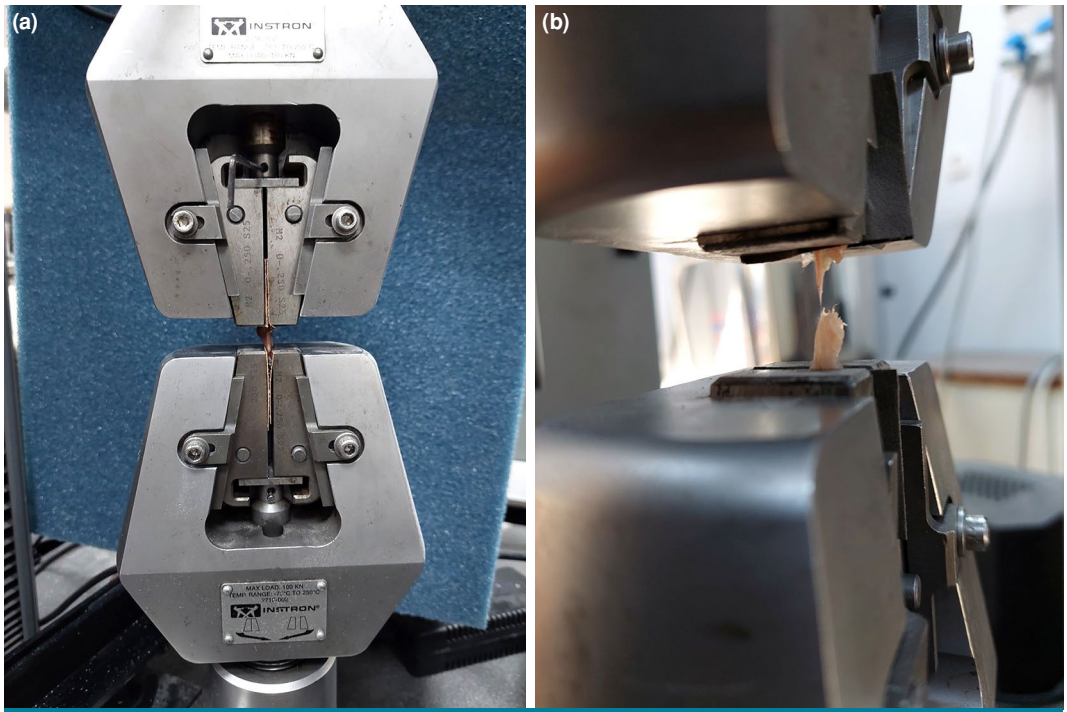

FIGURE 1. (a) Specimens attached on sandpaper are interposed between the tensile device probes. **(b)** Image of the torn tendon.

the Kruskal-Wallis H test, a non-parametric equivalent of one-way analysis of variance, while intergroup comparisons were performed with the Mann-Whitney U test.

The IBM SPSS version 21.0 software (IBM Corp., Armonk, NY, USA) was used for evaluating the average tensile breaking forces in each group. The statistical significance level was set at $p<0.05$. The Shapiro-Wilk test revealed that data normalization was not achieved ($p<0.05$), leading to the adoption of the nonparametric Kruskal-Wallis method for statistical data analysis in intergroup comparisons. Differences between the groups were assessed using the Mann-Whitney U post hoc test.

RESULTS

Histopathological findings

Histopathological assessments were conducted for samples collected on the 15th and 30th days. Upon analyzing the early-period results, no statistically significant differences were observed between the control group and the groups that received systemic and local papaverine (Table II).

TABLE II
Statistical analysis among the groups according to the modified Soslowsky, Svensson, and Cook classification on the 15th day

	Control (1a)		Systemic (2a)		Local (3a)		
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median	*p**
Fiber structure	1.25±0.5	1	1.75±0.5	2	1.75±0.5	2	0.28
Cellularity	0.5±0.57	0.5	0.5±0.57	0.5	0.75±0.5	1	0.73
Vascularity	0.25±0.5	0	0.25±0.5	0	0.25±0.5	0	1
Cartilage	0.5±0.57	0.5	1±0.81	1	0±0.0	0	0.10
Total	2.5±1.2	2.5	3.5±0.57	3.5	2.75±0.5	3	0.25

SD: Standard deviation; * Kruskal Wallis (Mann-Whitney U test) ($p<0.05$).

