Role of Platelet Rich Plasma in Tendon Healing

Mahmoud Hassan Maawad 1, Raafat Abd El Latif Anani 1, Mohamed Ali Nasr 1, Ahmed Mohamed Yehia 1

1Department of plastic surgery, Faculty of Medicine - Zagazig University, Egypt.

Corresponding author: Mahmoud Hassan Maawad Soliman,
E-mail: m_action_zamlkawy_o6u@yahoo.com

Abstract

Background: Tendons are dense connective tissues composed primarily of a highly organized type I collagen extracellular matrix (ECM). Which enables a tendon to perform its mechanical function of force transfer. Platelet rich plasma (PRP) serves as a growth factor agonist and has both mitogenic and chemotactic properties.

Aim: investigation the effect of PRP in tendon healing in rats, and evaluation the rate of possible complications.

Materials and Methods: The study included (35) rats, (5) rats only used as donors for PRP, while (30) rats separated as PRP group:(1); injection, group (2); spray, group (3); mixed. The left leg in all rats (control group) receives nothing, while the right leg receives PRP as separated groups. Each rat was kept in a separated cage and checked on until the end of the follow up period (15-30) day. After (15) and (30) days post-treatment, (5) rats of each group were sacrificed, and their Achilles tendons were extracted and examined histopathologically. Stained sections examined for the number of the inflammatory cells / HPF, the ratio between collagen 1 and collagen 3 and the arrangement of the collagen fibers.

Results: Stained sections shows differentiation in levels between collagen 1 and collagen 3, we did not observe altered level of collagen III in the PRP-treated tendons, an increased level of Collagen I was measured in the PRP treated tendons. Furthermore, PRP inhibits proliferation of macrophages could prevent an excessive inflammation during the early phase of healing, and could promote proliferation, metabolic activity, and differentiation of the mesenchymal cells into active tenocytes. Finally, it was obvious that PRP not only enhances healing, but also promotes the regeneration of the fibrocartilage zone in the wounded rat ATs and decrease adhesions. From this study, PRP treatment did high promoting the formation of fibrocartilage in wounded rat ATs. It did enhance healing by inducing collagen 1 expression. The results indicate that collagen 1 fibers formed in the wounded rats ATs due to PRP treatment. This may partially explain why PRP treatment increased the tensile mechanical strength of the wounded ATs. Compared with treatment without PRP, it contained scar tissue formation, characterized by the presence of excessive collagen (iii), which is typically disorganized, and weak in mechanical strength. Finally,
Histo-pathological analysis showed well organized arrangement of collagen fibers and proteoglycan formation in the wounded ATs in the PRP group.

Conclusion: With PRP treatment appeared to have healed the injured Ats, without, PRP treatment showed poor healing without fibrocartilage tissue formation in the ATs. We recommend using PRP treatment to human tendon pathologies.

Keywords: PRF, Tendon Healing, Platelet

Introduction

Tendons are dense connective tissues composed primarily of a highly organized type I collagen extracellular matrix (ECM). The structure of the collagen ECM allows tendons to transmit large forces between muscle and bone, facilitating movement of nearly the entire body. Acute traumatic injuries to the tendon are quite common due to the superficial anatomic location of many tendons. Following injury, tendons generally heal with a scar tissue response rather than regeneration of native tendon. This fibrotic healing response is particularly problematic in the hands, as excursion of the flexor tendons within the synovial sheath is restricted by both increased tissue bulk and the formation of adhesions between the tendon and surrounding tissues. Thus, adhesion formation and rupture are frequent complications of tendon injury repair (1).

Platelet have been used to treat wounds since 1985. Platelet rich plasma (PRP) serves as a growth factor agonist and has both mitogenic and chemotactic properties. It contains a high level of platelets and a full complement of clotting and growth factors (2).

The secretory proteins contained in the α-granules of platelets include platelet-derived growth factor (PDGF-AA, BB, and AB isomers), transforming growth factor-P (TGF-P), platelet factor 4 (PF4), interleukin-1 (IL-1), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), insulin-like growth factor (IGF), osteocalcin (Oc), osteonectin (On), fibrinogen (Ff), vitronectin (Vn), fibronectin (Fn), and thrombospondin-1 (TSP-1). These growth factors aid healing by attracting undifferentiated cells in the newly formed matrix and triggering cell division. PRP may suppress cytokine release and limit inflammation, interacting with macrophages to improve tissue healing and regeneration, promote new capillary growth and accelerate epithelialization in chronic wounds (3).

PRP is easy to produce with minimal effort and can be prepared as needed at the point of care. Clinically valuable PRP contain at least one million platelets per microliter. Lesser concentrations cannot be relied to enhance wound healing, and greater concentrations have not shown increase in wound healing (4).

