Increasing Platelet Concentration In Leukocyte-Reduced Platelet Rich Plasma Decreases Collagen Gene Synthesis In Tendons

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Disclosures:

Introduction: Platelet-rich plasma (PRP) is used for the treatment of tendinopathy. There are numerous PRP preparations, and the optimal combination of platelets and leukocytes is not known. The purpose of this study was to determine the effect of varying absolute platelet concentrations within leukocyte-reduced PRP (lrPRP) preparations for treatment of tendinopathy. The hypothesis was that within leukocyte-reduced PRP, there would be a plateau effect of platelet concentration with increasing platelet concentration being detrimental to extracellular matrix synthesis.

Methods: Different formulations of lrPRP with respect to platelet:leukocyte ratio were generated from venous blood of 8 horses. Explants of superficial digital flexor tendon were cultured in lrPRP products for 96 hours. Platelet-derived growth factor-BB (PDGF-BB), tumor necrosis factor-α (TNF-α), transforming growth factor-β1 (TGF-β1), and interleukin-1β (IL-1β) concentrations were determined in the media by ELISA. Gene expression in tendon tissue for collagen types 1 and 3 (COL1A1, COL3A1), matrix metalloproteinases-3 and -13 (MMP-3, MMP-13), cartilage oligomeric matrix protein (COMP), and IL-1β was determined using RT-qPCR. Data was divided into three groups of lrPRP based on the ratio of platelets:leukocytes (Group 1 &lt750:1; Group 2 750:1-2800:1, and Group 3 &gt2800:1), based on natural breaks in a plot of platelet:leukocyte ratios. Gene expression of COL1A1, COL3A1, COMP, MMP-3, MMP-13, IL-1β in lrPRP-treated tendons (all 3 groups combined) was compared to control tendons using t-tests to verify that lrPRP had a treatment effect. A general linear model (GLM) was then used to evaluate the relationship between independent variables (platelet or leukocyte concentration (continuous) and ratio groups (categorical variable)) and gene or protein expression dependent variables. Using this approach, the platelet:leukocyte ratio is controlled for, so that the effects of platelet concentration of protein and gene expression can be determined. P≤ 0.05 was considered significant. Student’s t-tests were performed with Statistix 9 software (Analytical Software, Tallahassee, Florida). The general linear model was performed using SPSS version 20 software (IBM, Armonk, New York).

Results: Complete blood counts verified leukocyte reduction by a mean of 93.6% (range 90.1 - 97.9%, SE, 9.6x10-3) and platelet enrichment by a mean of 31.9% (range, 12.6 - 81.0%, SE 8.07). Although the results were significant, there was only a modest increase in growth factor concentration with increasing platelet concentration as indicated by regression coefficients of 1.71x10-6 (PDGF) and 7.09x10-7 (TGF-β). The ratio of platelets:leukocytes was not significantly associated with growth factor concentration. Concentration of the catabolic cytokine IL-1β was modestly but significantly decreased with increasing platelet concentration at time 0 (regression coefficient -8.49x10-7), but not at 96 hours after culture. No significant relationships were found between the concentration of TNF-α and either platelet concentration or platelet:leukocyte ratio. Both COL1A1 (p<0.001) and COMP (p<0.001) gene expression were significantly increased in lrPRP treated tendons while COL3A1 was decreased (p=0.005) compared to controls indicating that lrPRP enhanced normal tendon matrix homeostasis (Figure...
There was a significant inverse linear relationship between platelet concentration and COL1A1 gene expression. Neither platelet concentration nor the ratio of platelets to leukocytes was significantly associated with MMP-13, MMP-3 or IL-1β expression.

**Discussion:** A maximum threshold of benefit appears to exist for platelets, suggesting that moderate increases in platelet concentration can be effective to augment tendon repair and excessive platelets can be detrimental to matrix synthesis. Increasing the platelet concentration within lrPRP preparations results in delivery of more anabolic growth factors and less pro-inflammatory cytokines, but the biological effect on tendons is diminished metabolism as indicated by a decrease in synthesis of both COL1A1 and COL3A1. Together, this information suggests that minimizing leukocytes in PRP is more important than maximizing platelet numbers with respect to decreasing inflammation and enhancing matrix gene synthesis.

**Significance:** A maximum biologic threshold of benefit was demonstrated with regard to the number of platelets, where further increases in platelet concentration did not result in further anabolic upregulation. Continuing in vivo investigations documenting the use of platelets for the treatment of tendinopathy are justified, as well as further in vitro characterization of the ideal PRP product for the treatment of tendinopathy and other musculoskeletal applications.

**Acknowledgments:** Harry M. Zweig Fund for Equine Research (LAF)

**References:**

*ORS 2014 Annual Meeting*

*Poster No: 0515*