TABLE III
Statistical analysis among the groups according to the modified Soslowsky, Svensson, and Cook classification on the 30th day

	Control (1a)		Systemic (2a)		Local (3a)		
	Mean±SD	Median	Mean±SD	Median	Mean±SD	Median	*p**
Fiber structure	0.5±0.57	0.5	1.25±0.5	1	0.75 ±0.5	1	0.49
Cellularity	1±0.0	1	0.75±0.5	1	0.25 ±0.5	0	0.040
Vascularity	0±0.0	0	0±0.00	0	0±0.00	0	1
Cartilage	2 ±0.0	2	0.75 ±0.5	1	0.75±0.95	0.5	0.046
Total	3.5±0.57	3.5	2.75±1.25	3	1.75±0.95	1.5	0.036

SD: Standard deviation; * Kruskal Wallis (Mann-Whitney U test) (p<0.05).

Upon examination of the late-period results, a significant advantage was demonstrated in the 30th-day samples for the local papaverine group compared to the control group. As shown in Table III, this superiority was evident in terms of cellularity, cartilage formation, and the total modified Soslowsky, Svensson, and Cook scores (p<0.05). Based on Tang et al.'s[18] criteria, there were no significant differences among the groups regarding adhesion.

Biomechanical findings

When comparing the results on the 15th day, statistically significant differences were found between Group 1a (control) and Group 3a (local papaverine), as well as between Group 2a (systemic papaverine) and Group 3a (p<0.05). However, no statistical difference was observed between Group 1a and Group 2a. Upon examining the results on the 30th day, statistically significant differences were identified between Group 1b and Group 2b, Group 1b (control) and Group 3b, and Group 2b and Group 3b (p<0.05, Figure 2).

In terms of tensile strength, when analyzing the results on the 15th day, statistically significant differences were found between Group 1a and Group 2a, as well as between Group 1a and Group 3a (p<0.05). However, no statistical difference was noted between Group 2a and Group 3a. Upon examining the results on the 30th day, a statistically significant difference was observed solely between Group 1b and Group 3b (p<0.05). No statistical difference was identified between Group 1b and Group 2b nor between Group 2b and Group 3b (Figure 3).

Regarding the elasticity modulus, significant statistical differences were observed when comparing the results from the 15th day. Specifically, differences

