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Regenerative medicine with platelet rich plasma: a therapy for tendonitis in equines

ARTÍCULO DE REVISIÓN

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ABSTRACT: Tendonitis is considered one of the most frequent conditions which affect the soft tissues. This condition may appear in horses of any kind of the equestrian discipline, resulting in significant economic loss for the equine industry. Nowadays, a variety of medical techniques area available to correct tendon injuries but, in spite of the multiple treatments that have been proposed, they have not obtained a full functionality. Within this context, regenerative medicine offers the perspective of repairing the structure and normal or almost normal function of the injured tendon, resulting in the satisfactory reestablishment of the activity without the risk of reinjuring. Recently, the use of platelet-rich plasma (PRP) has been considered as therapy for tendonitis in equines, due to its nature, its apparent biological effect and its low local reaction. All of these beneficial effects make of this treatment one of the most studied in these past years. This bibliographical compilation summarizes the advantages and disadvantages of PRP treatment as well as the techniques presently proposed for its attainment and application method for the treatment of tendonitis in equines.

Key words: horse, injury, treatment, tendon, tissue

Medicina regenerativa con plasma rico en plaquetas: Una terapia para la tendinitis en equinos

ABSTRACT: La tendinitis es considerada como una de las enfermedades más frecuentes que afecta a los tejidos blandos. Esta condición puede generarse en caballos de todas las disciplinas, lo que resulta en pérdidas económicas significativas para la industria equina. Actualmente, una variedad de técnicas medicas están disponibles para corregir las lesiones de tendón, sin embargo, a pesar de los múltiples tratamientos que han sido propuestos, estos no han logrado una funcionalidad completa del mismo. En este contexto, la medicina regenerativa ofrece la perspectiva de restituir la estructura y función normal, o casi normal del tendón lesionado, resultando así en un restablecimiento satisfactorio de la actividad sin riesgo de reinjuria. Recientemente, ha sido considerado el uso de plasma rico en plaquetas (PRP) como terapia para la tendinitis en equinos, debido a su naturaleza, aparente efecto biológico y poca reacción local. Estos beneficios hacen que este tratamiento sea ampliamente investigado en los últimos años. Esta revisión bibliográfica resume las ventajas y desventajas de la terapia con el PRP, así como las técnicas actualmente propuestas para su obtención y su método de aplicación para el tratamiento de la tendinitis en equinos.

Key words: caballo, injuria, tratamiento, tendon, tejido

Introduction

Tendonitis is considered to be the most frequent condition suffered by the soft tissue; it may occur to horses in all equestrian disciplines and contributes to a significant economic loss among this industry, due to the high level of risk of reinjuring (43% to 93%) and the time that is required to heal this kind of condition (Genovese *et al*., 1996; Dyson, 1997; Balesdent *et al*., 2008; Abellanet, 2009; Rindermann *et al*., 2010). Additionally, tendonitis is considered a decisive factor for low endurance in equines, especially racing, show-jumpers and event horses (Wallis *et al*., 2010).

There are numerous treatment options for tendonitis, for example, the administration of NSAIDs, sodium hyaluronate, oral supplements, cold therapy, phlebotomy, plaster bandage, the administration of intralesional β-aminoproprionitrile fumurate, tendon splitting with or without superior check ligament desmotomy, rest and diverse variety of physical therapy options, such as ultrasound, laser, and magnetic therapies. However, despite of the above treatments listed, the scar tissue formed during the healing phase is functionally deficient compared with a normal tissue (Abellanet, 2009; Wallis *et al*., 2010; Alves *et al*., 2011).

Taking in account the above concerns, present ongoing studies are focused on discovering new therapeutic alternatives capable of improving both time and final results in the healing phase of this condition (Balesdent *et al*., 2008; Abellanet, 2009). Lately, cell-based therapy has been considered as a viable strategy in the replacement, repair, or enhancement of the biological function in a damaged tissue by using autologous or allogenic cells (Bordignon *et al*., 1999). In fact, regenerative medicine offers a chance of restoring completely or partially, both, structure and function of an injured organ, by the early granulation of defects, the maximization in the production of collagen type I and minimization of the formation of scar tissue, thereby, resulting in a successful restoration of activity without the risk of reinjuring (Sutter, 2007; Fortier *et al*., 2008; Alves *et al*., 2011).

