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Platelet-rich plasma for enhancing surgical rotator cuff repair: evaluation and comparison of two application methods in a rat model

Ali Ersen · M. Demirhan · A. C. Atalar · M. Kapicioğlu · Gokhan Baysal

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Abstract
Purpose Platelet-rich plasma (PRP) is a natural concentrate of autologous growth factors now being widely tested in different fields of medicine for its potential in enhancing the regeneration of tissue with low healing potential. However, studies of PRP in enhancing rotator cuff repair have been contradictory, perhaps because of how PRP is administered. The purpose of this study is to evaluate the effect of PRP and compare two different application methods of PRP on rotator cuff healing.
Methods The supraspinatus tendons of 48 mature, male Wistar–Albino rats were detached from their insertion on the humerus. The animals were divided into four groups: (1) no repair, (2) primary repair, (3) repair plus PRP injections into the tendon–bone interface, and (4) repair plus PRP absorbed from a sponge carrier to the tendon–bone interface. The tendons were evaluated biomechanically and histologically at week 8.
Results Cuffs repaired with PRP had significantly greater mean (SD) load-to-failure rates [11.1 (6.5) and 11.6 (3.9) N; $P < 0.05$] and stiffness [3.5 (2.3) and 1.6 (0.75) N; $P < 0.05$] than did cuffs repaired without PRP. The groups receiving PRP did not differ significantly on these variables. Histological evaluation showed no significant differences among the four groups.
Conclusions The application of PRP, independent of the application method, significantly improved biomechanical properties at the rotator cuff tendon–bone interface. The type of application, injection or absorption from a sponge did not influence the effect of PRP on rotator cuff healing.

Keywords Rotator cuff · Platelet-rich plasma · Rat model

Introduction

Mechanical improvements in rotator cuff repair, such as new suture materials and the double-row technique [1–3], have greatly improved clinical results. However, failure rates remain between 5 and 90 % [4–7]. These high rates have stimulated research into new biologic methods to enhance bone-tendon healing [8–10].

Platelet-rich plasma (PRP) is increasingly used in orthopedic surgery, especially in sports medicine, because it is believed to accelerate healing, tissue regeneration, and return-to-play. Several studies show that PRP can enhance healing in soft-tissue injuries, cartilage defects, osteoarthritis, and even fractures and non-unions [11–16]. In general, PRP accelerates the natural healing cascade by elevating the concentrations of cytokines that are released during platelet degranulation, probably through stimulating capillary regeneration [17–19].

Studies of PRP in enhancing rotator cuff repair have been contradictory [20, 21]. These contradictory results may be

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This study was approved by the Istanbul University Animal Studies Ethical Committee.

A. Ersen (✉) · A. C. Atalar · M. Kapicioğlu
İstanbul Medical Faculty Department of Orthopaedics and Traumatology, Istanbul University, Topkapı, 34390 Istanbul, Turkey
e-mail: ali_ersen@hotmail.com

M. Demirhan
School of Medicine, Department of Orthopaedics and Traumatology, Koc University, Istanbul, Turkey

G. Baysal
Faculty of Mechanical Engineering, Department of Strength of Materials, Istanbul Technical University, Istanbul, Turkey

caused by the lack of a standard definition and use of PRP. Several PRP products are available, and they have different platelet concentrations, contents, and different application methods (percutaneous injection [23], topically with a sponge [24], or post-arthroscopy [21, 22]). In a systematic review of in vivo studies evaluating the effect of PRP in tendinopathies, Achilles tendon injuries, ACL reconstruction and rotator cuff repair, differences in PRP preparation and applications methods were reported as remaining controversies for the use of PRP in sports medicine [25].

The effect of these differences on surgical outcomes has not been studied.

Only two randomized clinical trials have assessed the efficacy of PRP in arthroscopic rotator cuff repair. One of these trials, by Castricini et al. [21], does not support the use of autologous PRP for augmenting rotator cuff repair. Another randomised controlled study by Randelli et al., showed no statistical difference in the risk of retear between patients treated with PRP and those treated without PRP. Therefore, more in vivo animal studies are necessary to determine the optimal application method and dosing of PRP.

In this study, we aimed to evaluate the biomechanical and histological effects of two methods for applying PRP—direct injection and absorption from a sponge—on healing after surgical repair of rotator cuffs in a rat model.

