



The Effect of Platelet-Rich Plasma Application on Recovery of Contusional Muscle Injury in Trauma Induced Rabbits

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KEYWORDS Dystrophic Calcification. Muscle Injury. Platelet Rich Plasma. Rabbits. Trauma

ABSTRACT The purpose of this paper was to evaluate the effect of platelet-rich plasma (PRP) application after the contusion muscle injury. In the study, New Zealand type rabbits were placed into a trauma model. The rabbits were divided into three groups. In the 1st group PRP was applied just after the trauma, in the 2nd group PRP was applied one day after the trauma and in the 3rd group PRP was not applied. Fibrosis decreased in the groups in which PRP was applied in the first day or in the first hour but fibrosis increased in the control group steadily. Dystrophic calcification developed less in the group in which PRP was applied in the first hour compared with the control group. It is concluded that particularly with its effect on dystrophic calcification, PRP may reduce the possibility of re-injuries and help athletes in terms of returning to sports more quickly.

INTRODUCTION

Muscle contusions are one of the most common injuries in athletes and the frequency ranges between 10 to 50 percent. Muscle injuries occur in the form of contusions, strains or lacerations. Contusions and strains constitute 90 percent of all injuries. Contusions occur when muscles are exposed to a sudden, severe and compressive force (Jarvinen et al. 1993; Crisco et al. 1994; Garrett 1996).

Almost any tissue in the body heals with scars following a wound. Healing of injured muscle tissue, regardless of the nature of the injury, displays a particular pattern (Hurme et al. 1991). The healing process consists of the destruction, repair and remodeling phases. The cells reaching first to the injured region are activated platelets. There are several growth factors (GFs) in the alpha (α) granules found in platelets (Nurden 2011). Platelet derived growth

factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), basic fibroblasts growth factor (bFGF), insulin-like growth factor-1 (IGF-1) and transforming growth factor beta-1 (TGF-β1) are some of them (Beiner 2001). It has been shown that GFs in platelet-rich plasma (PRP) regulated the inflammatory phase and improved recovery (Nisbet et al. 2009). There is not one overarching mechanism of action of PRP given the differences in platelet content, resulting growth factor concentrations, and varying downstream cellular responses. What is known is that PRP contains a high concentration of platelets and that these platelets, once activated, release numerous growth factors into the surrounding environment. The resulting cellular effects are both pro-inflammatory and anti-inflammatory in nature and appear to be dependent on many different factors including the stage of the natural healing process, the site of the injury, and cellular environment (Kellie et al. 2012). Neutralization of TGF-β1 within PRP is a promising approach to specifically neutralize TGF-β1 within PRP and the injured skeletal muscle while eliminating potential side effect that can occur after a systemic neutralization of TGF-β1 (Hongshuai et al. 2016). Neutralization of TGF-

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β 1 within PRP is a promising approach to improve PRP's beneficial effect on skeletal muscle repair while decreasing deleterious fibrosis (Justin et al. 2016).

During the 2nd day after injury, the macrophages start clearing necrotized muscle fibers. Fibroblasts initiate the scar formation of connective tissue. During the 3rd day, satellite cells become active in the regeneration zone. During the 5th day, myoblasts form myotubes by joining in the regeneration zone (RZ). Connective tissue become intense in the RZ. During the 7th day, the regenerated muscle cells extend towards the regeneration zone and penetrate into the scar tissue. During the 14th day, scar tissue in the RZ shrinks and starts to integrate with the normal tissue by the 21st day (Hurme et al. 1991).

Muscle contusion causes pain in athletes and leads to lack of training accordingly. Although several treatment options exist, the time to return to play (RTP) is about six weeks (Wright-Carpenter et al. 2004). Injuries increase in the intensive training period of athletes. This weakens the athlete physically and psychologically and delays getting into the competitions before being ready. Repeated injuries tend to become chronic. Hence, methods to decrease the time to RTP by reducing the inflammation process are needed during these injuries.

In this paper, the effect of PRP application during the 5th day after the contusion injury was evaluated in terms of fibrosis and necrosis. Additionally, the effects in the muscle, when PRP was given immediately after and the day after the injury was investigated.

METHODOLOGY

This study was held in the Experimental Animal Production and Experimental Research Laboratory with the decision of the Ethics Committee for Animal Experiments of Suleyman Demirel University. In this study, 20 New Zealand type rabbits weighing between 2400-2700 g were used. All of the rabbits were fed with standard rabbit feed and kept separately in cages of the same scale. Their health status was frequently checked. During the study, the rabbits were fed with the same food in same amount.

