IJVS 2017; 12(2); Serial No:27

IRANIAN JOURNAL OF VETERINARY SURGERY (IJVS)
WWW.IVSA.IR

Original Article

Comparison of Platelet-Rich Plasma (PRP), Bone Marrow-Derived Mesenchymal Stem Cells and their Combination on the Healing of Achilles tendon in Rabbits

Farnoosh Bagheri[1], Abbas Tavasoly[1]*, Mohammad Mehdi Dehgahan[2], Farhang Sasani[1], Mina Raji[3], Saeed Farzad Mohajeri[2]

Abstract

Objectives- The purpose of the study was to observe whether PRP, Mesenchymal Stem Cells (MSCs) and combining them could help make improvements the healing of Achilles tendons in rabbits.

Design- Experimental study.

Animals-Sixteen male, healthy and mature white New Zealand rabbits.

Procedures- The animals were randomiaed into 4 groups: Control, PRP, MSCs with fibrin glue and MSCs + PRP therapy. A 3 mm diameter defect was created in the midsubstance of the medial M. gastrocnemius tendon in all rabbits. After 4 and 8 weeks, animals were euthanized (each time point, 2 rabbits of the each group) and samples were examined in term of histologic scoring with H & E and Masson's Trichrome.

Results- Histologic investigations confirmed that all 3 methods of treatment in Achilles tendon defect had suitable reparative effects. Final histologic scoring for control group at 4th week after operation was 4.5 in contrast to treatment groups (PRP =6, MSCs = 5.5 and MSCs + PRP= 8). Final histologic scoring in all 4 groups improved at 8th week after operation (control=6.5, PRP =9.5, MSCs = 8.5 and MSCs + PRP= 14.5).

Conclusion and Clinical Relevance- This study indicated that using PRP or MSCs treatment alone and combination of them in injured tendon improved healing process and decreased time of repair. Reparative effect of PRP in tendon defect was better than use of allogenic MSCs treatment. However, the combination of them caused the best results.

Keywords : Tendon histologic scoring, PRP, MSCs, Rabbit

Received: 2 August 2017; **Accepted:** 7 October 2017; **Online:** 3 March 2018

Introduction

The tendon, which is the strongest in the human's body and is called the Achilles tendon, is around 8.2 times body weight when running tolerated.

It transmits forces from the *M. gastrocnemius* and *M. soleus* complex to the *Calcaneus* and therefore, allows movement.[1] About 30 to 50 percent of sports injuries constitute tendon injuries.[2] Compared to other soft-tissues, the tendon has weaker vascular system and therefore heals gradually.

Therefore, treatment is usually long, final results are varied, and generally re-injury might occur.[3-4-5-6] The initial stage of repair of tendons and ligaments involves formation of scar tissue to provide continuity at the injury site. For tendons in particular, mobility must be maintained during healing to prevent or at least decrease the formation of adhesions and to increase strength. The sequence of repair includes three phases:

[1]Department of Pathology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2]Department of Surgery, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[3]Orthopedic surgeon, Moayeri Hospital, Iranian Social Security Organization, Tehran, Iran
Correspondence: Address all correspondence to Abbas Tavasoly (DVM, DVSc). E-mail: atavasoli@ut.ac.ir

DOI:10.22034/ivsa.2018.94255.1121

(a) tissue inflammation, (b) cell and matrix proliferation, and (c) remodeling and maturation. The inflammatory stage usually includes a hematoma formation, thereby triggering the discharge of chemotactic factors such as IGF-1(Insulin-like growth factor 1), TGF-β (Transforming growth factor beta), platelet-derived growth factor (PDGF), and also basic fibroblast growth factor (bFGF). Cells which have inflammation in them are drawn from the tissues around there to absorb the clot, remaining cellular particles, and the materials coming from outside. Fibroblasts are taken to the location to start synthesis of elements of the extracellular matrix. Also, angiogenic factors discharged in this stage trigger the development of a vascular network. As fibroblasts are necessary for synthetizing proteoglycans, collagens, and some other extracellular matrix elements, proliferation of these cells goes on through the stage of cell development. At this stage in the process of repair, the extracellular matrix is mostly made up of type III collagen. When the proliferative stage has ended, the repaired tissue is remarkably cellular and consists of almost big portions of water and a great deal of extracellular matrix elements. The remodeling stage will start 6 to 8 weeks following injury which is recognized by a drop in cellularity, lowered matrix synthesis, a drop in type III collagen, and a rise in synthesis of type I collagen. The fibers of type I collagen are arranged in a longitudinal way standing alongside the tendon axis which are necessary for the mechanical power of the tissue regeneration. In spite of numerous continuous stages of remodeling, the repaired tissue will hardly ever obtains the features of normal tendon.[7] In latest years, many different therapies for tendon injuries have been utilized or tested including bone marrow-derived mesenchymal stem cells (MSCs) and platelet-rich plasma (PRP) which has revealed remarkable results in modulation of Achilles tendon repair.[8,9] The majority of studies have confirmed that platelet-rich plasma (PRP) is useful in lowering inflammatory processes which happen after injury and speeding up soft tissue recovery. Studies previously conducted demonstrated that PRP assist to generate blood vessels and improve collagen fiber depositing in the injury location.[10,11].On the other side, PRP, which is an autologous concentrate taken from blood platelets, has been presented as a completely new treatment for tendon injury. Through providing growth factors in the location of lesion, Platelets play a vital role in the cascade of tissue recovery.[12] when platelets are activated, they discharge growth factors, including insulin-like growth factor -1(IGF)-1 vascular endothelial growth factor (VEGF), and platelet-derived growth factor (PDGF) coming from their granules.[13]According to the research results, PRP therapy in tendon injuries leads to much better histological qualities of the tissue repaired.[14] Yet, a number of studies confirmed the beneficial impact of PRP or MSCs on regenerating tendon, but utilizing the combination of these treatments have not been studied before. The purpose of present study was to examine the impacts of using bone marrow-derived mesenchymal stem cells and PRP, when used alone and in combination, on recovery processes after partial tenotomy. The final results of this study can be useful in clinical setting.