Studies in this field suggest that the platelets interact with leucocytes and endothelial cells and provide auto-activation with autocrine and paracrine effects, as result of which inflammatory response emerges, and situations such as chemotaxis, atherothrombosis, coagulation, and cellular differentiation occur. When compared with whole blood, PRP contains a much greater concentration of growth factor (GF) and other glycoproteins. The
effectiveness of PRP depends on the dose, concentration, and technique by which it obtained. Based on these biochemical properties, PRP can used clinically to accelerate wound healing. So injured tendons would be benefit after repair by addition of PRP to accelerate healing and decreases adhesions (5). So, we aimed to investigate the effect of PRP in tendon healing in rats

**Material and Methods**

**Experimental Design:**
All experimental procedures and protocols for animals conformed to the Institutional Animal Care and Use Committee Zagazig University ZU-IACUC. The study included (35) Sprague-Dawley male rats with an average weight of 300–350 gm. Five rats were used as donors for PRP, while (30) rats were separated into three groups (PRP group).

**Preparation of PRP:**
About 10 cm fresh bloods from (5) rats treated with 10% sodium citrate; anticoagulant solution was obtained in a sterile tube from inferior vena cava by open approaches after anaesthetizing them. Blood was centrifuged around on (2000) for 10 minutes at room temperature. The upper layer transferred to another tube without anticoagulant and re-centrifuged at a higher speed on (4000) for 5 minutes. Then PRP were collected in one tube and prepared for use after activation with 10% calcium chloride.

**Surgical Procedure:**
We used 30 young adult white male rats weighing 300–350 g. The rats anaesthetized by intraperitoneal and/or intramuscular injection Ketamine of 0.005 mg/gm body weight. Hair shaved from both hind limbs and the rats were placed in a prone position on a rodent operating board. The complete surgical procedure performed under aseptic conditions under a dissecting microscope. the area sterilized with povidine iodine 10 %. The skin of the Achilles tendon incised laterally and exposed after dissection of the surrounding fascia. Achilles tendons of both legs in all rats cut transversely 5 mm proximal to its calcaneal insertion. Administration of PRP to the three groups with 0.5ml PRP only by 28 G syringe: In group (1); injection, In group (2); spray, In group (3); mixed spray and injection. The left leg in all rats (control group) receives nothing, while the right leg receives PRP as separated groups. All cut Achilles tendons were repaired with 4/0 polypropylene with modified Kessler technique and running epitelen sutures with 5/0 polypropylene and skin with Vicryl 6/0.

**Clinical follow up:**
After recovery, each rat kept in a separate cage and checked on every day. Ceftriaxone 0.2 mg/gm antibiotic for 7 days once daily IM given as a treatment post-surgical procedure. After complete skin healing, rats left to live normally in groups until the end of the follow-up period.

**Evaluation Method:**
15- and 30-days post-surgery, (5) rats of each group euthanized with pentobarbital. The healing tendon dissected from surrounding tissues. a full range of motion of the joint, observed when dissecting tendons at the two time points. Then rats revised and killed with an overdose of anesthesia. Also (5) rats of each group were sacrificed, and their Achilles tendons were extracted and examined histo-pathologically.
Histopathological Work Up

Stained sections examined for calculating the number of inflammatory cells, the ratio between collagen 1 and collagen 3, the arrangement of the collagen fibers and any other pathological changes in the examined tissues.

Statistical Analysis:

According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD, the following tests used to test differences for significance; difference and association of qualitative variable by Chi square test (X2). Differences between quantitative multiple by ANOVA. P value was set at <0.05 for significant results & <0.001 for high significant result. All the data collected throughout the study were analyzed using Statistical Package software for analysis (SPSS version 20.0).

Results

Treatment with Plasma-Rich –Platelets (PRP) revealed a much more rapid recovery, normalization, and rehabilitation of the treated- injured tendons compared with the untreated ones at 15 days post-surgery (PS).

The average number of inflammatory cells (5HPFs/section) were estimated using image analysis software. It was (53.4/HPF) with predominance of lymphocytes (24.2/HPF), followed by macrophages (12.6/HPF), polymorph cells (10.4/HPF) and finally giant cells (6.2/HPF).

Treatment groups pointed out a progressive healing and tissue remodeling processes with deposition of more intense collagen type I intermixed with a lesser deposition of collagen type III as proved by the histochemical technique. The inflammatory, vascular changes, granulomatous, degenerative and tissue destructive reactions were mild particularly in the PRP-mixed treatment and injection groups (Figure 1).

A characteristic decline in the average numbers of inflammatory cells was seen in the different treatment groups, the least number was seen the mixed –treatment group (14/HPF) followed by the injection –treatment group (32/HPF) and finally the spray treatment group (35/HPF). At the end of the 4th week following PRP treatment, there was an enhancing promoting tendon recovery with gradual disappearance of the deformed chronic granulomatous reaction in tendons of treated rats as compared with the granulomatous deformed changes in the tendons of control untreated rats (Figure 2).

