Eurasian Journal
of Veterinary Sciences

www.eurasianjvetsci.org

RESEARCH ARTICLE

Comparison of steroid and platelet-rich plasma (PRP) applications in the treatment of collagenase induced tendinopathy in rabbit

Nuriza Zamirbekova[1], Nuri Yavru[1]

[1]Selcuk University, Veterinary Faculty, Department of Surgery, Konya, Turkey

Received:22.12.2020, Accepted: 30.03.2021
*zbnuriza@gmail.com

Tavşanların tendon yangılarının sağaltımında steroid ve trombositten zengin plazma (TZP) uygulamalarının karşılaştırmalı araştırılması

Eurasian J Vet Sci, 2021, 37, 2, 82-92
DOI: 10.15312/EurasianJVetSci.2021.330

Öz

Amaç: Bu çalışmada, tavşanlarda kollajenaz (Kollajenaz Tip I) ile oluşturulmuş aşil tendinopatilerinde trombositten zengin plazma (PRP) ve MPA (metilprednizolon asetat) enjeksiyonlarının terapötik etkilerini histopatolojik olarak değerlendirmeyi amaçlamıştır.

Gereç ve Yöntem: Aşil tendinopatisi, 23 tavşanın sağ ve sol aşil tendonlarının proksimal ve distal bölgelerine intratendinöz tip I kollajenaz enjeksiyonu ile oluşturuldu. Kolajenaz enjeksiyonundan bir hafta sonra, sağ aşil tendonlarının distaline PRP ve sol aşil tendonlarının distaline MPA enjekte edildi. Tüm aşil tendonlarının proksimal kısımlarına %0,9 serum fizyolojik enjekte edildi. Tavşanlar, terapötik enjeksiyonlardan 3, 6 ve 8 hafta sonunda ötenazi edildi. Tendonların histolojik dejenerasyon derecesi Modifiye Movin skorlamasına göre yapıldı.

Bulgular: Tedavi grupları ile % 0,9'luk serum fizyolojik enjekte edilen grup arasında incelenen tüm dönemlerde istatistiksel açıdan önemli farklılıklar tespit (p<0,05) edildi. Altıncı hafta sonunda tedavi grupları arasında tüm parametrelerde istatistiksel olarak önemli olmayan orta düzeyde iyileşme gözlenirken, 8. hafta sonunda MPA uygulaması yapılan tavşanlarla kıyasla PRP enjeksiyonu uygulanan hayvanların aşil tendonlarında kollajen ipliklerin paralel düzenlenmeleri ve demetleşmenin daha iyi olduğu gözlendi. Ayrıca tedavi grupları 8. hafta sonunda karşılaştırıldığında, PRP grubunun vaskülarizasyon ve tenosit çekirdek parametrelerinde istatistiksel olarak önemli düzeyde iyileşme gözlendi (p<0,05).

Öneri: PRP enjeksiyonlarının, MPA enjeksiyonuna göre tendinopati tedavisinde daha erken iyileşme sağlayacağı kanısındayız.

Anahtar kelimeler: Aşil tendinopatisi, kolajenaz Tip I, metilprednizolon, PRP, steroid

Abstract

Aim: The aim of this study was to evaluate histopathologically the therapeutic effects of platelet-rich plasma (PRP) and MPA (methylprednisolone acetate) injections in rabbits achill tendinopathies induced by collagenase (Collagenase Type I)

Materials and Methods: Achilles tendinopathy was formed with intrathendinous injections of type I collagenase into the proximal and distal regions of the right and left achilles tendons of 23 rabbits. PRP was injected distal to the right Achilles tendons and MPA to the distal of the left Achilles tendons. A 0,9% saline solution was also injected into the proximal parts of all achilles tendons. Rabbits were euthanized 3, 6, and 8 weeks after the therapeutic injections. The degree of histopathological degeneration of the tendons was made according to the Modified Movin scoring.

Results: Statistically significant differences were detected between the treatment groups and the 0.9% saline injected group (p<0.05). In the histopathological evaluations performed at the end of the 6th week no statistically significant difference was detected between the treatment groups (p>0.05) without tenocyte nuclei (p<0.05). At the end of the 8 weeks vascularization and tenocyte nuclei (p<0.05) parameters was observed better in the Achilles tendons of the rabbits undergoing PRP injection compared to rabbits with MPA application.

Conclusion: In conclusion, we consider that PRP injections tend to provide an earlier improvement in the treatment of tendinopathy compared to MPA injection.

Keywords: Achilles tendinopathy, collagenase Type I, methylprednisolone, PRP, steroid

Introduction

In veterinary medicine, tendinopathy and tendon diseases generally occur in racehorses. Tendinopathy in horses is more common in flexor digitalis superficialis tendons and in the forelegs than in the hind legs (Williams et al 2001). It is thought that environmental factors such as training changes, previous injuries, false horseshoes application, working on hard, slippery, or inclined grounds are extrinsic factors that may predispose Achilles tendinopathy (Luscombe et al 2003).
High-dose anti-inflammatory agents, hyaluronic acid, steroids, low-intensity ultrasonography, low-frequency infrared laser therapies are used in the treatment of tendinopathy. Lately, bone marrow-derived mesenchymal stem cells or PRP products have been included in the treatment of tendinopathy (Sànchez-Ibàñez et al 2015). PRP is defined as the plasma fraction of autologous blood which contains high concentrations of platelets (Marx 2001). The use of PRP in tissue regeneration is an emerging field for clinicians and researchers. The working mechanism of growth factors released by platelets is yet to be discovered. However, PRP has become a practical and easy application in the clinic due to its role in reducing the bleeding, bone regeneration, and rapid regeneration of tissues. PRP is also known to be important in increasing tendon tenoblasts and collagen productions (type I and type III) (Klein et al 2002).

The presented study was designed with the hypothesis that PRP can provide earlier recovery compared to steroid injections in the treatment of experimentally induced of rabbits Achilles tendinopathy. The results of PRP and methylprednisolone acetate injections was determined by histopathological evaluation.

Material and Methods

The study material consisted of 24 female and male New Zealand rabbits (about 3-5 kg and an average age of 6 months). The study was conducted according to the Ethics Committee of Seçuk University Experimental Medicine and Research Center, dated 27.04.2018 and numbered 2018/12.