Most recently, it has been considered particularly the use of growth factors (GFs) as a tool in therapy used for tendonitis amongst regenerative medicine practioners (Schnabel *et al*., 2009). Growth factors (GFs), also known as anabolic cytokines, are protein molecules that regulate cellular metabolism (Fortier & Smith, 2008), their effects are mediated primarily via autocrine and paracrine mechanisms, which provide the rationale for local administration of exogenous growth factors, in order to influence cellular metabolism (Sporn & Roberts, 1990). GF's are available as recombinant purified proteins or within less defined slurry of bone marrow (BM) aspirate or platelet-rich plasma (PRP) (Fortier & Smith, 2008).

The therapeutic bases of platelet-derived products arose in the late 1970s to 1980s, as multiple growth factors were discovered within the alpha granules of platelets (Kaplan *et al*., 1979; Karey & Sirbasku, 1989). These granules contain the following cytokines: platelet derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor, (VEGF), insulin-like growth factor-1 (IGF-1) and epidermal growth factor (EGF), which are released once the platelet is activated into a wound (Schnabel *et al*., 2007).

The term PRP describes a blood plasma with a high concentrated platelet count, generally greater than two to four times base line ($1x10^5$ to $3.5x10^5$ platelets/µl), and some traces of erythrocytes, leukocytes and serum proteins, such as fibrinogen, fibrin, fibronectin, vitronectin, and thrombospondin, also play a significant role in the healing process of the injured tendon (Sutter, 2007a; Fortier *et al*., 2008; Abellanet, 2009).

The advantages of using PRP are its autologous nature, low cost (relative to stem cells), and its easy preparation/injection. What is more, the provisional matrix (scaffold) provided by PRP and its apparent biological effect, shows an

increment in the production of collagen type I, tenocyte proliferation, neovascularization, increased early strength, organization and fiber pattern alignment, ideal for injured tendons (Sutter, 2007a; Fortier, 2009). Furthermore, the intratendinous PRP administration doesn't cause a local reaction (Pimenta *et al*., 2009).

On the other hand, Fortier (2009), stated that one of the primary disadvantages of PRP therapy is the lack of stem cells within its preparation; though, in spite of this, in vitro studies done in humans have demonstrated the mitogenic stimulation of mesenchymal stem cells (hMSCs) by releasing PRP, taking him to the conclusion that approximately 90% of the mitogenic activity in PRP is derived by the release of platelet (Haynesworth *et al*., 2002).

Residual leukocyte content is considered to be another problem in the production of PRP (Fortier, 2009), due to its potential detriment during the healing phase, caused by the release of pro-inflammatory cytokines (Sutter, 2007a). Additionally, Sundman *et al*. (2011), proved that the GF's and catabolic cytokine concentrations were influenced by the cellular composition of PRP, in fact, while platelets increased anabolic signaling, leukocytes increased their catabolic signaling molecules. Hence, PRP products should be previously analyzed for content of platelets and leukocytes, given that both can influence the biologic effects of PRP (Sundman *et al*., 2011).

How can we obtain the PRP?

The use of Platelet concentrates (PC) as a therapy used within the healing phase, is focused on determining, both, the cellular population (mainly platelets, white blood cells (WBCs), and red blood cells (RBCs)) and the levels of GFs (Argüelles *et al*., 2006; Textor *et al*., 2011). These concentrations may vary widely between preparation systems in order to obtain the PRP. Equivalently, results can differ between the separation techniques, the centrifugation (Ionita *et al*., 2010), the type of anticoagulant and the activated form (Sutter, 2007a; Textor, 2011).

Up to this date there are 3 techniques applied in order to separate platelet concentrates (PCs) which consist in: buffy coat, apheresis, and tube (Argüelles *et al*., 2006). The goal of these techniques are separating and isolating platelets from other blood cells.