Four weeks PS, H&E-stained sections from PRP treated tendons denoted a characteristic collagen crimp, which was started to synchronize within big collagen bundles (collagen type 1) as compared with the injured tendons which showed disorganized, thin ,loosely arranged bundles (collagen type III)with intervening cystic granulomatous reaction, interstitial edema , many foci of aggregated inflammatory cells, mainly lymphocytes and macrophages with unorganized hyaline – cartilageinization. The estimated average number of inflammatory cells in this group was (46.4/HPF) with predominance of lymphocytes (23/HPF), followed by macrophages (16.2/HPF) then polymorph cells (4.8/HPF) and finally giant cells (2.4/HPF).
Tendons of PRP treated rats showed characteristic orientally arranged collagen fibrils, they were clearly apparent, visibly thicker, with parallel arrangement and a more bundled appearance (collagen type 1). Numerous fibrocytes and some fibroblasts were - 54 - Results still found between the collagen fibrils. There was a distinct overall tendon-like appearance with thick collagen bundles and cells, oriented along the tension fibers. Occasional vascular conglomerates with some macrophages and foreign body giant cells could be seen around the sutures particularly in the spray treated group. Moreover, tendons of PRP-treated rats had a smooth appearance resembling very much normal tendons with orderly arranged fibrocartilaginous areas and normal vascularization. Such changes were clearer in mixed-treated group rather than other groups (Figure 3).

A peculiar change in the average numbers of inflammatory cells was seen in the different treatment groups, with a comparable decrease in the number of such inflammatory cells, the least number was seen the mixed treatment group (8/HPF) followed by the injection treatment group (18/HPF) and finally the spray-treatment group (34.2/HPF).

Sirius red staining, 2 weeks post-surgery revealed established formation of tendon-like tissue at the healing hot points (junction ends of the sutured tendon) with densely packed bundles of collagen fibers, built from type I collagen, type III collagen occupied small areas. There was a distinct increase of thicker mature red-orange collagen fibers (type1) with the best result obtained in the mixed treatment group.

The estimated ratios of collagen type 1 in the different experimental groups were calculated in the control, spray treated, injected and mixed-treated groups and described in (Figure 4). The deposited collagen appeared well organized, with parallel orientation and minimal traumatic inflammatory reactions, the number of inflammatory cells and or granulomatous reaction were minimal in the mixed treatment group meanwhile animals with injured tendons at this time showed deposition of lesser amount of collagen type 1 (coarse red-59 - Results orange fibrils) and greater amount of collagen type III (faint yellowish green fibrils), such fibrils were disorganized and randomly distributed with associated inflammatory and granulomatous reactions and infiltration of different populations of inflammatory cells, mainly lymphocytes and macrophages. Similar findings were recorded using Masson Trichrome stain, but the collagen fibers type I was coarse spiral dark blue while collagen type III was thinner, regular faint blue. The former was greater in the treatment groups with the best result in the mixed treatment group and the latter were minimal in such schedule and greater in the injured-sutured tendons (Figure 5). At the same time the inflammatory reactions, cellular infiltrations and foreign body granulomas were of greater densities in the control group and of lesser degrees in the treatment groups.

By the end of the 4th week, histochemical staining reactions were peculiar in control group. Tissue repair was unorganized with less amount of the tense collagen 1 deposition and persistence of the chronic inflammatory and foreign body granulomatous reactions, denoting delayed healing processes. Meanwhile treated groups revealed organized healing process of the tendon with parallel orientation of collagen fibers which were of more collagen type 1 (Figure 6).
The estimated ratios of collagen type 1 and collagen type III, using image analysis software, in different experimental groups (control, injection and mixed treatment) were described in (Figure 7). Rats survived the operation and the follow-up period without major complications. The healed tendon had very little adhesions and had the ability to glide smoothly in its sheath, and a full range of motion of the joint was observed when dissecting tendons at the two time points.

Figure 1. Rat’s tendon Achilles of PRP-treatment groups spray group; (A), injection group; (B) mixed group; (C), showing healing and tissue remodeling processes with deposition of more intense collagen type 1 (black arrows) and lesser deposition of collagen type III (yellow arrows). Inflammatory and granulomatous reactions are mild particularly in the PRP- mixed treatment groups (C, yellow stars). A granulomatous reaction with predominant macrophages is seen in the injection-treatment group (B, yellow star). Scale pars 20, 40 ums. /400 HPF.