Methodology and Materials

Design

Sixteen healthy and mature male white New Zealand rabbits (weight: 2-2.4 kg and around 11 weeks of age) bought from the Razi Institute of Iran were used in this study. The animals were held in standard cages under steady temperature of 18-22 °C, and humidity level of 40-50%, with12 h/12 h light and dark cycles, with easy access to standard food (the ration set for rodents) as well as filtered water from tab. This study was done according to guidelines of animal care review board of the University of Tehran and the law of ethics comity in Faculty of Veterinary Medicine.

Experimental groups

The animals were randomly divided into 4 experimental groups which with 4 rabbits:
Control: animals with partial Achilles tendon defect and no treatment.
PRP: animals with partial Achilles tendon defect and PRP treatment.
MSCs: animals with partial Achilles tendon defect and MSCs treatment with fibrin glue.
MSCs +PRP: animals with partial Achilles tendon defect and combined treatment of MSCs and PRP.

Anesthesia

The animals were anesthetized by intramuscular injection of ketamine hydrochloride 10% (35mg/kg ,Alfasan, Woerden-Holland) and xylazine hydrochloride 2% (8 mg/kg, Alfasan, Woerden-Holland). Anesthesia were continued in the rabbits with the application of inhalation machine and isoflurane 1% with tracheal tube size of 2 mm.

Experimental procedures

The surgery of Achilles tendon in these rabbits were performed at Department of Small Animal Surgery of Faculty of Veterinary of Medicine, University of Tehran. Following anesthesia, the rabbits were put on a

table, in ventral recumbency posture. Also their rear and fore limbs were gently immobilized by some thin ropes. The right hind paw was shaved and ready for sterile operative intervention. For this purpose, the skin was opened with a 2-cm longitudinal incision and a lateral Paramedian approach after a longitudinal splitting of the crural fascia and the Paratenon surrounding the Achilles tendon complex. Through this method, the Achilles tendon was accessible for subsequent procedures inducing defect or performing treatment. The medial *M. gastrocnemius* tendon was separated from surrounding tendons such as the lateral *M. gastrocnemius* tendon and the *M. flexor digitalis superficialis* tendon. Achilles tendon defect was induced with a 3 mm diameter round-shaped defect in the midsubstance of the medial *M. gastrocnemius* tendon utilizing a biopsy punch (Fig 1). The defect made in the control group remained untreated, but PRP treatment and MSCs prescription were used in PRP group and MSCs group, respectively finally, the combination of MSCs and PRP treatment was used in the last group (MSCs+PRP group). The tendon was situated in the normal site and the related fascia/Paratenon closed applying continuous suture and vicryl twine. The muscles and derma of skin were closed by 5.0 vicryl twine for the muscles and 5.0 prolene twine (Ethicon) for the skin. For reducing pain in these animals analgesic drug (Tramadol, 4 mg/kg IM, Chemi Daro-Iran) and for prevention of infection antibiotics (enrofloxacin, 10 mg/kg SC, Aburaihan-Iran) were prescribed. After 4 and 8 weeks for each group, all the rabbits were euthanized (thiopental sodium 100mg/kg IV) and then tissue specimens were extracted and submitted to laboratory. Tissue samples in each group were used for histopathological evaluation.

