

PLATELET RICH PLASMA (PRP) TREATMENT FOR TENDINITIS

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INTRODUCTION:

Tendon injuries are often recalcitrant to treatment. Platelet rich plasma (PRP) has been used for several years in oral and maxillofacial surgery and it has recently been investigated for use in defects of bone, cartilage, and tendon. The main rationale for the use of PRP arises from the growth factors released from platelet alpha-granules. In addition, PRP is readily available and is an autogenous product. However, there are several potentially catabolic proteins released by platelet granules including pro-inflammatory mediators such as thromboxane. Our hypothesis was that PRP and other blood products tested would have a dose related effect on DNA content and mRNA synthesis of tendon matrix molecules, being stimulatory at concentrations less than or equal to 50% and cytotoxic at 100%.

METHODS:

Venous blood was collected in acid-citrate dextrose (ACD) from 8 horses and processed using the SmartPRP2 system (Harvest Technologies) to obtain PRP and platelet poor plasma (PPP). Plasma was generated by centrifugation of whole blood. Sternal bone marrow aspirates were obtained from 3 horses using a Jamshidi bone biopsy needle. Complete blood and platelet counts were performed on all samples. Samples were stored at -20°C until used.

The tensile region of the flexor digitorum superficialis tendons from 3 adult horses were harvested. The paratenon was removed and tendons were minced into explants (3x5x5 mm). Cultures were established with five pieces/well of 6-well plates with two replicate wells/horse/treatment group. Culture media were whole blood, plasma, PRP, PPP, or bone marrow at concentrations of 100%, 50%, and 10% in serum-free DMEM supplemented with amino acids. Medium for control cultures consisted of DMEM with 10% fetal bovine serum and ACD anticoagulant. Cultures were maintained for 3 days at 37°C, 5% CO₂, and 90% humidity.

After harvest, explants were extensively rinsed in PBS, snap-frozen in liquid nitrogen and pulverized in a freezer-mill. DNA content was determined in papain digested samples using the bisbenzimidazole fluorometric assay. Total RNA was isolated using Trizol (Invitrogen) and further purified using RNeasy spin columns (Qiagen). Gene expression for MMP-13 and collagen types I and III were quantified by real time PCR and normalized to 18S RNA expression.

Release of growth factors from platelets was induced following a single freeze-thaw cycle. IGF-I, TGF-β1, and PDGF-BB concentrations were determined using the Active IGF-I ELISA (DSL Inc.), the Emax TGF-β1 ELISA (Promega), and the Quantikine PDGF-BB ELISA kits (R&D) respectively. Data were analyzed using a one-way ANOVA and Tukey's post-hoc test with significance set at p≤0.05.

RESULTS:

Platelet and cell counts: Platelets were enriched in PRP from whole blood by an average of 3.77-fold (n=8, range 2.25-4.95; STDV 0.86). Platelet counts were an average of 3.85-fold less in PPP compared to whole blood (n=6, range 3.00-4.17; STDV 0.53). Total white blood cell counts were variably increased in PRP (range 5.00-fold decrease-2.33-fold increase, mean 1.85-fold decrease of n=8; STDV 3.62). Hematocrit was an average of 23.77-fold less than whole blood (n=8, range 2.69-55.00, STDV 15.72).

Growth factor concentration: PRP had a significantly higher concentration of both TGF-β1 and PDGF-BB compared to other blood products (Figure 1). TGF-β1 concentration was an average of 1.83-fold greater in PRP compared to marrow (n=3, range 1.62-1.95; STDV 0.19). PDGF-BB concentration was an average of 3.24-fold greater in PRP compared to whole blood (n=3, range 2.61-4.08, STDV 0.76). No statistical difference was observed in IGF-I concentration between the treatment groups.

DNA concentration: PRP treatment groups at both 100% and 50% concentration as well as plasma at 100% had a higher concentration of DNA than the control group. Furthermore, tendons cultured with PRP and plasma at 100% concentration had a higher DNA concentration than those cultured with PRP at 50% concentration (Figure 2).

Gene expression: PRP and PPP treatment groups at 100% concentration and marrow at 100% and 50% had a greater expression of collagen type I than the control group. No statistical differences were

observed between these concentrations of PRP, PPP, and marrow. Plasma, PRP, and PPP treatment groups at 100% concentration as well as blood at 50% and 10% had a greater expression of collagen type III than the control group. Between these groups, blood at 50% had a greater expression than plasma at 100%, which had a greater expression than PRP and PPP at 100%. Tendons cultured in PRP and PPP at 100% had a greater expression than those in blood at 10%. The blood treatment group at 50% concentration as well as plasma groups at 100% and 10% had a greater expression of MMP-13 than the control group. In addition, tendons cultured in blood at 50% concentration had a greater expression of MMP-13 than those cultured in either plasma groups.

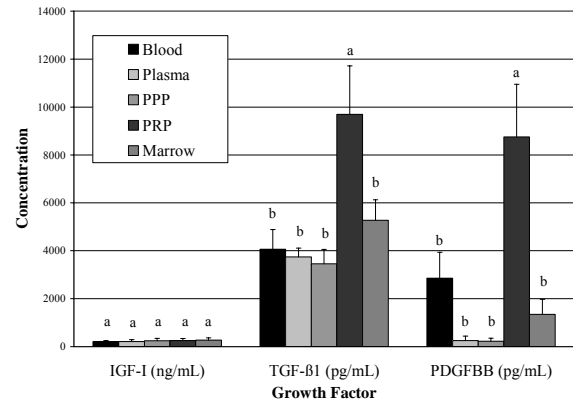


Figure 1. Growth factor concentration in venous blood, plasma, PRP, PPP, and bone marrow (mean of n=3 ±STDV). Letters indicate significant differences between the blood components (p≤0.05).

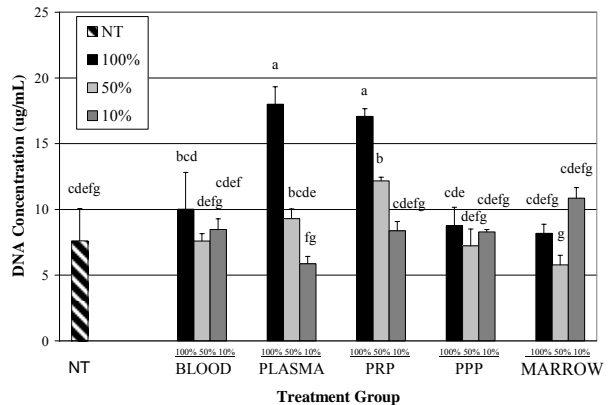


Figure 2. Mean DNA concentration (ug/mL) (n=3±STDV). Superscript letters indicate significant differences between the groups (p≤0.05).

DISCUSSION:

In these studies we found that PRP contains higher concentrations of TGF-β1 and PDGF-BB than all other blood products tested. In addition, we found that DNA concentration and gene expression of collagen types I and III were greater in the 100% PRP treatment groups than the control groups. These initial findings support that PRP is a promising new treatment for tendonitis and that it is most beneficial at 100% concentration. Although no deleterious side effects of high concentrations of PRP or any other blood product were detected, in vivo studies are still necessary prior to implementing PRP in routine clinical management of tendonitis.

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ACKNOWLEDGEMENTS: This work was supported by the Harry M. Zweig Memorial Fund for Equine Research Program.