Experimental method

Before starting the study, a sample biopsy was taken from one of the Achilles tendons of a rabbit to determined the normal tendon structure. Then the rabbit was taken into a bandage. To induce tendinopathy to the other Achilles tendon of the same rabbit, 0,3 ml of collagenase type I was injected intratendinously. One week after collagenase injection, a biopsy sample was taken from this Achilles tendon. The sections taken from the right and left achilles tendons were evaluated microscopically in terms of normal and tendinopathic tendon structure model after stained with Crossmon's triple (Culling et al 1985a) and Hematoxylin-Eosin staining methods (Culling et al 1985b).

Induction of inflammation in tendons and surgical procedure

A total of 23 rabbits were induced for general anesthesia with 5-10 mg/kg xylazine hydrochloride (Xylazin Bio® 2%, Bioveta, xylazin hydrochloride, 23.32 mg/mL) and 35-50 mg/kg ketamine hydrochloride (Ketasol® 10% (Ritcher Pharma, ketamine hydrochloride 100 mg/mL) injected intramuscularly. On the posterior face of both hind legs at the level of the Achilles tendon, the part from the knee joint level to 1-2 cm distal to the tuber calcanei was shaved to include the lateral and medial surfaces. Following the disinfection of the operation site, skin incisions were made directly on the Achilles tendon. Then, subcutaneous connective tissue and tendovagin of the achilles tendon were incised (Figure 1b). To the right and left Achilles tendon of the rabbits, at the 0.5-1 cm and 2.5-3 cm area in the proximal to the calcaneus, 0.3 mL collagenase type I enzyme was injected intratendinously (Collagenase Type I Sigma-Aldeich Co. Ltd, St, Louis, MO, USA SCR103, produced from Clostridium histolyticum) (Perucca Orfei et al 2016, de Cesar Netto et al 2018) (Figure 1c).

Tendovagina and subcutaneous connective tissues were sutured with 4/0 P.G.A. continuous stitching (Alcasorb®, Katsan Katizasyon Sanayi ve Ticaret a.ş.). The skin was closed with simple stitches 3/0 P.G.A. (Figure 1d). For postoperative pain relief, Paracetamol (Atabay Parol Plus, 50 mg/5mL) oral suspension was given at the dose of 300 mg/kg after the operation. Daily examinations and dressings of the operation area were performedin the following days. Sulfadoxin-trimethoprim was injected at a dose of 30 mg/kg to prevent the possible post-operative infections. Then, the animals were placed freely in separate cages. As antibiotics, Bakteral® (Sulfadoxin Trimethoprim 24%, Topkim Topkapı İlaç Premık San. Tic. A.S.) injectable suspension was injected at the dose of 48 mg/kg subcutaneously twise a day.

Preparation of platelet-rich plasma (PRP) and surgical procedure

After the collagenase injection, 5 rabbits died due to the anesthesia. The remaining 18 rabbits were randomly divided into 3 groups. After a week of the waiting period, as the rabbits were under general anesthesia, as in the previous procedure. To prepare PRP, 5-7 mL of blood was collected from the ear vein of each rabbit into the syringe containing 1.5 mL of ACD (Acid Citrate Dextrose). The blood was then transferred to Genesis PRP tubes (15:55:35 Genesis Autologous Cell System 2 branded PRP (15mL) preparation kits) and centrifuged at 1700 G (RCF) for 5 minutes. After centrifugation, PRP was obtained. 0.5 ml PRP distal to the right achilles tendons, 0.3 mL MPA (Prednol-L® 250 mg, methylprednisolone acetate, Mustafa Nevzat, Turkey) at a dose of 2.33 mg distal

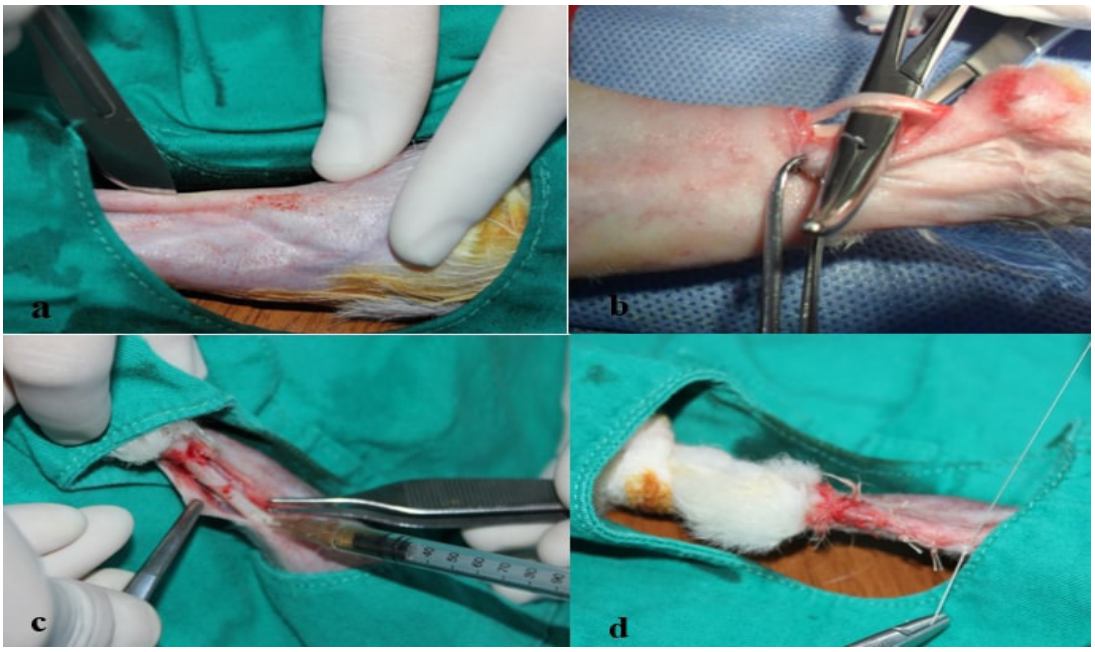

Figure 1. a) Establishing the Achilles tendon line and performing skin incision b) Defining the Achilles tendon, c) Injecting collagenase type I enzyme intratendinous d) Suturing the incision line after the procedure

to the left achilles tendons, and 0.5 mL 0.9% saline to both tendons proximally were injected. Treatments and control injections were made as a single dose. Tendovagina, subcutaneous tissue and skin closed as described previously. The extremities were then dressed and the dressings were changed daily.