A device similar to those used in the literature was designed to generate contusion injury. The rabbits were placed into the trauma model while they were under anesthesia and the part where the weight would fall was determined. The hair in that part were shaved. In the trauma mod-

el, an iron of 272 g was dropped on the rabbits' M. Gracilis muscle near the femur from a height of 40 cm while keeping their knees in hyperextension (Fig. 1).

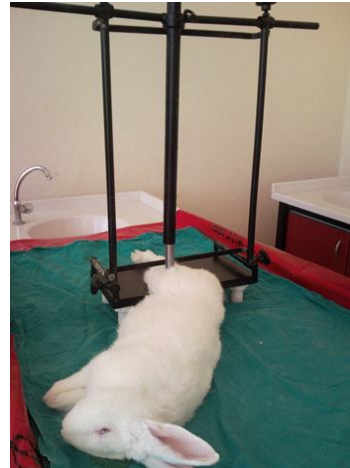


Fig. 1. Dropping of weight

PRP was obtained with the Curasan method. PRP of 0.6-0.7 ml was obtained by taking 9 ml of venous blood and centrifuging at 2400 rpm for 10 minutes at first and then at 3600 rpm for 15 minutes then (Ferreira et al. 2005) (Fig. 2).



Fig. 2. Heraeus Labofuge centrifuge device

The rabbits were divided into three groups. The 1st group was the study group which PRP was applied just after the trauma (Group A), the 2nd group was the study group which PRP was applied 1 day after the trauma (Group B), the 3rd group was the study group which PRP was not applied (Control group).

In this study, no death occurred in any of the groups. In the extremity of rabbits in which trauma was created, skin ulceration or wound infection did not occur. At the end of the fifth day, the euthanasia procedure was performed in the same session for all rabbits.

After the tissue sections were taken, they were stained with Masson's trichrome stain for the assessment of Hematoxylin Eosin (HE) and fibrosis (Fig. 3). The evaluation was performed in the binocular double-headed light microscope of Olympus BX50 brand.

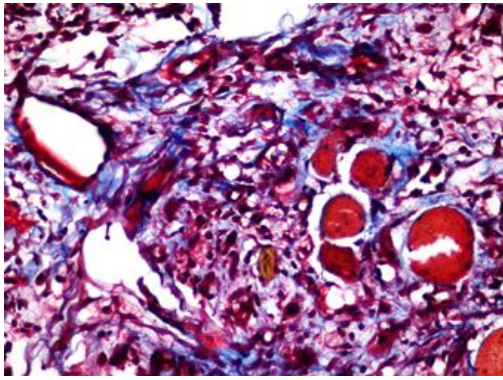


Fig. 3. Tissue sections stained with Masson's trichrome (Dense collagen fibers are seen as dark blue)

All statistical analyzes were performed with the SPSS 15 (Statistical Package for the Social Sciences, Chicago, IL) program. In the statistical evaluation, descriptive statistics (median [minimum-maximum]) and Chi-square tests were used. Where the chi-square standards were not met, test results of Fisher Exact test were considered. In the assessment, $p < 0.05$ was considered as significant.

RESULTS

The minimum age of the rabbits was 3 months, the maximum age was 9 months and the average age was 6 months in three groups. The average weight of the rabbits was 2650 g in Group A, 2400 g in Group B and 2100 g in the control group (Table 1).

Table 1: Descriptive characteristics of the groups Median (min-max) values

Groups	1 st hour	1 st day	Control
Age (months)	6 (3-7)	6 (4-9)	5 (3-6)
Weight (g)	2650 (2200-3100)	2400 (2100-2800)	2150 (1900-2300)

Necrosis occurred in all groups. There was no significant difference between the groups ($p = 0.50$ and $p = 0.72$). Similarly, fibrosis occurred in all groups. Fibrosis decreased in the groups in which PRP was applied in the first day or in the first hour, but fibrosis increased in the control group steadily. However, the difference was not statistically significant ($p = 0.27$). By comparing the groups in which PRP was applied in the first day and in the first hour, fibrosis was observed to decrease at the same rate in both groups. No statistically significant difference was found ($p = 1.00$).

Dystrophic calcification occurred in all groups (Table 2). When the group in which PRP was applied in the first hour and the control group were analyzed using chi-square, dystrophic calcification was observed to develop less (33.3%) in the former group compared with the control group (100%). When the unidirectional hypothesis was established, the result was found significant ($p = 0.03$). Compared with the chi-square test at slope, the results were more significant. While dystrophic calcification developed less (0%) in the group in which PRP was applied in the first hour, it developed more in the control group (83.3%) ($p = 0.02$).