The buffy coat technique consists in collecting a full blood sample into a quadruple bag which contains anticoagulant. First the sample is subjected to a "hard spin" centrifugation in order to separate the different type of blood components depending only in their level of density. After centrifugation, plasma with a low cell count is transferred to a satellite bag leaving a small portion of it, in order to cover the buffy coat. When the buffy coat is separated onto another container, it is again centrifuged in a bucket system which allows the separation of PRP from other cell components (Murphy, 2005; Soleimany, 2011). In the apheresis, blood is obtained directly from the bloodstream through a apheresis processors. The amount of platelets acquired depends on the following factors: type of cellular separator, weight, height, platelet count and hematocrit of the patient (Mendoza *et al*., 2007; Lozano *et al*., 2011). Meanwhile, with the tube method, the blood is aseptically taken of the jugular vein by hand and deposited in tubes with anticoagulant. The fraction of Platelet concentrate is obtained after two cycles of plasma centrifugation, both with different time and speed rates (Carmona *et al*., 2005), Argüelles *et al*. (2006) suggested that the advantages of the tube method compared with the other two are the low cost and the minimal technical requirements.

A diverse variety of protocols have been presented in order to obtain the PRP, using the tube method. Vendruscolo *et al*. (2012), evaluated the efficiency of the above method by application of different type of protocols that could be used in the preparation of the PRP, testing diverse time rates and relative centrifugal forces; protocol that was proposed by Carmona *et al*. (2007).

The most common anticoagulants used to preserve the integrity in the structure of platelets

and the prevention of spontaneous activation are the following components: acid citrate dextrose (ACD), sodium citrate, citrate-theophylline-adenosine-dipyridamole (CTAD) and heparin (Carmona *et al*., 2009a; Lei *et al*., 2009).
Lei *et al*. (2009) evaluated the microstructure of platelets collected with heparin, citrate, CTAD and ACD anticoagulants, they observed that ACD and CTAD anticoagulants had better results, rather than heparin and citrate, due to the fact that the structure of the platelet did not suffer any kind of alteration, which prevented it from spontaneous activation. Furthermore, they found that ACD and CTAD released more TGF-β1 and significantly enhanced the proliferation of human marrow stromal cells compared to heparin and citrate anticoagulants.

Centrifugation forms the basis of current methods in the production of PRP (Eppley *et al*., 2006). The relative centrifugation force and its duration, differs amongst the techniques described by diverse authors (Ionita *et al*., 2010). There are many PRP centrifuge systems which are commercially available; however, the system that has shown success throughout all scientific reports in regard to PRP therapy in equines are: Harvest SmartPReP2, Secquire, and GPSII Biomet.

The advantages of these systems are; it's simplicity, promptness in the production of PRP and its free of any kind of possibility of being contaminated (Textor, 2011). On the other hand, it is possible to use a standard laboratory centrifuge to produce PRP, but generally it requires of a double spin and multiple manipulation of it, consequently, its sterility may be compromised (Eppley *et al*., 2006).

The administration of PRP in tendonitis

Once the condition is diagnosed through the use of a ultrasound and the damaged located in the injured tendon is documented by evaluation of the cross sectional area (CSA; cm^2), the echogenicity score (ES) of the lesion (range: 0 to 4) and the fiber alignment score (FAS: range 0 to 4) (Rindermann *et al*., 2010), the injected volume (2 ml to 4 ml) can be calculated depending on size and type of the lesion (Rindermann *et al*., 2010).

Sutter *et al*. (2007b), proposed a method to calculate the injectable volume, this consisted in injecting approximately 1ml/3% lesion calculated on transverse images (**Figura l**).