Collection and processing of tissue samples

At the end of each experimental period of 3, 6, and 8 weeks, the rabbits, were euthanased under general anesthesia, with administered injected intracardiac 75-150 mg/kg of 10% KCL (Potassium Chloride® ISOLAB chemicals) (Saxena 1988). Achilles tendons of rabbits were dissected after euthanasia. Tissue samples for histopathological evaluations were fixed in a 10% buffered formol-saline (pH 7.4) solution, followed by routine histological methods and then blocked in paraffin. Crossmon's triple staining method (Culling et al 1985a) and Hematoxylin-Eosin staining methods were applied to 6 µm thick sections taken from Paraffin blocks using Leica RM2125RT model microtome (Culling et al 1985b). Stained tissue samples were evaluated under Leica DM-2500 model light microscopesuited with the DFC-320 model camera attachment, scored according to the modified Movin Scoring System (Table 1) (Maffulli et al 2000; Minkwitz et al 2017). Digital images of the required regions were recorded.

Statistical analyses

SPPS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical package program was used to evaluate the data. Mean ± standard deviation, median (maximum-minimum) percentage, and frequency values were used to express the results. The suitability of the data for repeated measures variance analysis was evaluated by Mauchy's Sphericity Test and Box-M Variance Homogeneity Test. For comparisons of means, one of the factors in factorial order was the repeated measures variance analysis. If parametric tests (repeated measures variance analysis in factorial order) did not meet the prerequisites, one of the Greenhouse-Geisser (1959) or Huynh-Feldt (1976) tests with a degree of freedom correction were used. Multiple comparisons were made with the corrected Bonferroni test. For the significance level of the tests, $p < 0.05$ value was accepted.

Results

The stained tendon tissue samples of a rabbit without collagenase enzyme treatment were evaluated as a control. In these sections, it was observed that the collagen bundles were tightly packed, stained blue-dark blue with aniline blue, and arranged parallel to each other. Few small diameter vessels were parallel to the long axis of the collagen bundles. As fat cell accumulations were not detected, tenocytes were seen in their flat and shuttle shapes (Figure 3a).

The tendon sections taken 1 week after the collagenase type I application of the rabbit was used as a reference to the tendinopathy model. The damaged tissue section revealed, under a light microscope, degenerations in collagen fibers, decreased collagen stainability, hypercellularity, neovascula-

Table1. Modified Movin scoring system

Parameter	Stain	0	1	2	3
Collagen fiber structure	HxE, Crossmon's triple staining	Collagen bundles packed tightly	Collagen bundles slightly separated	Collagen bunch structure is moderately lost	Tendon structure is completely lost
Collagen fiber arrangement	HxE, Crossmon's triple staining	Collagen fibers parallel to each other	Collagen fibers parallel to each other, but there is slight fluctuation	Collagen fibers maintain its parallelism, but the wavy appearance is quite evident and there are threads cross-over each other	Collagen fibers have completely lost their parallelism
Collagen stainability	Crossmon's triple staining	Dark blue	Blue	Light blue	Pale blue
Tenocyte nuclei shape	HxE, Crossmon's triple staining	Long spindle shape cells	Slightly rounding	Moderately rounding	Severely rounding
Cellular density	HxE, Crossmon's triple staining	Normal pattern	Slightly increase	Moderately increase.	Severely increase
Vascularity	HxE, Crossmon's triple staining	The blood vessels are only between the bundles and parallel to the long axis of the bundles	There are some vessels in the tissue	Several sets of vessels.	There are many and extremely hyperemic vessels.
Fat cells density	HxE, Crossmon's triple staining	There are almost no fat cells between the collagen fibers	There are only a few fat cells	There are some fat cells between the collagen fibers	Numerous fat cells are collected between the collagen fibers

rization, tendon cell nuclei, and fat cell densities (Figure 3b). At the end of the 3rd week of treatment, the histological examination showed that in the saline-treated group, the layout and structure of the fibers disappeared in both right and left achilles tendon sections. The collagen staining property was significantly reduced. The inflammatory reactions, which displayed an increase in cellular elements, were severe. The fat cell defined as the tendolipommatous between the collagen strands were observed, and a large number of hyperemic blood vessel assembled within irregular locations indicating to angiofibroblastic activity between the strands were significantly compared to the tendon sections obtained from the groups where PRP and MPA treatment procedures were applied (Table 2 and Table 3) (Figure 2).

