ALLOGENIC PLATELET’S GROWTH FACTORS INCREASED ACHILLES TENDON CALLUS STRENGTH IN RATS

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Abstract

The study was conducted on 64 adult male Wistar rats. The experimental group consisted of 32 animals with experimental cut of the Achilles tendon and local injection of allogenic platelet rich plasma. The control group comprised 32 rats with cut of the tendon and saline local injection. The animals were euthanized after 7, 14, 21, and 42 d. The mechanical testing of the tensile strength of the tendons was performed on an universal testing machine. Biomechanical testing was performed at 7, 14, 21, and 42 d after cutting the Achilles tendons. The maximal breaking force (Fmax), force at the end of proportional range (Fs), and stiffness of the tendons (H) were evaluated. At 7 d postoperative, there were no significant differences in Fmax (P=0.53) and Fs (P=0.48) of the Achilles tendons among the groups. Significant differences between the examined and control groups in the H (P=0.021) were found at day 7 of the experiment. Mechanical testing results in the mean Fmax, Fs, and H values were significantly higher in experimental group in comparison to controls at 14, 21, and 42 d (all P<0.05). Growth factors from allogenic platelet rich plasma administered locally increased significantly the tensile strength of rats’ Achilles tendon callus after experimental cut at 14, 21, and 42 d.

Key words: rats, tendon, platelet rich plasma, mechanical testing.

Clinical application of growth factors could be clinically effective with the use of platelet cells. These cells after activation release growth factors such as platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), epidermal growth factor (EGF), fibroblast growth factor (FGF), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), and connective tissue growth factor (CTGF). Each growth factor has its own characteristics and mode of action (1, 6, 10). The process of tendon healing is relatively slow because of reduced vascular supply in tendon and small amount (5%) of cells (21, 26). Although most tendons have the ability to heal spontaneously, the callus tissue, that is formed, is usually mechanically inferior, more susceptible to further injuries, and therefore much less able to perform the functions of a normal uninjured tendon (15). Improvement of callus tissue strength might be beneficial for the injured individuals.

Therefore the aim of the study was the evaluation of the influence of growth factors from allogenic platelet rich plasma administered locally on callus tensile strength of the rat Achilles tendon.

Material and Methods

The experiment was conducted on 64 adult, male Wistar rats. The guidelines for the care and use of laboratory animals were followed. The Local Ethics Committee approved the experiment. Licensed Laboratory Animal Farm (Warsaw) prepared all animals. Before the study, all the animals had 14 d acclimation to the experimental conditions. During the study, rats had stable conditions with unlimited access to food and water. Weight of the animals did not differ between groups both before and after the study.

The rats were anaesthetised using 97% 2,2,2–tribromethanol administered intraperitoneally (0.2 g/kg) (8, 18). The Achilles tendon was transversely cut 5 mm proximal to its calcaneal insertion and a 2 mm long segment was removed and left unrepaired (2, 17). The plantaris tendon was removed (2, 24). The animals were randomly divided into two groups. The animals from experimental group (n=32) received just postoperatively injection of 0.1 ml of allogenic platelet rich plasma (PRP) into resection area. The control group (n=32) was given injection of 0.1 ml of 0.9% NaCl into resection area. The allogenic platelet concentrate was prepared from the blood of 12 rats. Three donor rats were sufficient for an experiment on eight recipient rats. The groups of eight rats (both experimental and control) were euthanised after 7, 14, 21, and 42 d after the operation. The Achilles tendon with the attached calcaneal bone was dissected from surrounding tissues and removed (24). The specimen was mounted onto the universal testing machine (UTM, Lloyd, LRX, UK) with tool head 500 N. The tendons were then loaded to failure...
at a constant rate of 10 mm/min. The maximal braking force (Fmax, N), force at the end of proportional range (Fs, N), and lengthening (L, mm) were measured. The stiffness of the tendons (H, N/mm) was calculated as a tangens of linear region of force-lengthening curve. The Fmax was the value of highest point of force-lengthening curve and the Fs was the value of the end point of linear region of the same curve. It means the force value at the limit of resilience (16, 24).

Statistical analysis was performed with Statistica 8.0 PL. All results are expressed as mean ± SD. Differences among groups were evaluated using the t-test or the nonparametric Mann-Whitney U test, for parametric and nonparametric data, respectively. The difference at P<0.05 was considered statistically significant.

Results

The platelet concentration in PRP injected rats at 7, 14, 21, and 42 d was 7.6; 9.6; 8.8, and 7.6 times higher, respectively, in comparison to concentration observed in the donor blood.

During the entire experiment all animals were active, postoperative wounds healed without complications. On visual examination, the Achilles tendon in all groups showed signs of repair, with fibrous scar formation around the injury sites. No macroscopic differences between experimental and control groups were observed. The Fmax, Fs, and H of the Achilles tendon in each group increased in a time-dependent manner. At 7 d postoperative, there were no significant differences in maximal breaking force (P=0.53). At 14, 21, and 42 d, the mean Fmax values in tested group were significantly higher in comparison to control (P=0.014, P=0.004, and P<0.001, respectively). The mean value of the Fmax at 42 d in experimental group was two times higher than the value observed in the control group. The mean values of the Fmax (N) at 7, 14, 21, and 42 d are presented on Fig. 1. The mean value of stiffness of regenerated tendons in the experimental group was significantly greater at 7, 14, 21, and 42 d (P=0.021, P=0.007, P<0.001, and P<0.001, respectively) than that in control. The mean values of stiffness in the groups were as follow: experimental group - 10.21 ±3.41 at 7 d, 17.98 ±4.03 at 14 d, 22.54 ±3.35 at 21 d, and 48.42 ±19.22 at 42 d, control group - 6.6 ±1.93 at 7 d, 12.14 ±3.37 at 14 d, 15.08 ±2.39 at 21 d, and 15.88 ±4.18 at 42 d. At the end of experiment (42 d) the stiffness of tendons for experimental group was three times greater in comparison to control animals. Fig. 2 presented trend line of the mean stiffness during the study.