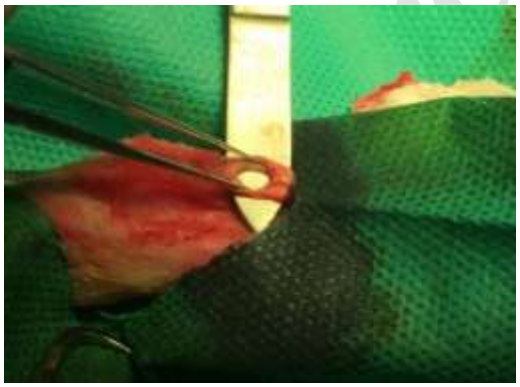

Figure 1. A round defect (3 mm diameter) was made in the mid-distance of the medial *M. gastrocnemius* tendon using a biopsy punch

PRP preparation

After inducing anesthesia, 10-mL of autologous blood was taken from the heart of each rabbit to prepare PRP. In this study, we used My Cells® harvesting kit. Blood was drawn and mixed gently with anticoagulant citrate dextrose solution (ACD). It was Centrifuged 10 min, 1450-2050 G, room temperature. Then PPP phase was removed carefully and plasma was drawn from the surface. Remaining PRP was drawn into syringe without touching the separation gel. Then Calcium gluconate (10%) activator was included in a ratio of 1:10 to get the total volume of PRP (as the instructions on the kit). In PRP treatment, regardless of its preparation method, the number of platelets must be 3-5 times more than the peripheral blood. In this study we used PLT count to understand if the number of platelets was in the mentioned range. It was about 1200000 per µl.

Bone marrow-derived mesenchymal stem cells

In this study, the cells used were allogenic (from two rabbits out of the study) mesenchymal stem cells (MSCs). Also, Bone marrow was aspirated from the iliac crest through a sterile surgical procedure. An incision was created over the iliac crest, periosteum was ruined, and a tiny hole was drilled via the cortex. Five milliliters of bone marrow were aspirated from each rabbit into a syringe which was covered with 3,500 IU of sodium heparin. Cells were then cultured in Dulbecco's modified Eagle's medium-high glucose, including 1% antibiotics and 20% fetal bovine serum, which, based on the method proposed by Lennon et al. [5],was particularly screened to increase proliferation of mesenchymal stem cells in rabbits with no differentiation. The cell culture medium were changed every 3 or 4 days, and were preserved for 10-14 days. Intact colonies were acquired from the flask with 3ml of 0.25% trypsin-EDTA. The cells were then gathered, washed, centrifuged with DMEM, counted, and replated to the first-passage. These colonies were used in the final-passage until cell count of mesenchymal stem cells reached to 2.5×10^6 cells per 75 cm^2 flask.

Confirmation of Mesenchymal Stem Cell being detached from bone marrow

The flow cytometery as a supplementary and safe method was performed for the confirmation and identification of the cell surface indicators of Mesenchymal stem cells of the rabbit's bone marrow. After performing this method, the samples were poured into the flow cytometer pipes and then it was read by FACS Canto II (BD Bioscience, USA) flow cytometer set analyzed by Flowjo software and displayed in histogram form. The flow cytometer results of Mesenchymal stem cells showed that these cells stated the cell surface indexes of CD29 (92%), CD90 (89%).

Meanwhile, CD45 (the indicator of Hematopoietic cells) and CD34 (the indictor of endothelial cells) were negative.

Fibrin glue preparation

In this research, fibrin glue was used as scaffold for MSCs prepared with method of Dresdale and et al. [15-16] with the least modifications. Fibrin glue was not used neither in PRP or PRP+MSCs groups because calcium in calcium gluconate can coagulate PRP which acts as scaffold in defect site of tendon by considering that use of fibrin glue has no effects on tendon healing process which was confirmed in some studies. [17] 10 mL of blood (from each rabbit) was centrifuged to produce the fresh frozen plasma (FFP), and kept frozen overnight at -18°C. The fibrinogen is prepared by thawing the plasma for a few hours at 4°C. The fibrinogen pellet is obtained by decanting the supernatant after centrifugation at 3500 rpm for 5 minutes. [18] The fibrinogen pellet can be precipitated in a small amount of FFP at the bottom of the tube. Total volume of the fibrinogen pellet and FFP, captured from the initial 10 mL of blood volume, is around one ml, which is more than the amount obtained by Drysdale's method. Perhaps this was caused by less dilution. These processes were used under a sterile condition. For confirmation of lack of microbial contamination, the final product was submitted for routine microbiological culture. Concentrated fibrinogen was used freshly after preparation of them. This production of blood was used after adding calcium chloride as well as commercial thrombin (Thrombin from bovine plasma, SIGMA, Germany). Lyophilized vials of thrombin with an activity of 500 units per vial are suspended in 0.2 ml distilled water. During surgery, 1 ml of fibrinogen is used with 0.2 ml of thrombin. [17]

Histological examination

All rabbits were euthanized at 4th and 8th week after operation (each time point, 2 rabbits of the each group) and the Achilles tendon of right leg was transected below its musculotendinous junction and over the calcaneal attachment of it. From each group, a harvested tendon was extracted and washed in physiological solution. They were then put in 10% buffered formalin and inserted in paraffin in order to submit them for histopathological analysis. Some thin sections (5 μm) were cut and stained with hematoxylin-eosin (H&E) and Masson's trichrome (MT) as a particular connective staining tissue used for histopathological evaluation. 10 fields were chosen randomly from each section under light microscopy. All the tendons used in this study were blindly evaluated by two investigators.