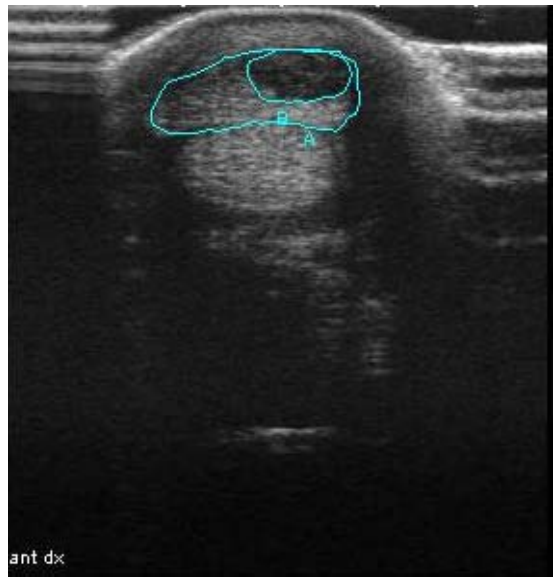


Fig. I. Transverse ultrasonographic image shows a lesion in the superficial digital flexor (SDF). A: Transversal area of SDF tendon B: Area of lesion.

Physiologically, platelets are activated by being exposed to damaged tissue, initiating a clotting cascade. The activated platelets are already within the blood clot or hematoma where they are de-granulate (Sutter, 2007a). Once platelet degranulation starts GFs are released, plus other substances that promote tissue repair, influences vascular reactivation and other blood cells in angiogenesis (Fortier & Smith, 2008).

Platelets can be activated through the use of pharmacologic agents (Maia, 2009), usually 1000 U of thrombin or a calcium chloride solution 10% is added to PRP (Schwartz & Martínez, 2011). It's been reported that thrombin stimulates fibroblast migration, cell migration, and the production of collagen at low concentrations (Sutter, 2007a). Following studies

have reported that the stimulation of one of the thrombin receptors (PAR-1) present an increment in pain threshold in laboratory animals via opioid pathways (Textor, 2011). This means that when activated PRP is administered, thrombin receptors throughout the area are also stimulated and could potentially induce an analgesic response (Sutter, 2007a). Taking in account the above, some authors recommend the inoculation of thrombin with the PRP to promote the release of GFs, also increasing effectiveness of the treatment. However, it has also been reported the formation of antithrombin antibodies and deficiencies in coagulation factor V associated with inoculation of thrombin (Anitua et al., 2004).

Some recommendations for PRP activation with freezing and thawing platelets has been proposed by Textor (2011), in particular the quantity of PDGF-BB released after freeze show it was equivalent to one that was released after treatment using a low dose of bovine thrombin (1 U/ml); applying this protocol, the platelets are partially activated and lyzed, causing passive leakage of GFs from the cells, and the PRP formulation remains liquid.

Upon activation, platelets are morphologically modified and release their granular contents into the surrounding environment (Sutter, 2007b), Manzano *et al*., 2009), with the release of presynthesized GFs starts within 10 minutes of clotting. Approximately 95% of the presynthesized GFs are released within the next hour. Following this initial burst, platelets continue to synthesize and secrete additional proteins for 5 to 10 days, or the balance of their life (Sutter, 2007b).

The reason why PRP treatment should be used in tendinopathy, is due to its ability delivering hyerphysiologic doses of beneficial GFs directly into the injured tendon (Skjong *et al*., 2012). Intralesional injection of PRP into the tendon, using an ultrasonographic guidance (Textor, 2011) **(Figura 2),** has gain popularity across the United States of America (Sutter, 2007a). The goal of intralesional therapy includes: minimizing damage to surrounding and injured tissue and accurate placement of the cell suspension within the lesion (Sutter, 2007a); ultrasounds can be used reliably to accurately place a needle within the lesion in most locations.

