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Equine suspensory ligament and tendon explants cultured with platelet-rich gel supernatants release different anti-inflammatory and anabolic mediators

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ABSTRACT

The aim of this study was to evaluate the release of pro- and anti-inflammatory as well as anabolic mediators stimulated by a leukocyte-reduced platelet-rich gel supernatant (Lr-PRGS) and a leukocyte-reduced plasma supernatant (Lr-PL) at two concentrations (25 and 50%) on normal equine suspensory ligament explants (SLEs) and tendon explants (TEs). SLEs and TEs from six horses were independently incubated for 48 h with Lr-PRGS and Lr-PL at concentrations of 25 and 50%, respectively. Samples were collected from the incubated tissues at 1 h and 48 h, which were employed for ELISA determination of interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), IL-4, IL-1 receptor antagonist (IL-1ra), platelet-derived growth factor isoform BB (PDGF-BB), transforming growth factor beta-1 (TGF-β_1, and hyaluronic acid (HA). Overall, 50% Lr-PRGS induced significantly less IL-1β release than the other hemoderivatives in both tissues. At 48 h, both Lr-PRGS and 25% Lr-PL induced significantly higher TNF-α concentrations in SLEs when compared to TEs, whereas both Lr-PRGS concentrations induced significantly higher IL-4 concentrations in SLEs in comparison to TEs. IL-1ra release was not different between tissues. However, this cytokine was significantly higher in tissue explants cultured with both Lr-PRGS concentrations. HA concentration was lower in tissue explants cultured with all hemoderivatives at two concentrations when compared to the control group. The positive effects observed for ligaments and tendons treated with Lr-PRGS may be mediated by the inhibition of IL-1β release of and increased release of IL-4 and IL-1ra. Furthermore, PDGF-BB could be a polypeptide responsible for mediating the release of anti-inflammatory cytokines in SLEs and TEs incubated with Lr-PRGS.

1. Introduction

Teno-desmic lesions are a frequent cause of lameness in horses, and these musculoskeletal disorders are responsible for reduced quality life in horses, which has a negative economic impact on the equine industry through medical expenses, long resting periods, and poor performance. Superficial digital flexor tendon (SDFT) tendinopathy and suspensory ligament (SL) desmopathy [1] are the most important teno-desmic diseases diagnosed in horses [2]. Comparatively, humans also suffer from similar soft tissue pathologies, such as Achilles tendinopathy and plantar fasciitis [3]; for this reason, further advances in the musculoskeletal pathophysiology of horses can impact our understanding of similar diseases in the context of human medicine [4].

Currently, a developing interest in regenerative therapies exists for the clinical management of teno-desmic degenerative diseases in horses [5]. One of these therapies includes the intralesional injection of platelet-rich plasma (PRP) in affected anatomical structures (tendons or ligaments) [6,7]. Clinical use of PRP is based on the higher concentration of growth factors (GFs) and cytokines, with anabolic and anti-inflammatory effects being naturally contained in this substance [8]. Furthermore, following PRP activation either with an activating substance (i.e. calcium salt or thrombin) [9] or by contact with the native collagen from the tendons or ligaments, it polymerizes in platelet-rich gel (PRG), which is a fibrin matrix that captures and gradually

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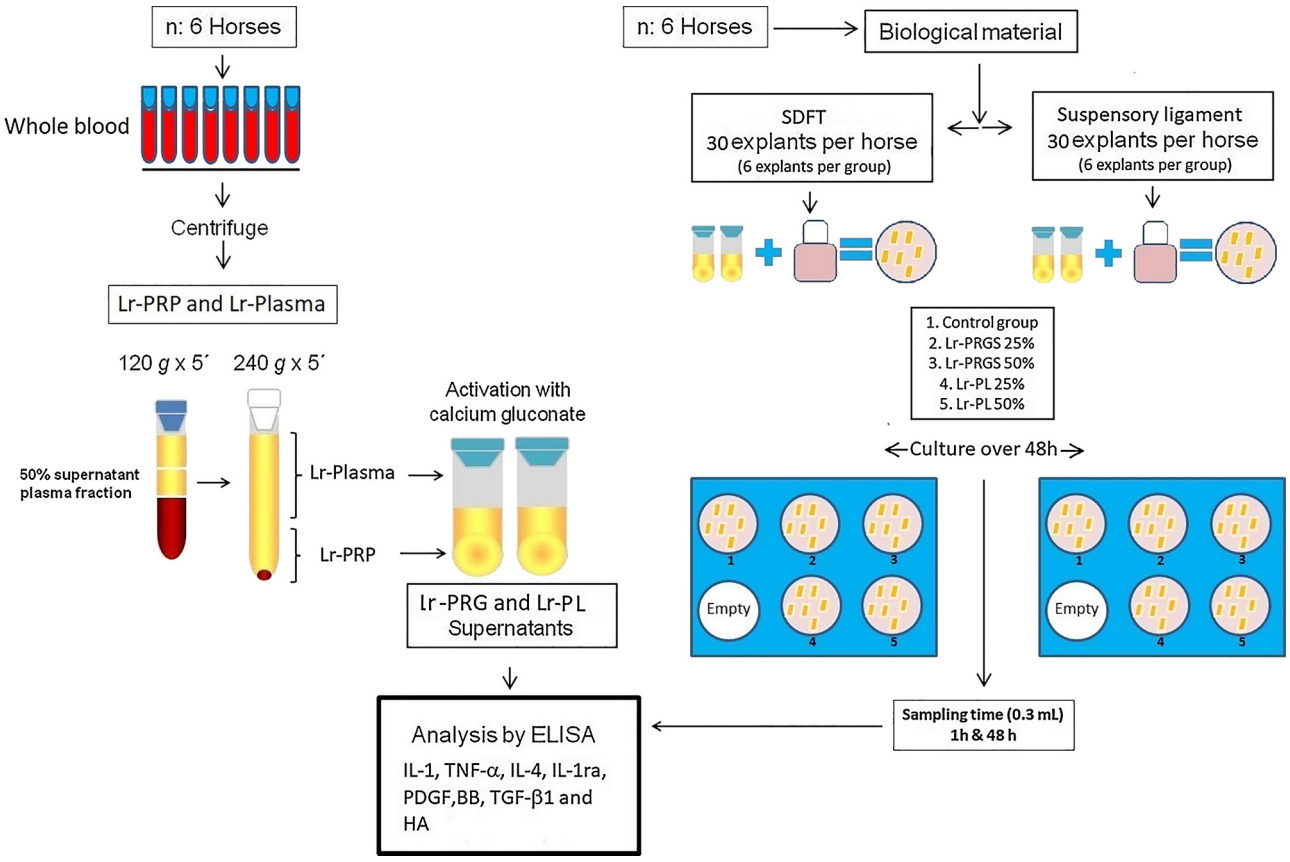