Scoring systems to assess tendon healing

Macroscopic criteria for evaluation of these tendons were occurrence of adhesion and inflammation. Histologic scoring system for quantitative evaluation of these tendons is based on the result of the study by Stoll et al.[3] with some minor modifications presented in Table 1. These criteria for scoring of these tendons included Extracellular matrix (ECM) organization of all the tendon, Cellularity/cell-matrix-ratio, cell alignment, Cell distribution, Cell nucleus morphology ,organization of the tendon callus, vascularization and inflammation.

Table 1. Histological scoring system (Adopted from Stoll et al. [3]).

	pt.
Extracellular matrix (ECM) organization of the whole tendon	
Wavy, compact and parallel arranged collagen fibers	2
In part compact, in part loose or not orderly	1
Loosely composed, not orderly ("granulation" tissue)	0
Cellularity/cell-matrix-ratio	
Physiological	2
Locally increased cell density	1
Increased cell density or decreased ECM content	0
Cell alignment	
Uniaxial	2
Areas of irregularly arranged cells (10–50%)	1
More than 50% of cells with no uniaxial alignment	0
Cell distribution	
Homogeneous, physiological	2
Focal areas of elevated cell density (10–50%)	1
More than 50% of elevated cell density (cell clustering)	0
Cell nucleus morphology	
Predominantly elongated, heterochromatic cell nuclei (tenocytes)	
10–30% of the cells possess large, oval, euchromatic or polymorph heterochromatic nuclei	2
More than 50% of the cells possess larger, oval, euchromatic or polymorph, heterochromatic nuclei	1
	0
Organization of repair tissue of the tendon callus	
Homogeneous (whole tissue with similar composition)	2
Locally heterogeneous tissue composition	1
Whole tissue composition completely changed	0
Vascularization in the defect area	
Hypo-vascularized, like surrounding tendon or less than 10% (small capillaries)	2
Hyper-vascularized (10–50% increased numbers of small or larger capillaries)	1
Hyper-vascularized (More than 50 %)	
0	
Inflammation	
No inflammatory cell infiltrates or less than 10%	
Infiltrating inflammatory cell types (10–50%)	2
Infiltrating inflammatory cell types more than 50% (neutrophils, macrophages, foreign-body/giant cell)	1
	0

pt.; points

Statistical analysis

In order to analyze the results, one-way analysis of variance (ANOVA) was performed using Tukey post hoc test. All statistical analyses were applied at a significance level of $P<0.05$. Results were reported as mean value.

Results

Clinical and macroscopic alteration of repaired tendons

After the operation, the rabbits had quite freely movement in their cages without any restriction of movement and behavior change. Rabbits had been tolerated well the surgical procedure, MSCs treatment, the PRP treatment and combination of them. No evidence of infection, suture dehiscence or other complications was observed in the rabbits.

Histological results

For correct and accurate histopathological evaluation of Achilles tendon in different groups, a numerical (quantitative) scoring suggested in material method was used. According to this scoring system, normal Achilles tendon score was number 16. In this study, the mean score for each group was obtained based on histopathology evaluation with H&E and MT stain calculation. Final histopathology scoring for each group was obtained based on gathering 8 different criteria scores. The results from each group were used for statistical analysis. Table 2 presents the mean value score in histopathological study. According to the table, the numerical difference between the control and treatment groups is obvious. One-way ANOVA test compared the groups separately for 4th and 8th weeks. No significant differences between control and other groups were seen at 4^{th} week ($P= 0/352$) and 8^{th} week ($P = 0/381$).