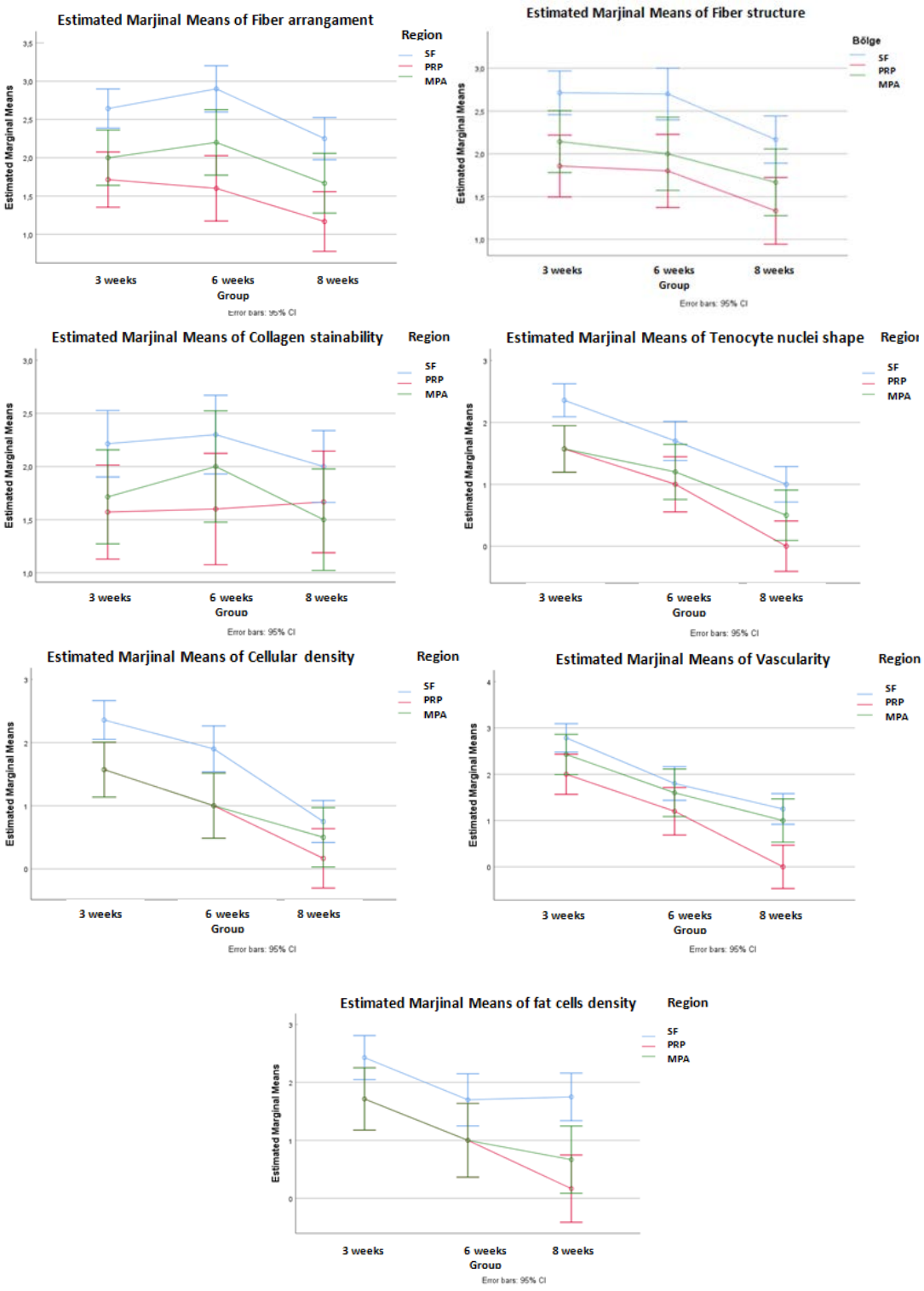

Figure 2. Collagen fiber structure, collagen fiber arrangements, collagen stainability, tenocyte nuclei shape, cellular density, vascularity, and fat cell infiltration values of the groups by weeks

Table 2. Collagen fiber structure, collagen fiber arrangements, and collagen stainability values among the groups in each experimental period (Mean ± Std deviation)

	%0.9 saline	PRP	MPA
Fiber structure	2.71±0.488^{Aa}	1.86±0.378^{B}	2.14±0.378^{B}
6th week	2.60±0.548^{Aa}	1.80±0.447^{B}	2.00±0.707^{B}
8th week	2.17±0.408^{Ab}	1.33±0.516^{B}	1.67±0.516^{B}
Fiber arrangements			
3rd week	2.71±0.488^{Aa}	1.71±0.488^{Ba}	2.00±0.000^{B}
6th week	3.00±0.000^{Aa}	1.60±0.548^{Bab}	2.20±0.837^{B}
8th week	2.25±0.408^{Ab}	1.17±0.408^{Bb}	1.67±0.516^{B}
Collagen stainability			
3rd week	2.43±0.787^{A}	1.57±0.535^{B}	1.71±0.488^{AB}
6th week	2.20±0.447^{A}	1.60±0.548^{B}	2.00±0.707^{AB}
8th week	1.83±0.408	1.67±0.516	1.50±0.548

A. B: Different letters in the same line are statistically significant (p<0.05. Corrected Bonferroni Test)

a. b: Different letters in the same column are statistically significant (p<0.05. Corrected Bonferroni Test)

Table 3. Tenocyte nucleus, cellular density, values of vascularity, and fat cell density among the groups in each experimental period (Mean ± Std deviation)

	%0.9 saline	PRP	MPA
Tenocyte nucleus	2.29±0.488^{Aa}	1.57±0.535^{Ba}	1.57±0.535^{Ba}
6th week	1.60±0.894^{Ab}	1.00±0.000^{Ba}	1.20±0.447^{Aab}
8th week	1.00±0.000^{Ac}	0.000±0.000^{Bb}	0.50±0.548^{Ab}
Cellular density			
3rd week	2.43±0.787^{Aa}	1.57±0.535^{Ba}	1.57±0.535^{Ba}
6th week	2.00±0.707^{Aa}	1.00±0.00^{Ba}	1.00±0.00^{Bab}
8th week	0.67±0.516^{Ab}	0.17±0.408^{Bb}	0.50±0.548^{ABb}
Vascularity			
3rd week	2.71±0.488^{Aa}	2.00±0.816^{Ba}	2.43±0.535^{ABa}
6th week	1.80±0.447^{b}	1.20±0.447^{b}	1.60±0.548^{b}
8th week	1.33±0.816^{Ac}	0.00±0.00^{Bc}	1.00±0.632^{Ab}
Fat cell density			
3rd week	2.57±0.787^{Aa}	1.71±0.488^{Ba}	1.71±0.756^{Ba}
6th week	1.60±0.548^{b}	1.00±0.00^{ab}	1.00±0.707^{ab}
8th week	1.83±0.983^{Ab}	0.17±0.408^{Bb}	0.67±0.816^{Bb}

A. B: Different letters in the same line are statistically significant (p<0.05. Corrected Bonferroni Test).

a. b: Different letters in the same column are statistically significant (p<0.05. Corrected Bonferroni Test).

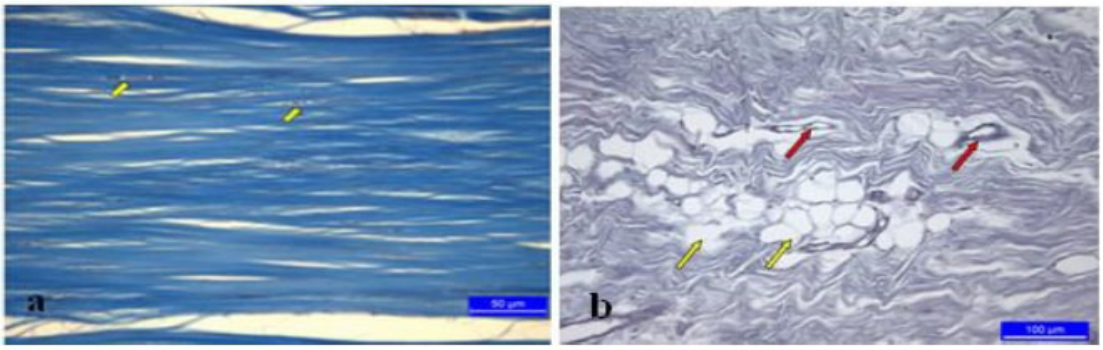

Figure 3. a) Achilles tendon without collagenase treatment, collagen fibers are arranged parallel to each other, dyed and stained blue-dark blue tone with aniline blue. Arrows: Small diameter vessels, located parallel to the long axis of the collagen bundles. Triple staining. b) An achilles tendon treated with collagenase shows a highly undulating appearance of collagen strands, the staining of collagen with a pale blue color, the presence of vessels, and multiple fat cells between the collagen fibers. Yellow arrows: Fat cells. Red arrows: Veins. Triple staining.