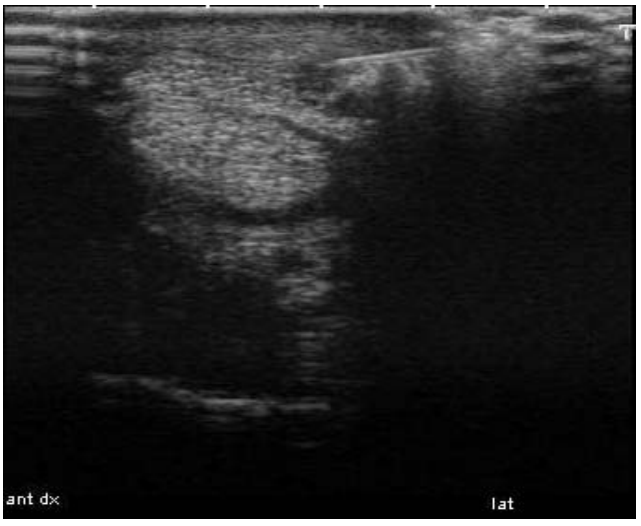
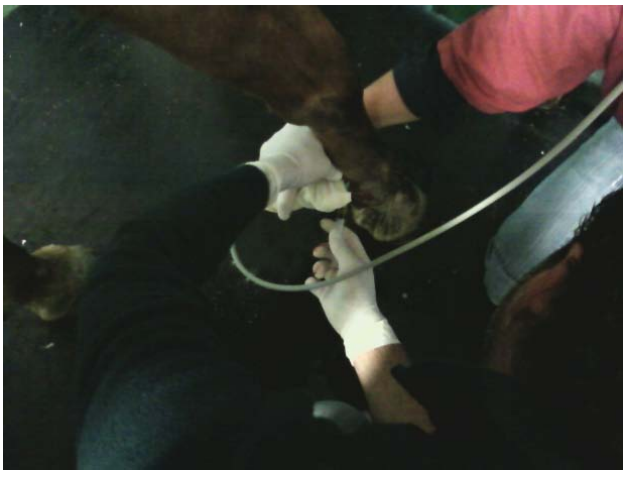


Figura 2. A) Intralesional injection of PRP into the tendon using ultrasonographic guidance. B) Ultrasonographic images during the injection of PRP in a superficial digital flexor (SDF) tendonitis



After PRP infiltration

After PRP infiltration, a regular ultrasonographic monitoring at 30- to 60-days intervals is recommended. Moreover, in order to recover full standard activity, it's recommended to take on a controlled exercise program based on quantitative and qualitative observations to the horse (Fortier, 2009; Sutter, 2007a).

In reference to the treatment, Carmona *et al*. (2009b) recommended continuing with the therapy with a 15 day intervals for 3 consecutive times. This theory was confirmed by (Abellanet, 2009). In this study, 191 horses were injected with PRP approximately 1 to 4 times with 10-15 day intervals; the percentages of therapeutic success and reinjures were 69-76% and 20-22% respectively.

Positives outcomes in the prognosis in athletic horses suffering of tendonitis is variable and primarily depends on the severity of the injured tendon, age, complexion and intended use of the horse (Fortier, 2009). Recent investigations in the biochemical and histological properties of the tendon being treated intralesionally with PRP, in a experimental placebo-controlled study, reflected a positive outcome in PRP-therapy after 24 weeks (Rindermann *et al*., 2010). A study released by Carmona *et al.* (2005), reported that horses with severe SDFT tendinopathy returned to complete exercise after 4.5 of their last PRP injection without reinjuring at 1 year of treatment.

Conclusion

Therapy with PRP has become a positive alternative within regenerative medicine in the treatment of soft tissue injuries in all equestrian disciplines. In the present, there diverse procedures to prepare PRP; however, these techniques are still on a quest for its ideal protocol which must be supported by further studies. It is important to identify the individual cellular reaction and its effect in the injured tissue, giving as the chance to make an accurate clinical decision. In fact, controlled quantitative data are rare, but absolutely necessary to determine the efficiency of the current "cell-based" techniques that are available. Therapy with PRP has an elevated cost in comparison with traditional therapy; however, these costs are not significant compared to the beneficial advantages of the treatment in equines. New strategies combining the different type of techniques could be viable way to find a better and much more reliable treatment for tendonitis; the combination of PRP with stem cells could be a possibility. The investigations in regard to PRP treatment and its applications in veterinary medicine continue guaranteeing effective results with the least risk level to the horses.

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