Materials and methods

The institutional animal care and use committee approved the protocol.

Skeletally mature, male Wistar albino ($n = 48$; 250–350 g) rats were housed individually before and after the surgery and had unrestricted access to water and their normal daily diet. The number of animals used for this was based on previous studies such as that of Galatz et al., which reported statistically significant results with similar sample sizes to ours [26].

We used an injury model that approximates an acute rotator cuff injury, as opposed to a chronic degenerative rotator cuff tear. After administering anesthesia with xylazine and ketamine we surgically detached the supraspinatus muscle in the right shoulder of each rat from its origin on the greater tuberosity. Briefly, an incision was made over the craniolateral aspect of the scapulohumeral joint. The deltoid muscle was detached sharply from the posterior, lateral, and the anterior aspects of the acromion and split distally. The supraspinatus was detached sharply at its insertion on the greater tuberosity (Fig. 1).

The rotator cuff was repaired by drilling a transverse, anteroposterior 0.5-mm hole through the proximal part of the humerus. Any fibrocartilage at the insertion was removed by scraping with a scalpel blade. The free end of the tendon was grasped with a double-armed 4-0 Prolene suture with a technique similar to the Mason–Allen method. The suture was passed through the drill-hole, and the tendon was reattached to its anatomic position.

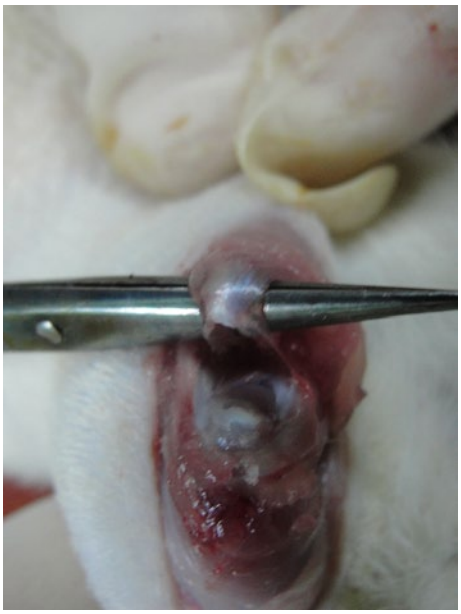

Fig. 1 Sharp detachment of the supraspinatus muscle from its origin

The rats were divided without known bias into 4 groups of 12. In an untreated control group, the supraspinatus tendon was cut from its origin and left without any treatment. In a primary-repair-only group, the tendon was cut and repaired with 4/0 polypropylene suture. In the injected PRP group, PRP 0.3 mL was injected directly into the repair site (Fig. 2). In the absorbed PRP group, PRP 0.3 mL was infused into a 0.5 cm × 0.5 cm-absorbable hemostatic

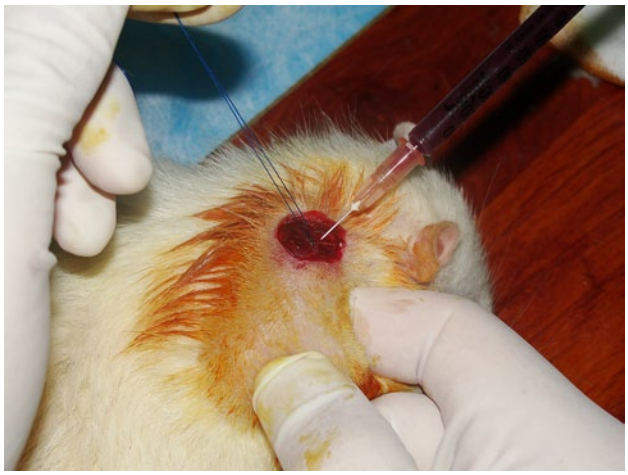

Fig. 2 Injecting platelet-rich plasma into the repair site

Fig. 3 Close-up of the tendon–bone junction

gelatine sponge (SPONGOSTAN, Ethicon, Inc.;), which was secured on the repair site.

All rats were killed at 8-week after surgery, and tendon-humerus unit of each was dissected. The deltoid muscle and acromion were removed. The supraspinatus muscle was removed from the supraspinatus fossa leaving the tendon–bone junction intact (Fig. 3). The supraspinatus tendon was tested for its biomechanical properties in 10 rats from each group and analyzed histologically in 2.

