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**THE USE OF PLATELET RICH PLASMA TO PROMOTE HEALING OF DAMAGED
TENDONS AND ITS EFFECT ON HYDROXYPROLINE LEVELS**

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ABSTRACT

Tendon healing is still a challenge for rehabilitation. Various treatments for tendon injuries are currently have been trialed. This study was established to find the effects of platelet rich plasma (PRP) treatment on the hydroxyproline levels of Achilles tendon. Twelve male white New Zealand rabbits were allocated randomly into two groups of six animals each: Control group: partial tenotomy with no treatment, only 1 ml normal saline was injected in days 1, 8 and 15 at the site of surgery; PRP group: partial tenotomy with PRP treatment. After 30 days the rabbits were euthanatized and tendon specimens were harvested and were submitted to lab for hydroxyproline levels measurement. The results showed that the treatment of rabbits with PRP has significant advantages over untreated animals ($P < 0.05$). We also used from the contra lateral tendons for comparison of hydroxyproline levels of normal tendons with healed ones. These results demonstrate that using PRP treatment will decrease in the time of tendon regeneration and accelerate the healing process.

Keywords: Platelet Rich Plasma, Rehabilitation, Hydroxyproline, Tenotomy, Rabbit

INTRODUCTION

Tendon healing of acute injuries occurs in three stages: inflammation, proliferation and remodeling. The first stage includes fibroblasts migration to the injured site. The proliferative stage includes fibroblasts increase in number and synthesizes collagen. The last stage involves cell and capillary number reduction and collagen fibers realignment [1].

Connective tissue injuries like tendon injury may require long term of rehabilitation. Although the early soft tissue healing process requires seven to ten days, complete tendon healing can take several weeks or months [2]. Tendon matrix is rich of collagens, such as types I and III collagens. Type I collagen is responsible for the tensile strength of the tendon tissue and type III collagen has main role in the healing process [3]. The type I collagen is primary collagen incorporated in the tendon structure, and increasing of this type collagen may enhance tendon healing [4].

In the last decade, platelet rich plasma (PRP) have been used in traumatology, orthopedics and sports medicine showing interesting results in modulation of tendon repair. However, optimal mechanisms and parameters behind these effects are not fully understood [5].

In some of studies PRP has been introduced as a possible new treatment for tendon Injuries that is effective at increasing of hydroxyproline levels. Platelets are known to play a crucial role in the cascade of tissue healing by delivering growth factors to the site of injury [6, 7].

Upon activation, platelets release growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)- β , insulin-like growth factor (IGF)-1, and vascular endothelial growth factor (VEGF), from their α -granules [8]. PRP treatment in tendon lesions results in better biochemical, mechanical, and histological properties of the repair tissue [6, 9].

The aim of this study was to investigate the effects of PRP on the healing of Achilles tendon based on hydroxyproline levels measurement.

MATERIAL AND METHODS

Twelve healthy mature male white New Zealand rabbits about 20 week-old with body weight varying between 2.5 to 3.5 kg were purchased from the Pasteur Institute of Iran.

All rabbits of this research were cared according to the norms of the Islamic Azad University Department of Clinical Sciences, Tehran, Iran, laboratory of animal experimentations; this investigation was

approved by the ethics committee in Islamic Azad University. The animals were kept in standard cages under constant room temperature of 18–22°C, and humidity of 40–50%, 12h/12h light/dark cycle, with *ad libitum* access to standardized food (ration for rodents) and filtered tap water.

Experimental Groups

The animals were divided randomly into two experimental groups of six rabbits each:

Control: Partial Achilles tenotomy with no treatment, only 1 ml normal saline was injected on days one, eight and fifteen at the site of surgery.

PRP: Partial Achilles tenotomy with PRP treatment on days one, eight and fifteen at the site of surgery.

Anesthesia

The animals were anesthetized with ketamine hydrochloride 10% (35 mg/kg IM) and xylazine hydrochloride 2% (8 mg/kg IM). For the maintenance inhalation machine and isoflurane with tracheal tube size 2 mm were used.