Fig. 1. Schematic representation of the study's design and methodology.

releases polypeptides (GFs and cytokines) while potentially permitting stem cell migration and differentiation [10].

Many methods and systems, yielding different platelet and leukocyte hemoderivatives with different concentrations, are used to collect PRP from horses, which influence the final composition and concentration of GF and cytokines released following PRP activation [11,12]. Classifications arranging PRP-related products according to several factors include leukocytes, platelets, GFs, or activating substances. However, Dohan-Ehrenfest et al. [13] classify PRPs for infiltration as leukocyte- PRP (L-PRP) and pure-PRP (P-PRP). L-PRP is hemoderivative containing variable concentrations of leukocytes and platelets, whereas P-PRP is a leukocyte-reduced PRP (Lr-PRP) because it only contains platelets with low concentrations of leukocytes [13].

Currently, a controversial debate is occurring regarding about the ideal PRP compound (either L-PRP or P-PRP (Lr-PRP)) for the treatment of tendon and ligament lesions [14]. Notably, several clinical and experimental *in vivo* studies have shown positive results using either L-PRP [6,15–18] or P-PRP [19] products. However, several *in vitro* studies performed in tendons and ligaments from humans and horses demonstrated that Lr-PRP hemoderivatives could be more suitable for tendinopathy and desmopathy treatment than L-PRP products [20–23]. Generally, these studies indicate that P-PRP products promote normal collagen matrix synthesis and decrease the cytokines related to matrix degradation and inflammation to a greater extent than L-PRP products [20–23].

We present a comparative study that evaluates the effects of fresh supernatants derived from the calcium gluconate activation of Lr-PRP and plasma with a reduced concentration of leukocytes (Lr-PL) at two concentrations (25% and 50%) in an *in vitro* system of isolated explant culture of normal equine SL and SDFT explants over 48 h. The aim of this study was to evaluate the release of pro- and anti-inflammatory as well as anabolic mediators stimulated by these substances through measurement of the concentrations of interleukin 1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), IL-4, IL-1 receptor antagonist (IL-1ra), platelet-derived GF isoform BB (PDGF-BB), transforming growth factor beta-1 (TGF-β_1) and hyaluronic acid (HA) released from incubated tissues (tendon and ligament) at 48 h.

2. Materials and methods

This study was approved by the Committee of Animal Experimentation of the Universidad de Caldas, Manizales, Colombia. An informed consent authorization was signed by the owners of the horses.

2.1. Blood collection

Six clinically healthy horses (3 gelding and 3 mares) between 7–10 years of age were used as blood donors. These animals were free from transmissible diseases, such as equine piroplasmosis and equine infectious anemia. All horses were stabled, fed and managed in a similar fashion. These horses were selected after an extensive physical examination alongside with a complete blood cell count and general blood chemistry panel. Only clinically healthy horses with platelet counts higher than $100 \times 10^3/\mu L$ were used. Whole blood from each horse was obtained by jugular puncture using a 21 G butterfly catheter.

2.2. Pure platelet-rich plasma/platelet rich gel supernatant (Lr-PRP/Lr-PRGS) and Lr-plasma (Lr-PL) preparation

Both hemoderivatives were obtained through a manual double centrifugation tube method [24] that was previously validated and used clinically in horses with SL desmopathy and SDFT tendinopathy [16]. Blood was deposited in 4.5 mL tubes with sodium citrate solution (BD Vacutainer®, Becton Drive, Franklin Lakes, NJ, USA). After