PRP preparation

After general anesthesia with xylazine and ketamine, a total of 30 mL of whole blood from three rats was collected through intracardiac aspiration and mixed with 3 mL of the anticoagulant, citrate dextrose A. PRP was prepared with the GPS 3 MINI SYSTEM (Biomet Biologics) according to the manufacturer's instructions.

After centrifugation at 3,200 for 15 min, red blood cells, PRP, and platelet-poor plasma were separated. This process produces 4 mL of PRP. We determined the total platelet count in 1 mL of prepared PRP and confirmed the count with a phase-contrast microscopic platelet count. We used this process twice, once for each of the two methods of application, and from each process divided 3 mL of PRP into ten, 0.3 mL portions for each rat in these two groups. The concentration of the platelets in two groups receiving PRPs was 609.000/μL (injected) and 710.000 μL (absorbed). Control platelet counts in the blood from which the PRP was prepared for this group, were 53.000/μL and 62.000 μL.

Biomechanical evaluation

All 10 specimens from each group were evaluated biomechanically on the same day as dissection.

Fig. 4 Biomechanical testing set-up

Specimens were thawed, and the humerus was embedded in an aluminium tube with chemical polyester putty and clamped with its long axis in the horizontal plane. The proximal end of the supraspinatus tendon was compressed between two pieces of sandpaper, and the sandpaper-tendon complex was clamped vertically in a soft tissue clamp. Testing was performed with the shoulders at 90º of abduction with a material testing machine (Universal Material Testing Machine, MTS 858 BIONIX II; (Fig. 4). Specimens were subjected to a preload of 0.2 N and tested to failure intension at a rate of 5 mm/min. Load-elongation graphs were drawn for each specimen, and failure load was determined as the peak values on these graphs.

Histologic evaluation

Two specimens from each group were also analyzed histologically. Blocks containing the tendon–bone complex were stored in 10 % formalin within a week for fixation. After fixation, tissues were stored in 8 % nitric acid for 36 h for decalcification. Specimens were embedded in paraffin and sectioned at 5 μm. Sections were stained with hematoxylin and eosin to examine cell morphology and with Mason-tricrom to better evaluate collagen and bone tissue. Tissue sections and tendon–bone junctions were evaluated by a pathologist for collagen fiber alignment, inflammation, fibrosis, and foreign body reactions.

The pathologist had 20 years of experience and was not informed of the purpose of the study.

Table 1 Biomechanical properties of surgically repaired supraspinatus tendons in a rat rotator cuff model, with and without the administration of platelet-rich plasma

Biomechanical property	Type of rotator cuff repair in mature male Wistar rats[a]			
	No repair[b] ($n = 10$)	Primary repair[b] ($n = 10$)	Repair + injected PRP[b, c] ($n = 10$)	Repair + topical PRP[b, c] ($n = 10$)
Failure load, mean (SD), N	3.58 (2.35)	1.66 (0.75)	11.12 (6.53)	11.64 (3.93)
Displacement mean (SD), mm	2.69 (2.0)	3.23 (0.96)	4.84 (3.78)	6.42 (4.41)

[a] The four groups differed significantly on both measures ($P < 0.005$)

[b] Post hoc analysis showed that failure load rates in the two PRP groups were significantly higher then those of the two control groups ($P < 0.005$)

[c] The two PRP groups did not differ significantly ($P > 0.5$)