Table 2. The mean value score in histopathological study

Group	Mean value (4week)	Mean value (8week)
Control	4.5	6.5
PRP	6.0	9.5
MSCs	5.5	8.5
MSCs + PRP	8.0	14.5

*The mean value score in Healthy tendon = 16

Histopathology changes in repaired tendon evaluated by the quantitative scoring system

The histopathology score was obtained from the results of H&E and MT staining. Histopathology scoring system evaluated the control tendon and the experimental groups with 8 criteria. Repaired tendon in histologic scoring of control group had significant difference with other groups at 4^{th} week after operation. This finding is clearly seen in pictures stained with H&E in Figure 2. (A to D). Cell and collagen fiber alignment in last group (PRP + MSCs) was clearly better than other groups. Investigating all repaired rabbit tendons in MT stain revealed that vascularization decreased from control to PRP + MSCs. And also inflammatory events were not visible in last groups. Only little inflammation was detected in control group after 4 weeks in healing tendon. Microscopic investigation revealed that repaired tendon treated by PRP + MSCs can accelerate the healing process. Histologic investigation also showed that applying PRP had better repairing effects than using MSCs.

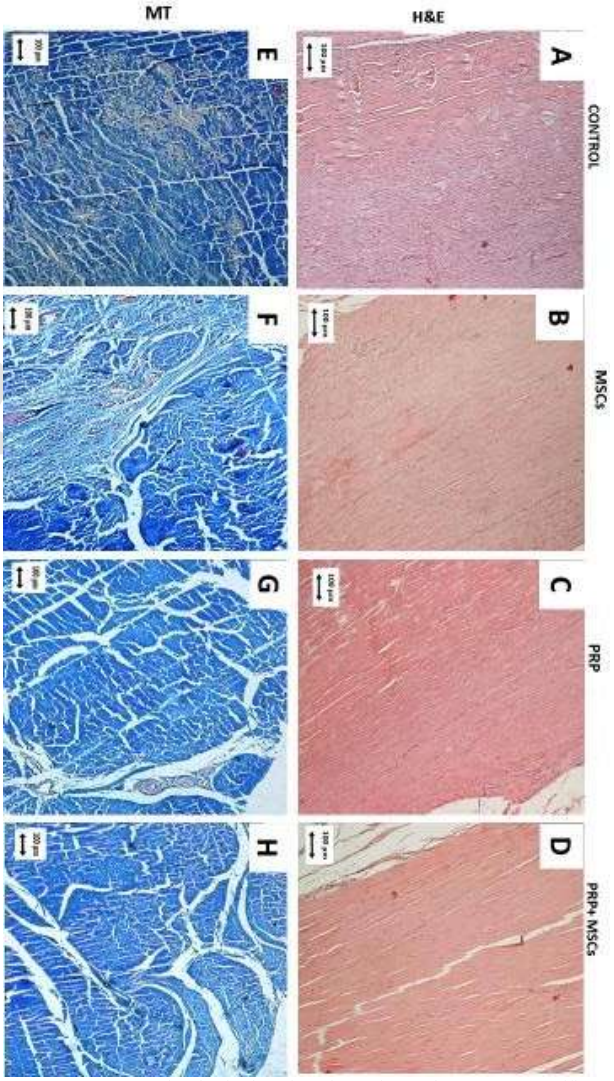

Figure 2. A-H. Histological stain of repair tendon.5 µm paraffin section of injured Achilles tendon stained with H&E (A-D) and MT (E-H) derived from: (A,E) control (untreated), (B,F) MSCs, (C,G) PRP, (D,H) MSCs+ PRP.4 weeks after tendon surgery. Scale bar 100 µm