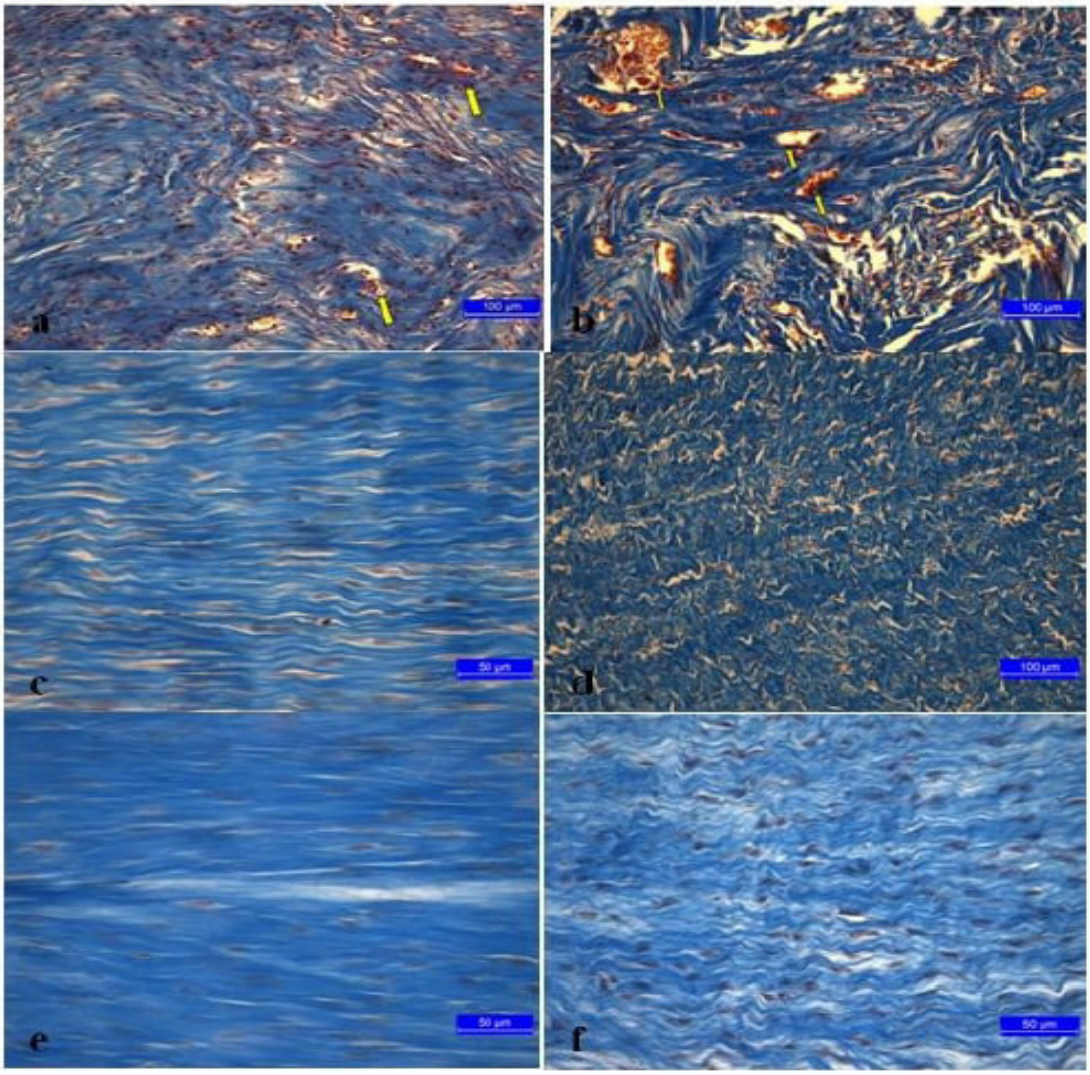

Figure 4. a) In the PRP-treated group at the end of the third week after collagenase application, the irregularly located vessels werefew and small in a cross-section taken from the right foot achilles tendon; the collagen fiber structure and collagen stainabilitywere better. Arrows: Veins. b) In the MPA treated group at the end of the third week collagen stainability was preserved in a section taken from the left foot Achilles tendon, but the collagen after collagenase applicationstructure and arrangementswere impaired and vascularity was increased. Arrows: Veins. c) In thePRP-treated group after the collagenase application, the right foot achilles tendon showeda prominent improvement in fiber structure and arrangements at the end of the sixth week. Triple staining. d) In the MPA-treated group after the collagenase application, the left foot Achilles tendon showed an improvement in the collagen bundles at the end of the sixth week, but healing was remarkable. e) In thePRP-treated group after the collagenase application, the right foot Achilles tendon showed that collagen bundles were arranged parallel to each other at the end of the eighth week. f) In theMPA-treated group after the collagenase application, the left foot Achilles tendon showed a significant improvement in fiber structure and arrangements at the end of the eighth week. Triple staining.