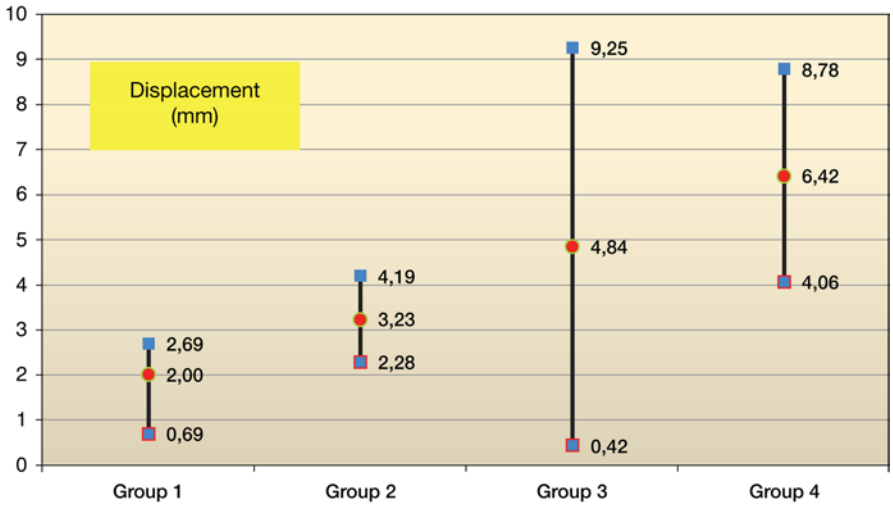

Fig. 5 Biomechanical results of four groups—Displacement

Statistical methods

Continuous variables are described as means and standard deviations. The Mann–Whitney *U* test (For comparison of nonparametric data between two independent groups) and the Kruskal–Wallis test (For comparison of nonparametric data between four independent groups) were used to evaluate differences among the four groups. Alpha was set at 0.05, and all tests were two-tailed. Histological results were not compared statistically. All data were analyzed with the SPSS statistical software package, version 11.0.1 for Windows.

Results

No complications related to the surgery or PRP application were observed during the treatment or the 8-week healing period.

Biomechanical results

Both failure loads and displacement were significantly higher in the two PRP-treated groups than in the non PRP-treated groups. The two PRP-treated groups did not differ significantly from each other on either biomechanical measure (Table 1; Figs. 5, 6). Suture pullout from the tendon was the mode of failure in all biomechanical evaluations.

Histological results

The tendon–bone complexes of two specimens from each group showed no obvious differences among the four groups in inflammation, fibrosis, or foreign body reactions, save for an irregularity at the attachment site of the tendon from the two groups not receiving PRP. The collagen fiber orientation in PRP-treated groups (group 3–4) was more mature oriented and aligned.

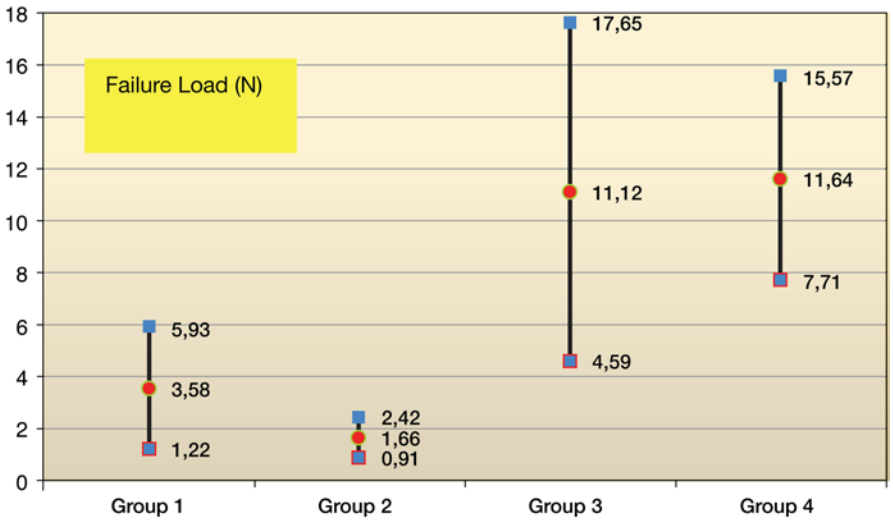

Fig. 6 Biomechanical results of four groups—Failure Load

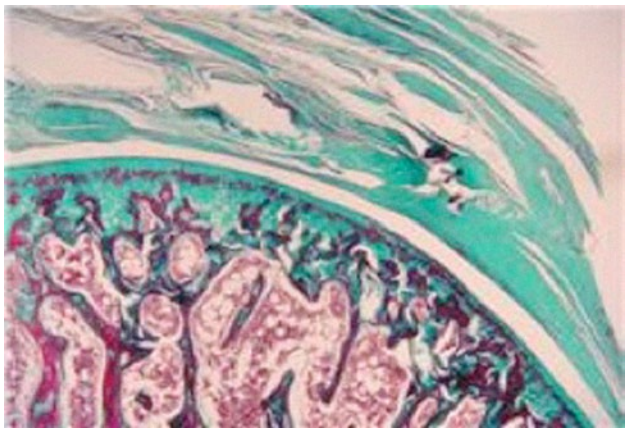

Fig. 7 Histological specimen from a rat undergoing only primary repair, without platelet-rich plasma

Perivascular inflammatory cells were visible around the suture material in the three surgically repaired groups; however, there was no inflammatory infiltration into the tendons. Sharpey-like fibers were present in all repair groups. (Fig. 7).