Discussion

At best, tendon repair requires a series of medical procedures such as physical modalities, growth factors, tissue engineering, mechanical stimulation, cell and gene therapy create optimum results. These treatments can improve the condition of the tendon tissue repair and increase the tensile strength. Also, it is important to choose the best type of medical treatment in order to return the tendon to normal activities and prevent its rupture and adhesion it to the adjacent tissues.[20] One of these treatment methods is the application of platelet-rich plasma (PRP). PRP has a lot of growth factors which can accelerate repair process and decrease inflammation. The important growth factors which release from PRP are vascular endothelial growth factor, connective tissue growth factor, transitional growth factor beta 1, platelet-derived growth factor (PDGF), fibroblast growth factor, insulin-like growth factor-1(IGF-1), epidermal growth factor, platelet thromboplastin, serotonin, fibrinogen, calcium, and hydrolytic enzymes.[21] The mechanism of PRP is not fully known. However, tumor growth factor beta (TGF-β), raised levels of PDGF, and IGF-1 might have a role in tendon recovery. PDGF triggers the creation of other growth factors in the intense phase of tendon injury. TGF-β prevents MMP activity during the inflammatory phase and has substantial effect on cell migration and proliferation. TGF-β starts proliferation of fibroblast and development of fibrosis. TGF-β lowers inflammatory process in recovery of tendon.[22] IGF-1 increases cell migration and proliferation as well as collagen production and thus enhances healing.[23] Hapa O. et al. have suggested that injecting PRP can lower the inflammation at the location of tendon injury, and lead to improve tendon strength after 2 weeks.[24] These findings are in line with the results of this study. A number of researchers mentioned applying PRP treatment which has the capability to speed up tendon healing, increases quality of repair, and organizes fibroblasts and collagen bundles in a better way. Cell therapy is an additional treatment recently proposed for tendon repair which can improve healing by using bone marrow-derived mesenchymal cells. Bone marrow is a source of pluripotent stem cells including mesenchymal stem cells (MSCs). These cells are actually progenitor cells having the ability of producing mesenchymal tissue like fat, bone, connective tissues, cartilage, muscle, and tendon [25]. Others reported that the using autologous bone marrow-derived mesenchymal stem cells in collagen gel can substantially enhance the structure of tendon after injury.[26,27] The majority of studies insisted on using autogenic cells in order to avoid immunogenic complications. However, in the present study allogeneic cells were used because they have regulatory and commercial advantages. A number of these advantages include large cell bank with greatest quality control and simple use. Since using autologous mesenchymal stem cells could be challenging and costly as well as possible infection problems, cell therapy as modern treatment will be minimally used. [28-29] It has been mentioned that the knitted PLGA biodegradable scaffold loaded with allogeneic bone marrow stromal cells has the ability to create and repair gap defect in Achilles tendon and to successfully restore function and structure.[8] This result is similar to our research findings. Additionally, Chen, L. et al. confirmed that combining tendon stem cells and PRP has synergistic effects on tendon healing under both loaded and unloaded conditions, and loaded conditions improve tendon healing.[30] Our study revealed that using stem cell with PRP had best result in healing of Achilles tendon defect. According to the histologic scoring results of Achilles tendon treated with MSCs+PRP had the highest score and significant difference with other groups (control, PRP, MSCs). Microscopic investigation for this group showed that cell alignment and ECM organization were suitable and inflammation and vascularization were at the least limitation. In this study, different evaluations such as histopathology testing found the final score of all groups which was obtained at 8th week after operation improved compared to the score of 4th week for each group. This finding confirmed that duration of time is important in tendon healing. Also, this study showed that using PRP or cell therapy (MSCs) had little difference in Achilles tendon repair. It was mentioned that using MSCs as cell therapy in repaired tendon may have side effects such as ectopic bone. They showed that 28% of cases had ectopic bones. In this research no ectopic bone was diagnosed.[31] The similar finding was reported by others.[32] They reported that the healing time of injured tendon decreases by using combining PRP and LLLT (Low Level Laser Therapy). Young et al. highlighted that delivering mesenchymal stem cell-contracted, organized collagen implants to large tendon defects can substantially enhance the structure, and the function of the tendon after injury.[26] For this reason, fibrin glue was used as scaffold in MSCs groups. In this study, Calcium gluconate (10%) was used for activation of PRP. Additionally, calcium can coagulate PRP which acts as scaffold in defect site of tendon. A histologic scoring system which was used in our research was based on C. Stoll et al with a little modification.[3] MT as a special staining for connective tissue was used for staining the Achilles tendon. Tendon stained with MT improved histologic scoring specially in vascularization. This study indicated that using PRP or MSCs treatment alone and combination of them in

injured tendon improved healing process and decreased time of repair. Use allogenic MSCs treatment caused no significant immunologic response [33]. Reparative effect of PRP in tendon defect is better than use of allogenic MSCs treatment.

Acknowledgment

This research was supported University of Tehran that is acknowledged by authors

Conflicts of interest

None

References

1. Scott SH, Winter D A. Internal forces of chronic running injury sites. Medicine & Science in Sports & Exercisec,1990; 22(3):357-69

2. Baksh N, Hannon CP , Murawski CD, Smyth NA , Kennedy JG. Platelet-rich plasma in tendon models: a systematic review of basic science literature. The Journal of Arthroplasty, 2013;29(3):596–607

3. Stoll C, John T, Conrad C, Lohan A, Hondke S, Ertel W, Kaps C, Endres M, Sittinger M, Ringe J, Schulze-Tanzil G. Healing parameters in a rabbit partial tendon defect following tenocyte/biomaterial implantation. Biomaterials, 2011; 32(21):4806-15.