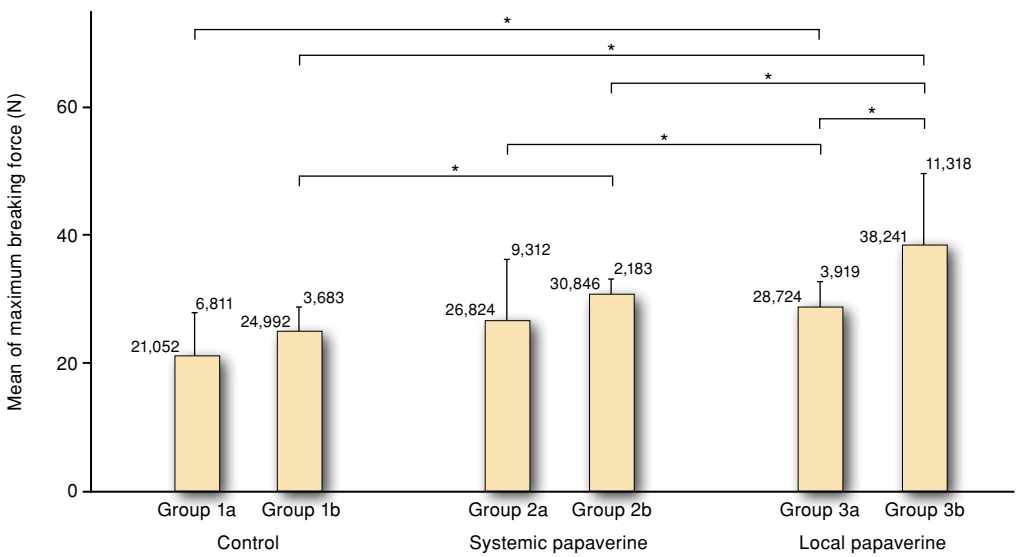

FIGURE 2. Mean maximum tensile breaking force measured in the groups. Group 1a (Day 15), Group 1b (Day 30), Group 2a (Day 15), Group 2b (Day 30), Group 3a (Day 15), Group 3b (Day 30) (*p<0.05).

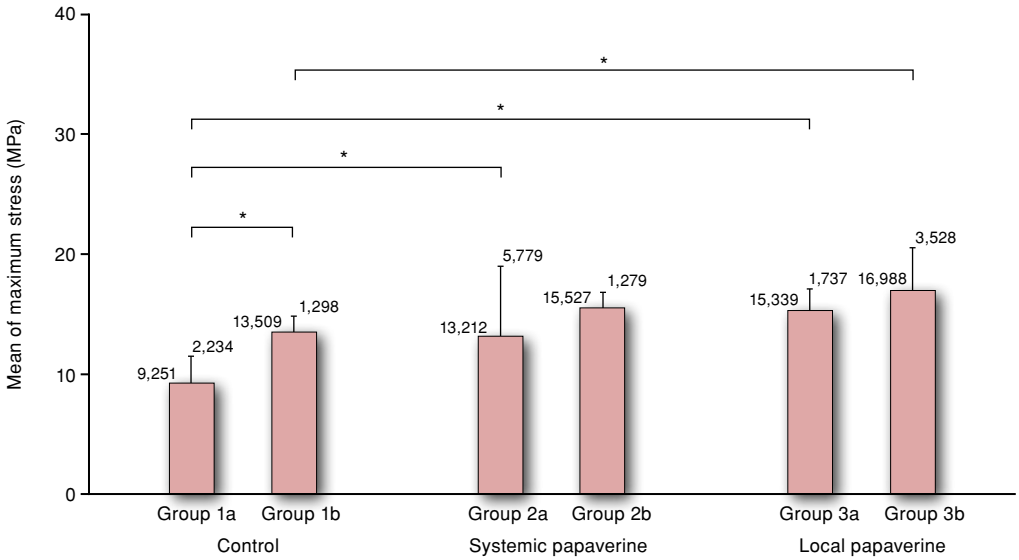

FIGURE 3. Mean maximum tensile strengths measured in the groups. Group 1a (Day 15), Group 1b (Day 30), Group 2a (Day 15), Group 2b (Day 30), Group 3a (Day 15), Group 3b (Day 30) (*p<0.05).