Discussion

We evaluated the biomechanical effects of PRP in a rat rotator cuff repair model applied either by direct injection to the repair site or by absorption from a sponge that kept the platelets and growth factors at the repair site longer than did the direct injection. Our results support the positive biomechanical effects of PRP in this rotator cuff repair model and indicate that the application method probably does not influence these positive effects.

The use of PRP to enhance bone regeneration and soft tissue maturation in patients undergoing maxillofacial and orthopedic surgery continues to increase. At first, PRP was used mostly to treat chronic tendinopathies. Compared to steroid injections, PRP provided superior results and marked symptom relief for treating lateral epicondylitis [27, 28], and it has also been useful in treating patellar tendinitis [29]. Acute tendon and muscle injuries healed faster with less fibrotic tissue when injected with PRP [30, 31]. Even in patients with osteoarthritis or chondral defects, intra-articular PRP treatment provided substantial pain relief [32]. Sports medicine researchers incorporated PRP into anterior cruciate ligament repair and reported substantially faster graft maturation in the tunnel [33]. MRI studies comparing the anterior cruciate ligament grafts with or without PRP augmentation showed faster graft maturation in PRP augmented groups [34, 35].

Repaired rotator cuffs heal through a scar tissue interface that makes them prone to failure. Functional results are correlated with the integrity of the rotator cuff [36].

New methods to improve the quality of tendon–bone healing in the rotator cuff are being developed. Recombinant human bone morphogenic protein-12 (Rh-BMP-12) augmentation accelerated healing in a sheep rotator cuff repair model [37]. The same researchers improved tendon attachment strength with osteoinductive growth factors in the sheep rotator cuff model [24].

Augmenting rotator cuff repair with PRP could hypothetically optimize the biologic environment at the repair site and allow for a more robust healing response at the osseous-tendon interface. The intraoperative use of PRP to augment rotator cuff repair is becoming popular among shoulder surgeons.

Controversies about the use of PRP center on the fact that most studies in humans are small case series without control groups. Preparations, dosing, and the composition of PRP are also not standardized, and the heterogeneity

of PRP preparation systems can influence the results. The presence of leukocytes in the end PRP product is one of the most important differences between PRP preparation systems. The destructive effects of proteinases derived from neutrophils are well known; therefore, PRP products containing leukocytes can increase inflammatory response and tissue damage. The PRP preparation system we used does not filter leukocytes. However, histological evaluation detected no exaggerated reactions.

The concentration of platelets in PRP can also influence the results. A platelet concentration of 1,000.000/μL provides the optimal biologic effects of PRP. This concentration is 3–5 times that of the platelet concentration in human blood. Lower concentrations are suboptimal and inhibit healing [38]. In our study, concentrations of platelets in PRP application groups are lower than the optimal concentration of 1,000.000/μL; however, the platelet concentrations were more than 10 times that of unprocessed rat blood. This increased concentration may be one reason of improved biomechanical properties.

The optimal application method of PRP remains to be determined. Direct injection and absorption are used in most studies. Injection is less invasive, easier, and can be done percutaneously, and in our study, injected PRP produced results similar to those of absorption from the sponge.

Our study does have some limitations. We histologically evaluated only two rats from each group. In a histological study evaluating the influence of PRP to sheep, Achilles tendons differences in morphometric features of fibroblast nuclei, mature collagen fiber orientation and lower vascular densities were shown as evidence of an advanced healing process [39]. In our study only a gross histological evaluation is performed instead of a detailed numerical histological analysis.

We found no obvious differences except for more mature orientation of the collagen fibers in PRP-treated groups (group 3–4), but a larger sample and detailed analysis might reveal more differences. We also repaired normal tendons rather than degenerated and retracted ones, which would have been more clinically relevant.

Conclusion

The application of PRP, independent of the application method, significantly improved biomechanical properties at the rotator cuff tendon–bone interface. The type of application, injection or absorption from a sponge did not influence the effect of PRP on rotator cuff healing.

Conflict of interest There is not any potential conflict of interest.

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