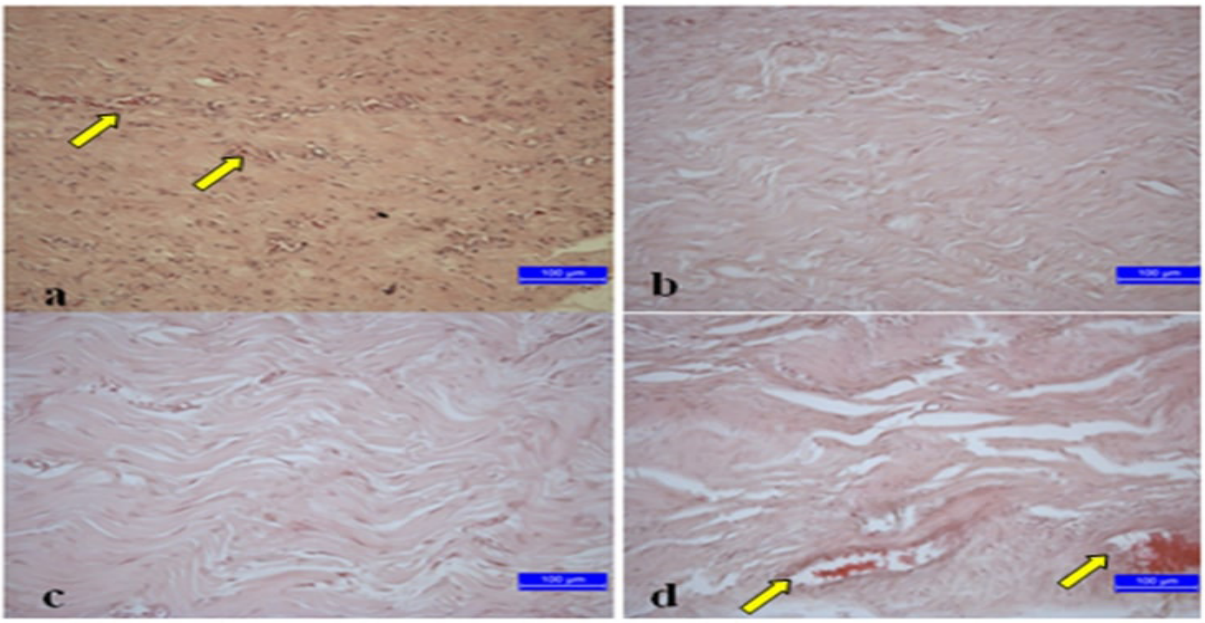

Figure 5. a) A cross-sectional inflammatory reaction in the left foot Achilles tendon at the end of the third weekwas observed in a section that was treated with MPA after the application of collagenase and taken from. Arrows: Veins. Hematoxylin / Eosin staining. b) A weak inflammatory reaction was observed in a section that was treated with PRP after collagenase application and taken from the right foot achilles tendon at the end of the third week. Hematoxylin / Eosin staining. c) The inflammatory reaction, fat cell accumulation, and angiofibroblastic activity disappear almost completely in the PRP group at the end of the sixth week. Hematoxylin / Eosin staining. d) İn the MPA group the severity of the inflammatory reaction was weakened at the end of the sixth week, but angiofibroblastic activity was still present. Arrows: Veins. Hematoxylin / Eosin staining.

In the MPA-treated tendon sections after the collagenase application, collagen stainability was preserved, increase in cellular elements were moderate, while it was observed that many irregularly located and relatively large diameter blood vessels were noticed, and the fiber structure and layout were disturbed (Figure 4 and Figure 5). It was noted that in the PRP-treated tendon sections after the collagenase application, the collagen stainability, the fiber structure and inflammation were found to be better than those of the other groups (Figure 4 and Figure 5). Concerning the long axis of the collagen fibers, irregularly located vessels were found to be both small in number and smaller in diameter (Figure 5).

Besides, the tenocyte nuclei in the saline-treated tendon sections were oval-round shaped (Table 2) (Figure 1). Taken into accounts all the parameters evaluated, there was no statistical difference between the treated groups ($p>0.05$).
At the end of the 6 and 8 week, significant improvements in the fiber structure, the fiber layout, collagen stainability, tenocyte nucleus shape, inflammatory reaction, tendolipommatous, and angiofibroblastic activity in all tendon sections obtained from animals in the treatment groups were noteworthy (Figure 4). While these improvements were more advanced especially in the PRP-treated animals, the parallel arrangements of the collagen bundles were comparable to that of healthy tendons (Figure 4).
There was a statistically significant outcome in the tenocyte nucleus and angiofibroblastic activity parameters in the PRP group ($p<0.05$) (Figure 5). However, collagen fiber structure, arrangements, and collagen stainability were not different among the treatment groups ($p>0.05$). Although the cellular elements and fat cell densities were rarely seen in the PRP-treated group, there was no statistically significant difference against the MPA-treated group ($p>0.05$).

Discussion

This study was designed to investigate the hypothesis that PRP treatment may provide an earlier recovery compared to MPA injections in the treatment of experimental achilles tendinopathy induced with collagenase application in rabbits. The histology findings provide an earlier recovery of PRP injection of the experimentally collagenase induced tendinopathy treament in this rabbit model than MPA injections.

In this study, the observations in the rabbit used for the control tendinopathy model were compatible with the microscopic findings specified in the earlier studies for tendinopathy induced by the application of collagenase type I (Movin et al 1997, Orfei et al 2016).

In the present study, PRP and MPA were injected immediately after the induction of tendinopathy. In the histopathological examinations, at the end of 8 weeks, it was observed that the PRP-treated group had improved collagen fibers structure and layout (Figure 4) and parallel structures of collagen

bundles were similar to healthy tendon structure. However, when the normal tendon collagen structure was evaluated as "0" according to the Modified Movin Scoring criteria, the collagen fibers were not observed to reach completely to the normal structure within 8 weeks (Table 2). Faisal et al. (2019) investigated the effect of early and delayed injections of PRP on the regulation of collagen fibers in experimental Achilles tendon damage. As a result, they found that there was no significant difference in receiving PRP either at early or late stages of the damage.

Therapeutic mechanisms of glucocorticosteroids are, unlike PRP, to eliminate pain in damaged tissue by reducing inflammation in the region (Smidt et al 2002). Glucocorticosteroids are used as pain relievers and/or antiinflammation in a wide range of musculoskeletal disorders, such as osteoarthritis, inflammatory arthritis, tenosynovitis, tendinopathy, and degenerative spinal disease. Evidence for the clinical efficacy of glucocorticosteroids is contradictory. In many studies (Arroll et al 2004) it is stated that glucocorticosteroids generally have a short-term relief effect. In another clinical study (Coombes et al 2013), most patients injected with glucocorticosteroids in the treatment of epicondylitis recurred after 1 year. In their experimental study, Hugate et al. (2004) showed that the corticosteroid injected in the Achilles tendon and the bursa adversely affected the biomechanical properties of the tendons of the rabbits. Besides, local steroid injections were shown to cause tendon rupture in many cases (Nichols 2005).

Previous studies also reported that steroids decreased the viability of tail and patellar cells and suppressed cell proliferation in rat tendons (Scutt et al 2006). In the present study 3 weeks after the MPA injection, suppression in the tendon cell proliferation was not observed. A decrease in the number of tendon cells was evident in the tendon sections examined in the 6th and 8th weeks. The structure and layout of the fiber were not completed within the 8-week treatment period and that the collagen strands maintained a more fluctuating appearance compared to the PRP group during the study. It is considered that the absence of tightly packed bundles and parallel structures of collagen fibers in the 6th and 8th weeks of the study may have resulted from the mechanisms by which steroids reduce the viability of tenocyte cells in the damaged region. However, no tendon rupture occurred in the MPA-treated group during the experimental period.

Stainability of collegen structure appears as dark blue in triple staining preparations. However, degenerated collagen losses its staining capacity and the color becomes pale (Movin et al 1997). In this study saline-treated group almost completely lost the stainability of the collagen and was dyed in pale blue color (Table 1).