Figure 2. Showing an enhancing rehabilitation effect on the tendon of treated rats (R) as compared with a granulomatous deformed change in the control untreated rats (L).
Figure 3. Rat’s tendon Achilles of PRP treatment groups 30 days PS. Spray group (A), injection group (B) and mixed group (C&D); showing vascular conglomerates with some macrophages and foreign body giant cells around the sutures particularly in the spray treated group (A, green and yellow stars). Normal vasculature (B, yellow star), apparently visible thick collagen, with parallel arrangement and a more orientally bundled appearance (collagen type 1) beside numerous fibrocytes some fibroblasts between the collagen fibrils (C and D, black and yellow arrows). Scale bars 60, 40, 20 ums. /400 HPF.

Figure 4. Percentage of collagen type 1 in different experimental groups at 15 (red star) and 30 days (yellow star) post-surgery using Sirius red and masson trichrome stains.
Figure 5. Rat’s tendon Achilles of control (C) and treatment groups (S, In, Mix), 15 days PS, stained with Sirius red and Masson trichrome stains showing unorganized repair of the injured tendons with less amount of the tense collagen I deposition and more collagen type III, (black arrow, yellow star).
Figure 6. Rat’s tendon Achilles of control (C) and treatment groups (S, In, Mix), 30 days PS, stained with Sirius red and Masson trichrome stain showing unorganized repair of the injured tendons with less amount of the tense collagen I deposition (Sirius red; black arrow), more collagen III (Sirius red; blue arrows) persistence of chronic inflammatory, granulomatous reaction (Trichrome; yellow arrow) and fibrous disorganization (trichrome; white arrow). Treated groups shows parallel orientation of collagen fibers which appears with more collagen type I; thick coarse spiraled red or dark blue fibrils (Sirius red; black arrows and Trichrome; yellow arrows) than type III; faint thin regular yellowish green or blue fibers (Sirius red; blue arrows and Trichrome; black arrows), less inflammatory reaction and inflammatory cells infiltrations, arranged, ordered fibro-hyaline-cartilageginization (faint bluish red fibrils and
cells) (Trichrome; yellow stars) and normal vascularization (Trichrome; brown arrows). Scale bars 15, 20 ums. /400 HPF.

Figure 7. Percentage of collagen type III in different experimental groups 15 (red star) and 30 days (yellow star) post-surgery using Sirius red and Masson trichrom stains.

Discussion

Platelets are critical cells during tissue injury particularly during the early inflammatory phase. Platelet degranulation as seen during exposure to clotting factors or by contact with connective tissue structures releases growth factors stored in granules such as platelet-derived growth factor Nurden et al., (6). These factors stimulate the synthesis of extracellular matrix macromolecules and mesenchymal cell proliferation, exert chemotactic activity toward circulating progenitor cells, and promote angiogenesis and cell differentiation (7).

In this study, PRP has been used in different ways (injection, spray and injection and spray) to regenerate wounded rat ATs. Delivery of PRP (inj. and spray) into wounded rat ATs effectively healed the injury and promoted better "quality healing" of wounded rat ATs than in the groups treated either, with PRP injection or spray. The PRP (inj. and spray) treated rat ATs appeared similar to the intact ATs. These results agree with De Mos et al., (8). The mechanical strength of the healed ATs was significantly high, these results indicate that PRP not only enhance healing, but also promotes the regeneration of the fibrocartilage zone in the wounded rat ATs.

At day 15, a great variability observed for PRP groups that explained by the inter-individual variation of responses to PRP. And these results show that a single intra-operative injection of PRP in ruptured Achilles tendon influences the early phase of healing and results in an ultimate stronger resistance. These data support previous observations that the very early improvement of the quality of the callus allows cells to perceive and respond to mechanical loading, a potent mechanical regulatory process of the metabolic activity of mesenchymal cells (9). Indeed, Aspenberg, (10) and his team observed that platelet injection could improve the mechanical properties of tendons as soon as 3 and 5 days, and they demonstrated that mechanical loading is important for growth of the callus but not its mechanical quality.
As we also observed, platelets improve the early phases of the healing process of tendons, allowing tendon cells to perceive and respond to mechanical loading more rapidly than in untreated tendons. This concept of mechanical stimulation after PRP treatment could be perhaps beneficial to human tendon pathologies such as ruptures or tendinopathies.

Although we did not observe altered level of collagen III in the PRP-treated tendons, an increased level of Collagen I measured in the PRP treated tendons at day 30, theses result agrees with McCarrel et al., (11).

Furthermore, it has been previously shown that PRP inhibits proliferation of macrophages could prevent an excessive inflammation during the early phase of healing, and could promote proliferation, metabolic activity, and differentiation of the mesenchymal cells into active tenocytes, these results agree with Lyras et al., (12).

Conclusion

With PRP treatment appeared to have healed the injured Ats, without, PRP treatment showed poor healing without fibrocartilage tissue formation in the ATs. We recommend using PRP treatment to human tendon pathologies.

References
