Platelet Rich Plasma Protects Rat Achilles Tendon From The Deleterious Effects Of Steroid.

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Introduction: Triamcinolone acetonide (TA) injections are widely used for treatment of enthesopathy with the possible side effects of tendon rupture and impairment. Many cases of tendon rupture after TA injections have been reported in the literature (1). Platelet-rich plasma (PRP) is a blood fraction containing high platelet concentrations that provides various growth factors, including platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), fibroblastic growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and epidermal growth factor (EGF) that participate in tissue repair processes (2). Previously we reported that in in vitro study TA decreased viability of human rotator cuff derived cells and caused apoptosis, and these deleterious effects were prevented by combination of TA and PRP. On the other hand, the effects of TA and PRP on tendon in vivo has not been elucidated yet. The purpose of this study was to investigate whether addition of PRP on TA can protest the deleterious effect of TA injection in rat Achilles tendon.

Methods: Animal model
Twenty four, 10-week-old, male, Sprague-Dawley rats were used. These were randomly divided into 4 groups: a saline injection group (control group), a TA injection group, TA and PRP injection group (TA+PRP group) and a PRP injection group. In each group, the ankles of each animal were shaved and 50 µL of each solution was injected percutaneously at Achilles tendon-calcaneus junction into the surface of the right Achilles tendon with a 26-gauge needle. At 1 week after injection, the rats were sacrificed and their Achilles tendons were harvested. Two specimens from each group were analyzed histologically and 4 specimens were stored at -80°C in saline solution-soaked gauze after isolation of the Achilles tendon-calcaneus complex until used for biomechanical testing.

PRP preparations
Whole blood was corrected from cardiac puncture from healthy rats then centrifuged at 2,400 rpm for 10 minutes to separate PRP and platelet-poor plasma portion from the red blood cell fraction. A second cycle of centrifugation followed at 3,600 rpm for 15 minutes to separate PRP from platelet-poor plasma (PPP) (3).

Biomechanical Testing
The specimens were thawed. The proximal Achilles tendons were covered with gauze and sutured with nylon yarn. The tendon and the calcaneus was placed in specially designed devices, using polymethylmethacrylate (PMMA) resin, and placed vertically in a tensile strength sensor. Before the tensile test was performed, the tissues were preconditioned with a static preload of 0.2 N for 1 minute, followed by 5 cycles of loading and unloading at a strain amplitude of approximately 0.5%, at 60 mm/min. Immediately after preconditioning, the maximum failure load was recorded at a uniaxial tension of 60 mm/min. The maximum failure load was measured as the primary outcome, and the tendon stiffness was calculated from the load-deformation curve.
Histological analysis
Tissue samples were fixed in 4% paraformaldehyde for 24 h, decalcified with 0.25 M ethylenediaminetetraacetic acid in phosphate-buffered saline (PBS), dehydrated in a graded series of alcohol solutions, and embedded in paraffin wax. Long-axis sections (6-μm thick) were H-E stained and assessed. The histological findings regarding the cellular response were assessed by using a light microscope. Picro-sirius red stain binds specifically to collagen fibers. Long-axis sections (6-μm thick) were stained by using a Picrosirius Red Stain Kit. Deparaffinized sections were stained in Picro-sirius Red for 60 min and washed in acidified water. Under polarization microscopy, collagen attenuation by corticosteroids were assessed. Type I collagen was stained in yellow and type III collagen was stained in green.

Results: Mechanical evaluation
The samples ruptured at the Achilles tendon-calcaneus junction. The maximum failure loads were 29.8 ± 0.9 N in the control group, 19.1 ± 2.2 N in the TA group, 31.29 ± 2.8 N in the TA+PRP group and 28.0 ± 5.5 N in the PRP group. The tendon stiffness were 10.7 ± 0.9 N/mm in the control group, 6.3 ± 0.9 N/mm in the TA group, 10.0 ± 2.0 N/mm in the TA+PRP group, and 10.5 ± 1.5 N/mm in the PRP group. The failure load and stiffness were significantly lower in the TA groups than in the control group. There was no significant difference between control group and the TA+PRP group. (Figure 1)

H-E staining
The saline injection control group and PRP group showed normal tendon appearances. Cell invasions and vacuolations at the surface layers of the tendons were seen in the TA group. However, the apparent changes in the tendons were not seen in the TA+PRP group. (Figure 2)

Picro-sirius red staining
In the TA group, collagen fiber bundles were aligned irregularly, and they appeared denser with fiber attenuation at the surface of the tendon. The saline injection control group, TA+PRP group, and PRP group showed normal collagenous fibers arranged in compact, parallel bundles. Under polarization microscopy, there were type III collagen expressions at the surface of the tendon in the TA group. However, in the TA+PRP group the type III collagen expression was not observed. (Figure 3)

Discussion: Previous report showed TA significantly decreased cell viability and caused cell apoptosis in cultured human rotator cuff-derived cells, and this deleterious effect was prevented by the simultaneous administration of PRP in in vitro study (4). However, there is no report on the effects of a combination of TA and PRP in in vivo model. This study showed that exposure to TA significantly decreased mechanical strength and caused histological changes, and these deleterious effects were prevented by the administration of PRP.

Significance: TA significantly decreased mechanical strength and caused histological changes in rat Achilles tendon, and this deleterious effect was prevented by the simultaneous administration of PRP. Therefore, the combination of TA and PRP could be considered as one of useful treatments for enthesopathy.

Reference:
(1)Kleinman Me et al; The Journal of bone and joint surgery American volume.1983
(2)Everts PA et al; The Journal of extra-corporeal technology. 2006
(3)Pantou AL et al; Cell and tissue banking. 2010
(4)Muto T et al; Journal of orhopaedic research. 2013
Figure 1

(a) Maximum failure load (N) for different groups. TA+PRP shows a significant increase compared to TA and Control. PRF also shows a slight increase.

(b) Stiffness (N/mm) for different groups. TA+PRP shows a significant decrease compared to TA and Control. PRF shows a slight increase.

Figure 2

(a) Control tissue showing normal histology.

(b) TA-treated tissue with increased cell proliferation and neovascularization.

(c) TA+PRP treated tissue showing a reduction in cell proliferation compared to TA.

(d) PRP treated tissue showing minimal changes compared to Control.
Figure 3

a) control

b) TA

c) TA+PRP

d) PRP

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