The extracellular matrix, long tenoblasts, and collagen-producing tenocytes extending between the collagen fibers formed the cellular structure of the tendon (Kannus 2000). There is an increase in these cells during the proliferation phase of the tendon healing process (Khan et al 1998). Moreover, morphological changes in the rounding of the tenoblast nuclei are associated with the regulation of the metabolism of these cells for the production of the extracellular matrix (Dahlgren 2007). At the end of the maturation stage of tendon healing, the number of tenocyte cells decreases (Hooley et al 1979). Takamura et al. (2017) investigated histologically the healing effects of PRP in the rabbit and found that the migration of tenoblasts increased both in the control and treatment groups in the first week after the PRP treatment in the Achilles tendon. In the 3rd and 4th weeks of the study, it was found that the nucleus of the group treated with PRP started to take shuttle-like shape along with the decrease in the number of tenoblasts. In another study by Gonzalez et al. (2016) the treatment of collagenase-formed achilles tendinopathy models with the leukocyte-deficient PRP helped tenoblast nuclei reach their normal morphological forms after 12 weeks in rabbits.

In this study, a high degree of rounding in the tenoblast nuclei and a relative increase in the number of tenoblasts were determined in the saline-treated group after 3 weeks. In the tendon sections of the PRP-treated group, it was observed that the tenoblasts began to decrease and the nuclei began to grow at the end of the 6th week and the decrease in the number of tenoblasts continued and the nuclei took the form of the normal shuttle at the end of the 8th week ($p<0.05$). When the tendon sections of the MPA-treated group was evaluated at the 6th week, the decrease in the number of tenoblasts and the shuttle shape of the nucleus was found to be less than the PRP tendon sections. The reduction of tenoblasts in the PRP group and the return of the tenoblast nuclei to the shuttle form were interpreted as the recovery to the remodeling stage of the tenoblasts reached the maturation stage of tendon tissue healing earlier than the other groups and therefore the extracellular matrix and collagen synthesis of the tenoblasts were decreased.

Angiogenesis the earliest events of wound healing help migrate inflammatory cells and tenoblasts to the wounded area and cells accumulate mostly in the perivascular region and are scattered in a small amount throughout the tissue (Ahmed et al 1998, Millar et al 2010). In tissue biopsies with tendinopathy, irregular hypervascularity and thick-walled vascular groups have been reported, and in some regions, the vessels have a nodular appearance while others are perpendicular to collagen fibers (Aström et al 1995, Chen et al 2011). In addition, some researchers indicate that hypervascularization in chronic tendinopathy can contribute to the pain and chronicity of the disease (Fenwick et al 2002). This study, an increase in large-scale hyperemic vessels in the tendon sections was observed in the tendon sections examined

at the end of the 3rd week in the saline- and MPA-treated groups. In the tendon sections examined after 3weeks of the treatment period in the PRP-treated group, it was observed that the vessels were small in numbers and their diameters were smaller than that of the other groups. In this study, hyperemic vascular structures were not observed in the tendon sections examined at the end of the 8th week of the PRP-treated group whereas tendon sections in the MPA-treated group had large vessels. In the 8th week when the study was terminated, the fact that the vascularization in the tissue samples of the PRP-treated group showed similar results to the normal tendon structure was interpreted as PRP shortening the inflammation phase of healing and accelerating the tendon proliferation and remodeling phase (Table 2).

Adipose cell accumulation was found in patients with Achilles tendinopathy and partial or complete rupture in Achilles tendon fibers (Hoffmann et al 2011). Fat cell accumulation prevents the tissue repair process by causing the release of local pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) in the fat cells, muscle, and tendon tissue. Accordingly, inflammation has been found to disrupt normal blood flow in the muscle and activate lipolytic pathways to cause an increase in intracellular glucose concentration subsequently resulting in insulin resistance (Addison et al 2014). In the present study, fat cells accumulated within collagen fibers were found not only in the chronic stage of tendinopathy, but also in the inflammatory stage of tendinopathy healing. In the tendon sections from the saline-treated group, density of cell infiltrations were found to be severe at the end of the 3rd and 6th week, but moderate at the end of the 8th week. Three weeks after the treatment of tendon sections with PRP and MPA, moderate levels of adipose cell accumulation were observed while these accumulations were less frequent in the PRP-treated group at the end of the 8th week ($p<0.05$) (Table 3).

Conclusion

In conclusion, 8 weeks after tendinopathy, histopathological examination showed that PRP injections provide an earlier improvement in the treatment of experimental tendinopathy compared to steroid injection. In this study, the limitations were that the Achilles tendinopathy model was experimentally induced and not formed naturally, the duration of the study was limited to 8 weeks so that further studies are warranted to investigate PRP applications regarding the timing of the injections, the amount of PRP, the frequency of the applications, its follow-ups in the settings of experimentally induced and controlled clinical studies.

Acknowledgement

This study was presented orally at the 2nd International European Interdisciplinary Scientific Research Conference and published as a summary text in the congress book. The presented study; It has been summarized from the PhD thesis data of the first author.

Conflict of Interest

The authors did not report any conflict of interest or financial support.

Funding

This study was supported by Selçuk University Scientific Research Projects (BAP) Coordinator within the scope of thesis project number 18202017.

References

Addison O, Marcus RL, LaStayo P, Ryan AS, 2014. Intermuscular fat: a review of the consequences and causes. Int J Endocrinol, 1–11.

Ahmed IM, Lagopoulos M, McConnell P, Soames RW, et al., 1998. Blood supply of the Achilles tendon. J Orthop Res, 16(5), 591-596.

Arroll B, Goodyear-Smith F, 2004. Corticosteroid injections for osteoarthritis of the knee: meta-analysis. BMJ, 328(7444), 869.

Aström M, Rausing A, 1995. Chronic Achilles tendinopathy. A survey of surgical and histopathologic findings. Clin Orthop Relat Res, 316, 151-164.

Chen J, Yu Q, Wu B, Lin Z, et al., 2011. Autologous tenocyte therapy for experimental Achilles tendinopathy in a rabbit model. Tissue Eng Part A, 17(15-16), 2037-2048.

Coombes BK, Bisset L, Brooks P, Khan A, et al., 2013. Effect of corticosteroid injection, physiotherapy, or both on clinical outcomes in patients with unilateral lateral epicondylalgia: a randomized controlled trial. JAMA, 309(5), 461-469.