4. Bosch G, Moleman M, Barneveld A, van Weeren PR, van Schie HTM.The effect of platelet-rich plasma on the neovascularization of surgically created equine superficial digital flexor tendon lesions. Scandinavian Journal of Medicine & Science in Sports, 2011;21(4):554–561

5. Lennon DP, Hayncsworth SE , Bruder SP, Jaiswal N, Caplan A. Human and animal mesenchymal progenitor cells from bone marrow: Identification of serum for optimal selection and proliferation , In Vitro Cellular & Developmental Biology - Animal, 1996;32(10): 602–611

6. MolloyT, Wang Y, Murrell G. The roles of growth factors in tendon and ligament healing. . Sports Medicine, 2003;33(5):381–394

7. James F Zacha. Pathologic Basis of Veterinary Disease (sixth Ed.). Elsevier. St. Louis, Missouri 2017;PP:970-971

8. Ouyang HW , Goh JC , Thambyah A, Teoh SH, Lee EH. Knitted poly-lactide-co-glycolide scaffold loaded with bone marrow stromal cells in repair and regeneration of rabbit Achilles tendon. Tissue Engineering, 2003;9(3):431-9

9. Kaniki N, Willits K, Mohtadi NG, Fung V, Bryant D.A retrospective comparative study with historical control to determine the effectiveness of platelet-rich plasma aspart of non-operative treatment of acute Achilles tendon rupture. Journal of arthroscopic & related surgery, 2014 ;(9):1139–1145

10. Lyras DN, Kazakos K, Verettas D, Polychronidis A, Tryfonidis M, Botaitis S, Agrogiannis G, Simopoulos C, Kokka A, Patsouris E. The influence of platelet-rich plasma on angiogenesis during the early phase of tendon healing. American Orthopaedic Foot and Ankle Society, 2009; (11):1101–1106.

11. Lyras D, Kazakos K, Verettas D, Polychronidis A, Simopoulos C, Botaitis S, Agrogiannis G, Kokka A, Patsouris E. Immunohistochemical study of angiogenesis after local administration of platelet-rich plasma in a patellar tendon defect. International orthopaedics, 2010;34(1):143-8

12. Molloy T, Wang Y, Murrell G. The roles of growth factors in tendon and ligament healing. Sports medicine, 2003;33(5):381–394

13. Antitua E, Andia I ,Sanchez M, Azofra J, Del Mar Zalduendo M, De La Fuente M. Autologous preparations rich in growth factors promote proliferation and induce VEGF and HGF productions by human tendon cells in culture. *Journal of orthopaedic research*, 2005;23(2):281–286

14. Kaux JF, Drion PV, Colige A, Pascon F, Libertiaux V, Hoffmann A .Effects of platelet- rich plasma (PRP) on the healing of Achilles tendons of rats. Wound Repair and Regeneration, 2012 ;20(5):748–756

15. Dresdale A, Bowman FO. Jr Malm JR, Reemtsma K, Smith CR, Spotnitz HM, Rose EA. Hemostatic effectiveness of fibrin glue derived from single-donor fresh frozen plasma. Annals of Thoracic Surgery, 1985;40 (4):385-7

16. Dresdale A, Rose EA, Jeevanandam V, Reemtsma K, Bowman FO, Malm JR. Preparation of fibrin glue from single-donor fresh-frozen plasma. Surgery, 1985;97(6):750-5

17. Gahanmehr S, Ahmadi SH, Mohagheghi MA, Amanpour Zeraeti H, Emami SA. Preparation Of Fibrin Glue As A Biological Sealant To Control Bleeding In Heart Muscle And Blood Vessels. Medical Journal of The Islamic Republic of Iran, 2004;17(4):289-292

18. Wright PA, Hughes VC. Donor selection and component preparation, In: Harmening DM, (Ed) Modern blood banking and transfusion practices 4th Ed. 1999;Philadelphia: Davis Co.pp:307

19. Faturechi R,Hashemi A, Abolfathi N. A tensile machine with a novel optical load cell for soft biological tissues application. Journal of medical engineering and technology, 2014;38(8):411-5

20. Jame R, Kesturu G, Balian G, Chhabra AB.Tendon: biology, biomechanics, repair, growthfactors and evolving treatment options The journal of hand surgery, 2008;33(1):102 –112.

21. Barbosa D, de Souza RA, de Carvalho WR, Xavier M, de Carvalho PK, Cunha TC, Arisawa EÂ, Silveira L, Villaverde AB. Low-level laser therapy combined with platelet-rich plasma on the healing calcaneal tendon: a histological study in a rat model. . Lasers in Medical Science, 2013; 28(6):1489–1494.

22. Robbins and Cotran .Pathologic Basis of Disease. Philadelphia, PA 2015;19103-2899

23. Bosch G, van Schie HT, de Groot MW, Cadby JA, van de Lest CH, Barneveld A, van Weeren PR. Effects of platelet-rich plasma on the quality of repair of mechanically induced core lesions in equine superficial digital flexor tendons: a placebo-controlled experimental study. Journal of orthopaedic research, 2010;28(2):211–217

24. Hapa O, Cakici H, Kukner A, Aygun H, Sarkalkan N, Baysal G. Effect of platelet-rich plasma on tendon-to-bone healing after rotator cuff repair in rats: an in vivo experimental study. Acta orthopaedica et traumatologica turcica, 2012;46(4):301–307

25. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science, 1997; 276(5309):71-4

26. Young RG, Butler DL, Weber W, Caplan AI, Gordon SL. ,Fink DJ. Use of mesenchymal stem cells in a collagen matrix for Achilles tendon repair. Journal of Orthopaedic Research, 1998; 16(4):406-13.