Table 2: Dystrophic calcification results of groups

Dystrophic calcification	No(%)	Yes (%)	P*
1 st hour	66.7	33.3	0.030
Control	0.0	100.0	
1 st day	50.0	50.0	0.091
Control	0.0	100.0	
1 st hour	66.7	33.3	0.500
1 st day	50.0	50.0	

*Fisher's exact test

When Fisher's exact test was applied to the control group and the group in which PRP was applied in the first day, while dystrophic calcification developed less in the former group (50%), it developed more (100%) in the control group. However, the result was not significant ($p = 0.091$). Compared with the chi-square test at

slope, it was observed to decrease for the group that PRP was applied in the first hour (16.7%) while it was observed to increase (83.3%) in the control group.

When the groups in which PRP was applied in the first day and in the first hour were compared with the chi-square test at slope, less dystrophic calcification was observed (0%) in the group that PRP was applied in the first hour. However, it increased in the group that PRP was applied in the first day (16.7%). No statistically significant difference was observed, though ($p = 0.50$).

According to the results of this paper, the effects of trauma could be interpreted in two ways, firstly, starting at the surface with irregular border and beginning to concentrate in the area close to the bone and extending deeper, and thereafter in deeper tissue, the impact trapped between the muscle and the bone starting to expand to the surface in the form of a lightning. In the samples, the areas with necrosis were overlapping with the edematous areas, but it was observed that edema may also occur away from necrosis.

DISCUSSION

Considering the difficulty in determining of muscle damage after injury and the evaluation of histopathologic response to treatment, the researchers preferred to work with animals. 8-10 ml of blood is needed to obtain PRP. Since this amount can not be obtained from small animals, rabbits were used.

So far, the main purpose of the studies regarding PRP, was to accelerate the healing process by applying condensed growth factors triggering wound healing (Lynch et al. 1994). In the literature, there are a few studies demonstrating the effects of GFs on muscles in animal models. In a study conducted on mice, bFGF effect on three injury types (crush-injured, denervated, dystrophic muscles) was investigated and no limiting effect on regeneration was shown (Mitchell et al. 1996). In another study, the effect of IGF-1, bFGF and nerve growth factor (NGF) on myeloblasts was investigated *in vitro* and it was reported that in muscle lacerated mice, GF application improved muscle recovery and provided an increment in fast-twitch muscle force compared with the control group in one month (Menetrey et al. 2000). Therefore in this study

the researchers have opted the Curasan method, which grants a higher increase in IGF-1 (Weibrich et al. 2003). In a study carried out in strained rats platelet-poor plasma was compared with PRP and during the recovery period, improvements were reported to occur only in the repeated muscle injury model, suggesting that PRP has a greater effect on myogenesis than sarcolemmal repair (Hammond et al. 2009). In another study, in experimentally contusional injury imposed mice, autologous conditioned serum (ACS) was administered to a group and saline was administered to another after 2 hours, 24 hours and 48 hours following injury and it was observed that the muscle recovery time was reduced in the ACS administered group. This approach has been suggested to be clinically useful in the treatment of sports injuries (Wright-Carpenter et al. 2004). Similarly, the researchers identified that PRP provided a better recovery following a contusional damage.

Muscle has a great recovery potential. In addition, scar tissue formation through the muscle healing process occurs spontaneously and is completed with muscle regeneration (Foster et al. 2003). Recovery takes place even without any interventions, however it would result in many cell losses. With PRP application the number of intact cells could be augmented. In the literature regarding PRP calcification is not mentioned. In a living tissue, necrotic cells and their waste disappear. If these can not be eliminated, calcium salts and other minerals in these fields form a precipitate and become calcified. When this calcification occurs in a dead or dying tissue, the process is named as dystrophic calcification (Trump et al. 1984). The researchers have demonstrated a reduction in dystrophic calcification through PRP application. Additionally it is possible to increase the number of intact cells by administering PRP. Thus, they conclude that PRP application boosts functional performance by reducing the risk of re-injury. In future studies, it may be helpful to examine dystrophic calcification in the classification of muscles.