Surgical Procedures

After the animals were anesthetized they were placed on a table, in ventral recumbency position, fore and rear limbs immobilized by thin ropes carefully. Before surgery begins clipping, disinfecting with antiseptic povidone-iodine solution, and draping were

done, then a 2 mm skin incision was made in the location of the Achilles tendon, and the right Achilles tendon of each rabbit was freed from surrounding tissues and sharply, longitudinally and full-thickness incision was made ten times with the blade number 11, midway between its calcaneal insertion and the musculotendinous junction. Subcutaneous tissue and skin were sutured with 4-0 absorbable sutures (vicryl) and 3-0 non-absorbable suture materials (nylon) respectively.

After surgery analgesic drugs (Tramadol 4 mg/kg IM) and antibiotics (Pantrisol 30 mg/kg IM, Enrofloxacin 10 mg/kg SC) used to reduce pain and prevent infection. After 30 days, all rabbits were euthanized and tissue specimens were harvested and were submitted to lab for hydroxyproline levels measurement. We also used from the contra lateral tendons for comparison of hydroxyproline levels of normal tendons with healed ones.

PRP Preparation

After anesthesia, the 10 ml of autologous blood drawn from each rabbit for PRP preparation. The blood was centrifuged in two steps, Step 1: fifteen minutes centrifugation at 2000 rpm; Step 2: twenty minutes centrifugation at 3000 rpm. Calcium chloride (5%) activator was added in a ratio of 1:10 for

obtaining the total volume of PRP. PRP for each rabbit was stored at 20°C until the exact time for use at the site of splitting.

Each animal in PRP group received a single dose of PRP (1 ml) on days one, eight and fifteen directly into the surgical site, on top of the tenotomy. The injection of PRP in group PRP was performed immediately after suturing the lesion and was repeated on days eight and fifteen at the site of splitting.

Hydroxyproline Content Measurement

To extracting tendons, each rabbit was euthanized by one intracardiac injection of anesthetic sodium thiopental (crystal, 0.05 mL per 100 g body weight), followed by intracardiac injection of 19.1% potassium chloride, with a single dose of 0.4 mL per 100 g body weight. After euthanasia Achilles tendons from both group with their contra lateral tendons, harvested to determine hydroxyproline levels. The processing was performed as previously described [10].

Data Analysis

For statistical analysis one-way analysis of variance (ANOVA) and Tukey post-hoc with SPSS software (version 18), were used. All statistical tests were performed at a significance level of $P < 0.05$. Results were expressed as the mean \pm standard deviation.

RESULTS

The surgical procedure and PRP treatment were tolerated by animals and there was no sign of suture dehiscence and infection in operated rabbits.

Hydroxyproline Results

The results showed that hydroxyproline levels in healthy and healed tendons had significant difference with untreated ones ($P > 0.05$), and the results also indicated that there is significant difference between healthy tendons and treated ones ($P > 0.05$). Figure one shows comparison of hydroxyproline content in healed tendons with hydroxyproline content in healthy tendons (contra lateral tendons), and figure two shows the comparison of hydroxyproline content between both groups.

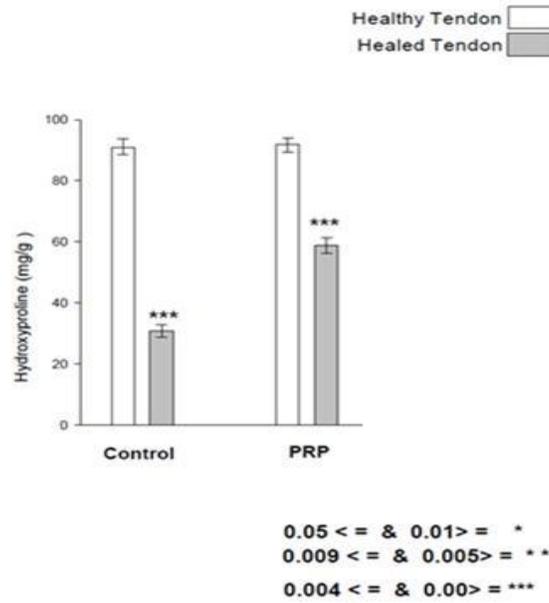


Figure 1: Comparison of hydroxyproline content in healed tendons with hydroxyproline content in healthy tendons (contra lateral tendons)