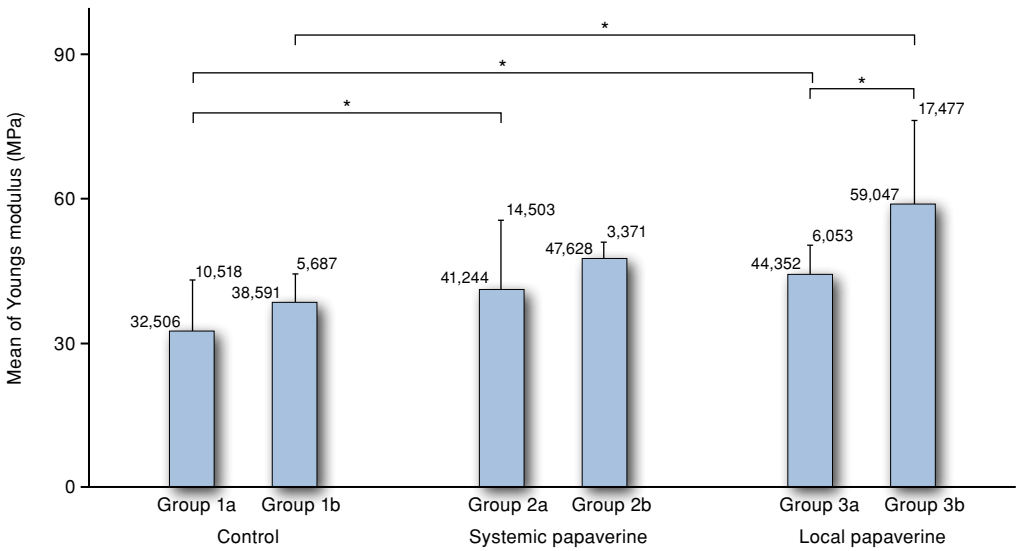

FIGURE 4. Mean elasticity modulus measured in the groups. Group 1a (Day 15), Group 1b (Day 30), Group 2a (Day 15), Group 2b (Day 30), Group 3a (Day 15), Group 3b (Day 30) (*p<0.05).

were noted between Group 1a and Group 2a, as well as between Group 1a and Group 3a ($p<0.05$). However, there was no statistically significant distinction between Group 2a and Group 3a. Upon examining the results on the 30th day, a significant statistical difference was found only between Group 1b and Group 3b ($p<0.05$). No statistical differences were detected between Group 1b and Group 2b nor between Group 2b and Group 3b (Figure 4).

DISCUSSION

Tendon injuries pose significant challenges in the daily practice of orthopedic and plastic surgeons, resulting in substantial healthcare costs due to limitations in patient mobility and increased individual morbidity. Despite advancements in treatment methods for these injuries over the past decade, the clinical outcomes of tendon injury surgeries remain a pressing concern.[19]

The tendon healing process is a multifaceted progression comprising three complex and interconnected phases: inflammatory, proliferative, and remodeling phases. Numerous factors, including stem cells, growth factors, and hemostatic agents, have been explored to facilitate a smoother tendon healing process.[20,21]

While angiogenesis is essential for tendon healing, hypervascularity in the long term after tendon injury does not always yield positive outcomes. In a study by Riggin et al.,[22] bilateral Achilles injuries were created in 344 Fischer rats, and VEGF (vascular endothelial growth factor), anti-VEGF, and saline were injected into the unrepaired Achilles tendons. The researchers demonstrated that reducing vascularity with anti-VEGF adversely affected early-stage healing, with a possibility of positive impact in the later stages.

Although the *in vivo* effects of papaverine have not been fully elucidated, it is known to increase cAMP (cyclic adenosine monophosphate) and cGMP (cyclic guanosine monophosphate) levels through the inhibition of phosphodiesterase enzymes.[23] These intracellular secondary messengers play pivotal roles in transmitting various physiological stimuli and regulating numerous physiological processes, including vascular resistance, cardiac output, visceral motility, immune responses, inflammation, neuroplasticity, vision, and reproduction.[24,25]

In a study, pirfenidone, a drug with antifibrotic and anti-inflammatory properties currently used in the treatment of idiopathic pulmonary fibrosis, was utilized to prevent tendon adhesion and tendon healing in rats with Achilles tendon damage.[26] The results indicated that pirfenidone decreased collagen synthesis and prevented the formation of peritendinous adhesion in rats; however, it did not impair tendon healing.

Another study involving 36 rats compared the effects of hyperbaric oxygen and pentoxifylline on Achilles tendon repair at the osteotendinous junction level.[27] In this experiment, 12 rats were subjected to 2.5 atmospheric pressure for one week, while an additional 12 rats received daily intraperitoneal doses of 50 mg/kg pentoxifylline. These two groups were then compared to a control group. While there were no histopathological differences observed among the groups, it was reported that energy absorption was significantly higher in the pentoxifylline group following biomechanical testing.

