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Effects of platelet rich plasma (PRP) on the repair of wounds on the distal limb in horses

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INTRODUCTION

Horses suffer from chronic non-healing wounds or the development of exuberant granulation tissue (EGT), especially when the wounds are located on the distal limb. Both conditions lead to extensive scarring which may limit the athletic career. Several mechanisms have been incriminated in problematic repair in horses including an inefficient inflammatory response to trauma. Platelet-Rich Plasma (PRP) represents a concentrated form of multiple cytokines released from platelet alpha-granules at sites of tissue injury. The principal purported therapeutic advantage of PRP over isolated purified cytokines is that it represents a physiologically natural mixture of mediators designed to exert synergistic biologic effects in a wound healing environment. Topical medications, as opposed to systemically administered drugs, are not required to undergo stringent FDA testing and approval. Consequently, a plethora of new products have recently hit the market, often with limited scientific evidence to support the company's claims. In an effort to improve the quality of wound management in horses, new methods should be critically evaluated in order to establish their efficacy, prior to commercialization.

The objective of our study was to evaluate the effect of topical application of autologous PRP to limb wounds in horses. We hypothesized that by increasing the concentration of mediators present in the wound bed via the addition of PRP, we might enhance the acute inflammatory response and consequently accelerate/improve the quality of wound repair and obviate the development of EGT.

FREE COMMUNICATIONS

MATERIALS AND METHODS

Six, 10-15 year old, clinically normal mares were used for the experiment, which was sanctioned by the University Council on Animal Care. Three, 6.25 cm^2 full-thickness wounds were created 2 cm apart on the dorso-lateral surface of each metacarpus of each horse under sedation and ring block. In each horse the three wounds on one randomly assigned forelimb were treated with 1.5 mL of PRP/wound (treated wounds "TW"; n=18) while those on the contralateral forelimb served as controls (control wounds "CW"; n=18) and received no topical treatment. PRP was prepared by the tube (manual) method in commercially designed platelet sequestration tubes then activated immediately prior to its application to the wound surface by adding 50 IU of human thrombin reconstituted in 1 mL of $CaCl_2$. Wounds, identified as A, B and C from distal to proximal location on the limb were then bandaged. Bandages were changed one week later at which time wounds A were sampled from the wound edge, under sedation and ring block, for histological evaluation and from the wound center for measurement of Transforming Growth Factor-beta 1 (TGF-#beta#1) protein concentrations by ELISA (DB100B; R&D Systems). A second dose of PRP was then applied to wounds B and C of the treated forelimb. From then on, bandages were changed a minimum of once weekly until complete healing. Wounds B and C served for macroscopic observation [incidence of EGT and measurement of wound surface area (WSA)] throughout the healing period. Once fully closed, wounds B were sampled, from the wound edge, for histological evaluation and from the wound center, for measurement of types I and III collagen mRNA by RT-PCR, while wounds C were sampled from the wound center for biomechanical evaluation of stiffness, stress and strain (model #5544 material testing system, Instron).

RESULTS

PRP, prepared by the tube method, showed a 3,5-fold increase in platelet count compared with whole blood ($p = 0.003$) while the mean concentration of TGF-#beta#1 protein was 2,8 times greater than in plasma ($p < 0.0001$). EGT was observed and resected in 5 of 6 TW and in 2 of 6 CW, though this difference was not statistically significant. A priori contrasts showed that the percent decrease in WSA was significantly inferior for TW compared to CW at location B one ($p = 0,048$) and two weeks ($p < 0,0001$), and at location C one ($p = 0,01$), two ($p = 0,04$) and three weeks ($p = 0,01$) post-treatment. Location on the limb (B or C) did not alter the speed of wound healing ($p = 0.33$). Mean TGF-β1 protein concentration was 1.6-fold higher

(p=0.09) in one week TW than in CW. Histological, biomechanical and gene expression data (Fig. 1) did not differ significantly between treated and control wounds.

CONCLUSION

Our findings do not support the hypothesis that, by increasing the presence of mediators in the wound via the addition of PRP, we might accelerate/improve the quality of repair and obviate the development of EGT, at least in small granulating wounds on the distal limb of horses. In conclusion, this therapy may better suit wounds suffering massive tissue loss or, alternatively, chronic wounds which would benefit from a fresh source of mediators to jump-start the healing process.

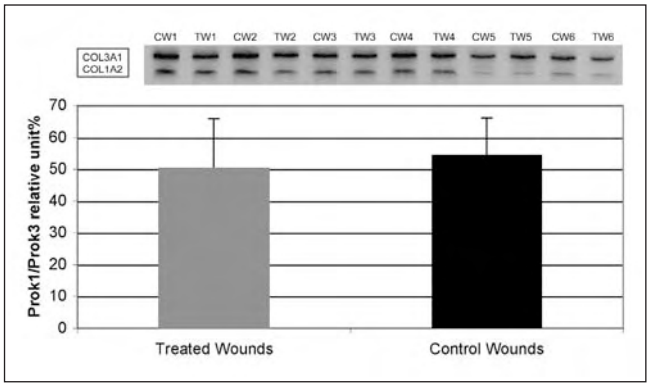

Figure 1 - *Regulation of equine COL1A2 and COL3A1 mRNA by wounding of the distal limb. Bar graphs represent the ratio of collagen types I:III mRNA levels in TW and CW. Top: COL3A1 mRNA [amplified fragment (AF) 225 bp] in CW and TW biopsies. Bottom: COL1A2 mRNA [amplified fragment (AF) 445 bp] in CW and TW biopsies. Signal intensity normalized to that of GAPDH.*

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