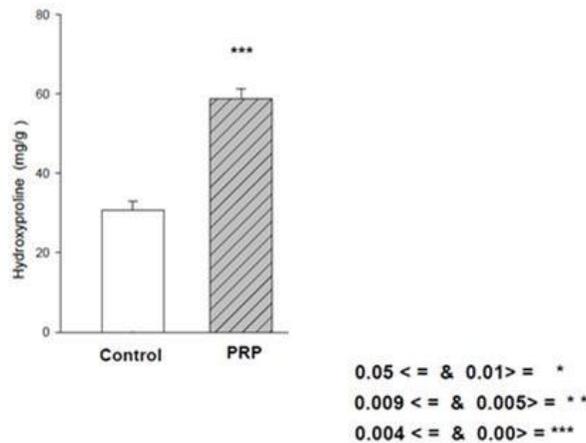


Figure 2: Comparison of hydroxyproline content between control and PRP groups

DISCUSSION

Tendon has special structure function and characteristics. Most injuries in tendon caused by trauma, that is very common and is a problem that requires a repair followed by an early mobilization. Some studies showed that,

healing of tendon takes weeks or even months to acquire the adequate resistance to effectively transmit the force generated by a muscle [11, 12]. There is a need for studies to focus in the improvement of tendon repair and reducing recovery time [13].

Animals are commonly used in tendon disorder research, the main case is that researchers can incorporate invasive evaluation methods, and there is possibility for detailed tissue examination. In animal studies, partial or total tenotomy is the most commonly technique for inducing defects and injuries in tendons [14-22]. In this study, tendon injury was induced by standard partial tenotomy as described before. In last years, interest in the use of PRP has been increased to promote healing in a variety of situations in podiatry. Platelets have been found to possess many important bioactive proteins facilitating tissue regeneration and wound healing. There is an in vitro dose-response relationship between the production of Type I collagen and the concentration of platelets. After placing of platelets in injured areas; growth factors release that can aid in activating the healing process. PRP can be very useful for posterior heel surgery involving the Achilles tendon. In this study PRP was used. Collectively, this findings support the previously proposed effect of PRP that may have a useful role during tendon healing [23-26].

In the past few years, Barbosa *et al* [5] investigated the effects of PRP. For that purpose, they induced partial tenotomy with 2 mm incision in the middle third of the tendon,

the medial to lateral. These authors administered a single dose of PRP (0.2 ml) directly into the surgical site. Animals were killed on the 13th day post tenotomy. They found that the deposition of collagen type I was higher with PRP treatment in comparison to untreated ones.

Hydroxyproline is a unique amino acid of collagen that can be used in biological samples to estimate the amount of collagen. There are few studies that investigate effects of different therapies, like PRP on hydroxyproline levels in injured tendon. In year 2010 Ibrahimi *et al*, analyzed data collected from hydroxyproline contents, they found significant different between PRP groups and the control groups [27]. In a study conducted by Nazhvani *et al* the effect of PRP on tendon healing was investigated, the results showed that using PRP treatment cause to hydroxyproline content increment, also the results indicated that there is significant difference between hydroxyproline content in PRP groups and control groups. These findings confirm our results about effect of PRP treatment on hydroxyproline levels [28]. The results of this research emphasize the importance of PRP treatment. These findings resemble those found by other authors who have reported similar effects

from PRP treatment during the process of tendon healing.

The results of this study indicate that treatment with PRP has beneficial effects on reconstruction of the Achilles tendon.

CONCLUSION

The results of this research suggest a decrease in the time of tendon recovery by using PRP treatment, accelerating the healing process. Accordingly, this form of treatment can be valuable for repair of ruptured and injured human tendons.

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