In the literature there are a small number of previous studies regarding the time needed for PRP to take effect. However the results are controversial. In a rat contusion model, a local injection of PRP into the injured muscle resulted in no significant differences in functional or histological outcomes, indicating no likely benefit to healing. Additionally, there was no significant

difference between immediate or delayed administration of PRP (Delos et al. 2014). In another injury model conducted on rats, samples from the PRP treated and untreated tissue were evaluated histologically 7 and 21 days after the procedures. PRP was found to promote complete tissue restitution between the 7th and 21st days (Marcelo et al. 2015). In this paper, researchers examined the application of PRP immediately after the injury and one day later to determine whether there is a difference in terms of healing. Studies on muscle injuries report that platelets are particularly active in the first two days (Weibrich et al. 2005). In research groups of this study, regarding recovery, there was no significant difference between the application of PRP one hour and one day following the injury. Considering the time until an injured athlete reaches a physician, the researchers conclude that it is no major issue to apply a one day delayed injection.

Theoretical concerns of researchers about utilisation of PRP mainly focus on muscle tissue healing following fibrosis (Kasemkijwattana et al. 1998; Huard et al. 2002; Chan et al. 2005). However there is no sufficient evidence to put this forth. Zhu et al. (2007) found that TGF and PGE were synergistic in the regulation of fibrosis levels during the recovery of the skeletal muscle, and this situation is particularly important for restoring total muscle function. It is shown that a treated injured muscle tissue with GF had a more increased load resistance as compared with the non treated one and GF has been demonstrated to provide healing. In addition, it is also shown that the combination of IGF-1 with TGF- β antagonist decorin, which is an inhibitor in the formation of fibrous scar, is very successful in the prevention of fibrous scar and in the acceleration of muscle recovery (Sato et al. 2003). In this study, fibrosis occurred in all groups. Fibrosis was even more intense in the non PRP administered group. In order to render this difference to be statistically significant, the “n” number in the groups should be increased. In this study, ethics committee approval for twenty rabbits prevented higher numbers. Nevertheless, what really matters in fibrosis is how long it continues and how it affects the functionality.

It is described that, with different mechanisms of injury, forces of external compression causes skeletal muscle injury and rupture occurs in or near the areas hit by the impact. If the damage is close to the bone, muscle becomes

compressed to bone surface with the pressure of impact, and the impact is conveyed to the muscle fibers (Crisco et al. 1996). The findings of this paper demonstrate that the resulting response may vary depending on the effects of trauma. For example, if the shock wave is superficial, necrosis becomes more, however if the shock wave is deeper, necrosis becomes less. Since fibrosis is more dense near the edges of muscle and in the areas near the bone, the effect of impact seems to take longer in these regions. Changes in the boundaries of muscle may be important in the clinical examinations. Fibrosis occurring in the fat and connective tissue around the damaged tissue is observed as a band in USG, which may contribute to the diagnosis and treatment phases.

There are very few clinical reports assessing PRP in muscle injuries. In one of these studies 14 professional athletes (eight football and six basketball players) with muscle injuries were examined and 16 muscle injuries occurring due to direct impact were classified. After the hematoma evacuation process, PRP was injected with the help of USG. In subsequent USG controls an acceleration in muscle healing and a shortening in the time to return to sports was observed (Cugat et al. 2005). Sanchez et al. (2005) prospectively evaluated PRP injection in muscle injuries of 20 top level professional athletes via USG. They found that all patients reached full function in half of the expected recovery time and no fibrosis was observed. In a study conducted on 30 male athletes, after muscle injury, PRP was applied together with the conventional treatment and improvement in pain, physical recovery and rapid regeneration were observed in the PRP applied group (Bubnov et al. 2013). In another study carried out on 28 athletes with Grade-2 hamstring injury, the ones treated with PRP were compared with those who received rehabilitation and the PRP group was found to make a return to the sport in a shorter time (Hamid et al. 2012).

The researchers could assess the results only histopathologically. Lacking of assessment of muscle recovery from a biomechanical aspect has been a limitation of the current paper. Small animal studies regarding PRP are not always archived, which is beclouding to reach the literature. Also, it is difficult to compare the results of animal studies due to application of different protocols.

CONCLUSION

The researchers believe that PRP is a quite promising treatment option for athletes and conclude that particularly with its effect on dystrophic calcification, PRP may reduce the possibility of re-injuries and help athletes in terms of returning to sports more quickly by increasing the number of intact cells.

RECOMMENDATIONS

Much of PRP effect on muscle injury has yet to be known. Therefore further studies regarding PRP are needed to determine in what kinds of injuries and at which doses it should be used.

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Paper received for publication on February 2016
Paper accepted for publication on December 2016