An increase in the Fs (N) on days 7, 14, 21, and 42 of the experiment was more prominent in the experimental group (16.17 ±5.87 at 7 d, 39.26 ±8.36 at 14 d, 38.86 ±4.25 at 21 d, and 70.45 ±18.63 at 42 d) in comparison to the control group (14.45 ±3.3 at 7 d, 27.78 ±6.85 at 14 d, 26.42 ±8.82 at 21 d, and 31.01 ±8.02 at 42 d). After day 7, there were no significant differences in the Fs (P=0.48). Statistically significant differences in the mean value of the Fs between both groups were observed on days 14, 21, and 42 (P=0.009, P=0.003, and P<0.001, respectively). After 42 d of the experiment, the Fs values in experimental group were over twice as high as the values of control group.

Discussion

During the last two decades several studies have been done analysing the effect of platelet rich plasma (PRP) on the tissue healing. First clinical studies described the use of growth factors from platelets to enhance bone defect healing in dentistry (13, 23). Encouraging results gave assumption to its use in orthopedic surgery, plastic surgery, and neurosurgery (12, 19). Recently clinical application of the PRP has gained popularity for stimulation of bone healing and stimulation of soft tissue repair. Platelet rich plasma is defined as a portion of the plasma fraction of autologous blood having a platelet concentration higher than in donor plasma. However, it is likely that the clinical effect of the PRP is a function of platelet concentration, there is no single recommendation for the degree of an increase in platelets in PRP over its baseline in donor blood (5, 7, 14). In our experiment, we prepared PRP with 7.3 to 9.6 fold increased platelet concentration over
baseline. This is consistent with other studies, which indicate that PRP should achieve a 2.5 to 8.5-fold increase in platelet concentration over that is observed in the donor blood (20, 22).

Numerous growth factors that are contained within the α-granules of platelets have strong influence on tendon repair. Anitua et al. (2) reported accelerated tendon cell proliferation, stimulation of synthesis of type I collagen, stimulation of endogenic HGF and VEGF synthesis, and promotion of neovascularisation after PRP application. The authors showed a potent effect on neovascularisation in sheep tendons as a result of PRP therapy.

In our study, we evaluated the effect of growth factors from PRP on mechanical strength of the regenerated tendon in a rat Achilles tendon model. To assess mechanical properties of regenerated Achilles tendon, the plantaris tendon was removed. Zhang et al. (26) in their experiment on effect of VEGF on rats’ Achilles tendon healing preserved the plantaris tendon, which is more developed in rats than in humans, to act as an internal split. The authors proved that preservation of the plantaris tendon enhances mechanical properties of the newly formatted tendon callus during first 2 weeks.

In our study, we eliminated the potential effect of the plantaris tendon on mechanical properties of the Achilles tendon. It is well described that mechanical stimulation improves tendon repair (3, 4); however, animals used in our study were not immobilised. The proper method of immobilisation is technically demanded and has undesired side effects. We believe that analogous lack of immobilisation for rats from both groups creates better experimental model for assessing the effect of PRP. Hou et al. (9) demonstrated that injured Achilles tendons in rabbits treated with TGF-β1 BMSCs healed more rapidly and more completely than non-treated, and demonstrated higher strength of the callus and faster matrix remodelling. We have also shown that the values of maximal breaking force and force at the end of proportional range in the examined groups were significantly higher than those in control group, at the same time points (2, 3, and 6 weeks). This finding leads to the conclusion that the application of plasma growth factor has a positive effect on tendons resistance to stretching. The higher mean values of maximum breaking forces in experimental group were reflected by an accelerated fibroblast proliferation, fibrillogenesis, and matrix remodelling as a result of the growth factors action. An increase in stiffness of healing tendon is a result of progressive fusion and collagenous fibers parallel organisation (25). In previous studies it has been shown that the maximal breaking force and stiffness of the healing tendon were increased by TGF-β1 after 2 and 4 weeks (11). In our experiment stiffness increased in both groups but it was considerably higher in experimental group, reaching the statistical significant differences since the first week. The values of maximal breaking force and force at the limit of resilience from biomechanical testing of the Achilles tendons were similar to those from Kashiwagi’s study (11). We found the values to be significantly higher since the second week of the experiment. It should be mentioned that we used the PRP in opposite to isolated TGF-β1. Zohar et al. (27) demonstrated that platelet derived growth factor (PDGF-BB) and TGF-β could act synergistically on the migration and proliferation of rat bone marrow derived cell (RBMC) with other growth factors found in PRP.

In our study, significantly bigger mechanical callus strength in the experimental group was apparent at 14 and 21 d. This is in accordance to data published by Aspenberg and Virchenko (3). The biggest differences were seen at 42 d, where the mean force at failure was more than 2-fold bigger in experimental group in comparison to control. It should be emphasised that our study had a longer follow-up.

We would stress that the tendon injuries in our study were induced under controlled and standardised conditions. This does not represent the clinical situation where tendons are usually injured by uncontrolled tensile loading. Another difference is the presence of predisposing factors. In the nature, tendon’s rupture usually follows the degenerative changes. However, our results have shown that local allogenic PRP application can significantly improve the callus strength of an experimentally transected tendon in which two ends are left non-repaired. The results of our study may serve as a guide for future studies on the clinical PRP treatment approach either in cases of partial rupture or after surgical repair for total rupture.

References