Culling CFA, Allison RT, Barr WT, 1985a. Connective tissue. Eds: CFA Culling, RT Allison, WT Barr, In: Cellular Pathology Technique. 4th edn. London, Butterworths and Co Ltd, pp; 164-80.

Culling CFA, Allison RT, Barr WT, 1985b. Haematoxylin and its couterstains. Eds: CFA Culling, RT Allison, WT Barr, In: Cellular Pathology Technique, 4th edn. London, Butterworths and Co Ltd, pp; 155-63.

Dahlgren LA, 2007. Pathobiology of tendon and ligament injuries. Clin Tech Equine Pract, 6(3), 168-173.

De Cesar Netto C, Godoy-Santos AL, Pontin PA, Natalino RJM, et al., 2018. Novel animal model for Achilles tendinopathy: Controlled experimental study of serial injections of collagenase in rabbits. PloS ONE 13(2), e0192769.

Faisal T, Qamar K, Asjid R, Umbreen F, et al., 2019. Effect of Autologous Platelet-rich Plasma on Appearance of Tenocytes at Injured Achilles Tendon Entheses in Rabbits. Jcpsp-J Coll Physıcı, 29(11), 1029-1033.

Fenwick SA, Hazleman BL, Riley GP, 2002. The vasculature

and its role in the damaged and healing tendon. Arthrıtıs Res Ther, 4(4), 1-9.

González JC, López C, Álvarez ME, Pérez JE, et al., 2016. Autologous leukocyte-reduced platelet-rich plasma therapy for Achilles tendinopathy induced by collagenase in a rabbit model. Sci Rep, 6, 19623.

Hoffmann A, Mamisch N, Buck FM, Espinosa N, et al., 2011. Oedema and fatty degeneration of the soleus and gastrocnemius muscles on MR images in patients with Achilles tendon abnormalities. Eur Radıol, 21(9), 1996-2003.

Hooley CJ, Cohen RE, 1979. A model for the creep behaviour of tendon. Int J Biol Macromol, 1(3), 123-132.

Hugate R, Pennypacker J, Saunders M, Juliano P, 2004. The effects of intratendinous and retrocalcaneal intrabursal injections of corticosteroid on the biomechanical properties of rabbit Achilles tendons. JBJS, 86(4), 794-801.

Kannus P, 2000. Structure of the tendon connective tissue. Scand J Med Sci Sports, 10(6), 312-320.

Khan KM, Cook JL, Bonar F, Harcourt P, et al., 1999. Histopathology of common tendinopathies. Sports Med, 27(6), 393-408.

Klein MB, Yalamanchi N, Pham H, 2002. Flexor tendon healing in vitro: effects of TGF-β on tendon cell collagen production. J Hand Surg-Am, 27(4), 615-620.

Luscombe KL, Sharma P, Maffulli N, 2003. Achilles tendinopathy. Trauma, 5(4), 215-225.

Nichols AW, 2005. Complications associated with the use of corticosteroids in the treatment of athletic injuries. Clın J Sport Med, 15(5), E370.

Maffulli N, Ewen SW, Waterston SW, Reaper J, et al., 2000. Tenocytes from ruptured and tendinopathic Achilles tendons produce greater quantities of type III collagen than tenocytes from normal Achilles tendons: an in vitro model of human tendon healing. Am J Sports Med, 28(4), 499-505.

Marx RE, 2001. Platelet-rich plasma (PRP): what is PRP and what is not PRP? Implant Dent, 10(4), 225-228.

Millar NL, Hueber AJ, Reilly JH, Xu Y, et al., 2010. Inflammation is present in early human tendinopathy. Am J Sports Med, 38(10), 2085-2091.

Minkwitz S, Schmock A, Kurtoglu A, Tsitsilonis S, et al., 2017. Time-dependent alterations of MMPs, TIMPs and tendon structure in human achilles tendons after acute rupture. İntJ Mol Sci, 18(10), 2199.

Movin T, Gad A, Reinholt FP, Rolf C, 1997. Tendon pathology in long-standing achillodynia: biopsy findings in 40 patients. Acta Orthop Scand, 68(2), 170-175.

Perucca Orfei C, Lovati AB, Viganò M, Stanco D, et al., 2016. Dose-related and time-dependent development of collagenase-induced tendinopathy in rats. PloS ONE 11(8), e0161590.

Sànchez-Ibàñez JM, Fernández ME, Monllau JC, Alonso-Díez A, et al., 2015. New treatments for degenerative tendinopathy, focused on the región-specific of the tendón. Rheumatology (Sunnyvale), 5(173), 2161-1149.

Saxena K, 1988. Death from potassium chloride overdose. Postgrad Med, 84(1), 97-102.

Scutt N, Rolf CG, Scutt A, 2006. Glucocorticoids inhibit tenocyte proliferation and Tendon progenitor cell recruitment. J Orthop Res, 24(2), 173-182.

Smidt N, Assendelft WJ, Van der Windt DA, et al., 2002. Corticosteroid injections for lateral epicondylitis: a systematic review. Pain, 96, 23-40.

Takamura M, Yasuda T, Nakano A, Shima H, et al., 2017. The effect of platelet-rich plasma on Achilles tendon healing in a rabbit model. Acta Orthop Traumatol Turc, 51(1), 65-72.

Williams RB, Harkins LS, Hammond CJ, Wood JLN, 2001. Racehorse injuries, clinical problems and fatalities recorded on British racecourses from flat racing and National Hunt racing during 1996, 1997 and 1998. Equine Vet J, 33(5), 478-486.

Author Contributions

Motivation / Concept: Nuri Yavru, Nuriza Zamirbekova
Design: Nuri Yavru, Nuriza Zamirbekova
Control/Supervision: Nuri Yavru
Data Collection and / or Processing: Nuri Yavru, Nuriza Zamirbekova
Analysis and / or Interpretation: Nuri Yavru, Nuriza Zamirbekova
Literature Review: Nuriza Zamirbekova
Writing the Article : Nuri Yavru, Nuriza Zamirbekova
Critical Review: Nuri Yavru, Nuriza Zamirbekova

Ethical Approval

The presented study was conducted with the approval and permission of the Selcuk University Experimental Medicine and Research Center Ethics Committee, dated 27.04.2018 and numbered 2018/12.

CITE THIS ARTICLE: *Zamirbekova N, Yavru N, 2021. Comparison of steroid and platelet-rich plasma (PRP) applications in the treatment of collagenase induced tendinopathy in rabbit. Eurasian J Vet Sci, 37, 2, 82-92*