27. Awad HA, Butler DL, Boivin GP , Smith FN, Malaviya P, Huibregtse B, Caplan AI. Autologous mesenchymal stem cell-mediated repair of tendon. Tissue Engineering, 1999;5(3):267-77.

28. Eaglstein WH, Alvarez OM, Auletta M.Acute excisional wounds treated with a tissue-engineered skin (Aligraft). Dermatologic surgery, 1999;25(3):195-201

29. Flabella AF, Schachner LA, Valenia IC, Eaglstein WH. The use of tissue- engineered skin (Apligraft) to treat a new born with epidermolysis bullosa. Archives of dermatological research ,1999135(10):1219-22

30. Chen L, Dong SW, Liu JP, Tao X, Tang KL, Xu JZ. Synergy of Tendon Stem Cells and Platelet-Rich Plasma in Tendon Healing. Journal of orthopaedic research, 2011;30(6):991-7

31. Harris MT, Butler DL, Boivin GP, Florer JB, Schantz EJ, Wenstrup RJ. Mesenchymal stem cells used for rabbit tendon repair can form ectopic bone and express alkaline phosphatase activity in constructs. Journal of orthopaedic research, 2004;22(5):998-1003

32. Allahverdi A, Sharifi D, Takhtfooladi MA, Hesaraki S, Khansari M, Dorbeh SS. Evaluation of low-level laser therapy, platelet-rich plasma, and their combination on the healing of Achilles tendon in rabbits. Journal of lasers in medical sciences. 2015;30(4):1305-13

33. Aggarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood, 105:2005;1815–1822.

نشریه جراحی دامپزشکی ایران
سال۲۰۱۷ ، جلد ۱۲ (شماره ۲)، شماره پیاپی ۲۷

چکیده

مقایسه اثرات پلاسمای غنی از پلاکت (PRP) ، سلولهای مزانشیمی مشتق از مغز استخوان و استفاده توامان آنها در التیام تاندون آشیل در خرگوش

فرنوش باقری*[۱]، عباس توسلی[۱]، محمد مهدی دهقان[۲]، فرهنگ ساسانی[۱]، مینا راجی[۳]، سعید فرزاد مهاجری[۲]

[۱]گروه آسیب شناسی، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران
[۲]گروه جراحی و رادیولوژی، دانشکده دامپزشکی، دانشگاه تهران، تهران، ایران
[۳]متخصص ارتوپدی، بیمارستان معیری، سازمان تامین اجتمایی، تهران، ایران

چکیده

هدف- هدف از این مطالعه، تعیین اثر بخشی استفاده از پلاسمای غنی از پلاکت(PRP)، سلول های بنیادی مزانشیمی مشتق از مغز استخوان(MSCs) و ترکیب آنها در بهبود روند ترمیم تاندون آشیل خرگوش است.

طرح- مطالعه تجربی.

حیوانات- ۱۶ عدد خرگوش نر سالم و بالغ سفید نیوزیلندی.

روش کار-در این مطالعه۱۶ عدد خرگوش نر سفید نیوزیلندی به ۴ گروه درمانی تقسیم شدند :کنترل، PRP، MSCs همراه با چسب فیبرین و MSCs + PRP .پس از ۴و ۸ هفته بعد ازجراحی ، حیوانات معدوم و نمونه ها به روش هیستوپاتولوژی به روش رنگ آمیزی با H & E و تری کروم ماسون مورد بررسی قرار گرفتند.

نتـایـج- در بررسی هیستولوژیک مشخص شد که هر ۳روش درمانی درترمیم نقصیصه تاندون آشیل موثر بوده است. نتایج نهایی هیستوپاتولوژی در ۴ هفته بعد از درمان عبارت است از : (control = 4.5، PRP =6 ، MSCs = 5.5 و MSCs + PRP= 8) و ۸ هفته پس از جراحی شامل : (control=6.5 ، PRP =9.5، MSCs = 8.5 وMSCs + PRP= 14.5).

نتیجـه‌گیـری و کاربرد بالینی- استفاده ازروش درمانی با PRP یا MSCs به تنهایی و ترکیب آنها باعث بهتر شدن روند ترمیم تاندون می شود.اثر ترمیمی PRP نسبت به MSC های غیرخودی ارجحیت دارد ولی بهترین نتایج در درمان ترکیبی حاصل می شود.

کلمات کلیدی- ارزیابی هستوپاتولوژی تاندون، PRP ، MSCs ، خرگوش.