In our study, during the first 15 days, we did not observe any significant histopathological differences among the rat groups. However, from a biomechanical standpoint, we noted that the group treated with local papaverine exhibited higher force tolerance before tendon rupture. In contrast, the late period results on the 30th day demonstrated a significant and positive improvement in the group that received local papaverine in terms of histopathological indicators, including total score, cellularity, and cartilage formation. Additionally, biomechanically, the tensile strength in the systemic papaverine and local papaverine groups exceeded that of the control group. Notably, the local papaverine group displayed superior strength compared to the control group. In this context, when considering histopathological comparisons, the contribution of systemic papaverine administration to tendon healing does not appear to be as significant as systemic pentoxifylline. Biomechanically, there was an increase in strength compared to the control group, albeit not to the extent observed with local drug administration. Similar to previous studies, we found that locally applied papaverine had positive histopathological effects in the late period and biomechanical benefits in the early and late periods.

Research has demonstrated that elevated cAMP levels can stimulate the migration and rapid differentiation of stem cells to wound sites, facilitating wound healing.[28] Another study showcased the ability of topically applied endogenous stem cells to accelerate wound healing significantly in a diabetic rat model.[29] In a study by Martynov et al.,[9] a 4×1 cm wound was created, and aerosol forms of papaverine and dipyridamole, another phosphodiesterase inhibitor, were investigated for their effects on wound healing at various time points. The results confirmed the superiority of papaverine and dipyridamole over dexpanthenol in terms of epithelialization, indicating their positive effect on wound healing and pluripotent CD34 cell stimulation. In a pig skin study, the topical application of papaverine cream on expanded flaps increased flap survival and enhanced blood flow in the expanded skin tissue.[10] The current study aligns with these findings, revealing positive histopathological effects from local and systemic papaverine use, consistent with prior studies demonstrating the positive impact of topical papaverine application on wound healing.

Researchers also assessed the impact of caffeic acid on the healing process of repaired rat tendons.[30] The study findings indicated that the caffeic acid contributed positively to tendon healing

histopathologically and biomechanically in rats with an Achilles tendon injury model. Additionally, Güleç et al.[31] conducted a study to explore the antioxidant and anti-inflammatory properties of curcumin in the context of Achilles tendon injury and repair in rats, revealing favorable outcomes. Notably, these earlier studies primarily evaluated the effects of these factors on tendon healing either through systemic or local administration, typically over a single period. In contrast, our study provided a comprehensive assessment by considering the systemic and local administration and assessing early and late-period results on the 15th and 30th days.

Papaverine is also widely used in urology for diagnosing and treating impotence.[23] Interestingly, a study evaluating the effects of sildenafil, another phosphodiesterase inhibitor used in impotence treatment, on Achilles tendon healing reported positive histopathological effects.[32] In our study, while biomechanical effects were evident in the late period, positive differences were observed in all groups receiving papaverine treatment. From a histopathological perspective, the group that received local papaverine administration in the late period exhibited favorable outcomes.

This study had some limitations, including the absence of an immunohistochemical examination and a longer-term evaluation for assessing adhesion. On the positive side, our study stands out as the first to investigate the effects of papaverine on tendon healing, offering both short- and long-term results, in addition to histopathological and biomechanical assessments, setting it apart from similar studies in the literature.

In conclusion, although systemic papaverine shows positive biomechanical results in the late period, it becomes evident that the local application of papaverine yields positive and correlated effects, as indicated by histopathological and biomechanical assessments. While we recognize the necessity for further investigations with larger sample sizes, we believe that the localized use of papaverine holds promise for enhancing tendon healing.

Ethics Committee Approval: This research, conducted at the Tekirdağ Namık Kemal University - Experimental Animals Application and Research Center, received approval from the Tekirdağ Namık Kemal University Animal Experiments Local Ethics Committee on September 26, 2022, under decision number T2022-979.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Idea/concept: E.C., Y.M.D.; Design: E.C.; Control/supervision: Y.M.D.; Data collection and/or processing, analysis and/or interpretation: E.C., D.K, S.K., Y.Z.A., Writing the article, references and funding: E.C.; Literature review: E.C., Y.M.D, S.K.; Critical review, materials: Y.M.D., D.K., Y.Z.A.

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