Platelet Rich Plasma (PRP) Enhances Anabolic Gene Expression Patterns in Flexor Digitorum Superficialis Tendons

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Received 17 February 2006; accepted 28 June 2006

Published online 14 November 2006 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jor.20278

ABSTRACT: Platelet rich plasma (PRP) has recently been investigated for use in tissue regeneration studies that seek to utilize the numerous growth factors released from platelet α-granules. This study examined gene expression patterns, DNA, and collagen content of equine flexor digitorum superficialis tendon (SDFT) explants cultured in media consisting of PRP and other blood products. Blood and bone marrow aspirate (BMA) were collected from horses and processed to obtain plasma, PRP, and platelet poor plasma (PPP). IGF-I, TGF-β1, and PDGF-BB were quantified in all blood products using ELISA. Tendons were cultured in explant fashion with blood, plasma, PRP, PPP, or BMA at concentrations of 100%, 50%, or 10% in serum-free DMEM with amino acids. Quantitative RT-PCR for expression of collagen type I (COL1A1), collagen type III (COL3A1), cartilage oligomeric matrix protein (COMP), decorin, matrix metalloproteinase-3 (MMP-3), and matrix metalloproteinase-13 (MMP-13) was performed as were DNA and total soluble collagen assays. TGF-β1 and PDGF-BB concentrations were higher in PRP compared to all other blood products tested. Tendons cultured in 100% PRP showed enhanced gene expression of the matrix molecules COL1A1, COL3A1, and COMP with no concomitant increase in the catabolic molecules MMP-3 and MMP-13. These findings support in vivo investigation of PRP as an autogenous, patient-side treatment for tendonitis. © 2006 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 25:230–240, 2007

Keywords: platelet rich plasma (PRP); tendonitis; collagen; cartilage oligomeric matrix protein (COMP); matrix metalloproteinase (MMP)

INTRODUCTION

Tendon injuries are common amongst athletes and are often recalcitrant to treatment.[1–7] Platelet rich plasma (PRP) has been used for several years in oral and maxillofacial surgery to accelerate peri-implant soft tissue and bone healing,[8–12] and it has recently been investigated for regeneration of bone,[13–16] cartilage,[16,17] and ligament.[18] The main rationale for the use of PRP arises from the growth factors released from platelet α-granules, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), fibroblastic growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-I (IGF-I), and epidermal growth factor (EGF).[19–25] All of these growth factors have been evaluated for their ability to enhance tenocyte mitogenesis and synthesis of matrix molecules such as collagen types I and III.[23,26–38] Bone marrow aspirate and bone marrow–derived mesenchymal stem cells have also been investigated for use in regeneration of tendon.[18,39] Bone marrow aspirate and PRP are clinically attractive therapies for tendonitis due to their autogenous nature and their accessibility at the time of diagnosis for immediate treatment.

PRP is typically used at 100% concentration for in vivo studies and clinical applications,[9,11,14] but in vitro studies to date have used PRP at concentrations less than 40%, with the remainder being serum-free tissue culture medium.[16–18] During the centrifugation process used to make PRP,

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mononuclear white blood cells are also concentrated compared to whole blood; although to a lesser degree than platelets.[22] This may pose a concern in tissue regenerative efforts due to the pro-inflammatory mediators such as neutral proteases and acid hydrolases contained in white blood cells as well as thromboxane released from platelets.[21,25,40] Because previous in vitro experiments utilized concentrations of $\leq$40% PRP, either maximal beneficial or catabolic effects of 100% PRP may have been overlooked due to dilution.

The objectives of this study were to examine gene expression patterns, DNA concentration, and total collagen content of tendon explants cultured in media consisting of blood products at varying concentrations. Our hypothesis was that PRP and other blood products tested would have a dose-related effect on mRNA synthesis of tendon matrix molecules and DNA content of tendon explants; being stimulatory at concentrations less than or equal to 50% and nonstimulatory or suppressive at 100%.

MATERIALS AND METHODS

This project was approved and performed according to guidelines of the Institutional Animal Care and Use Committee of Cornell University. For these studies, blood and tendon samples were obtained from young adult horses ranging from 2 to 4 years of age ($N = 6$).

Preparation of Blood Products

Blood products at concentrations of 100%, 50%, and 10% were used as culture media for tendon explants. Venous blood was collected into acid citrate dextrose (ACD) anticoagulant[25,41,42] and PRP and PPP were produced according to the manufacturer's directions using the SmartPReP2 system (Harvest Technologies, Plymouth, MA) with disposables containing a 1.046 density shelf. From each 60 mL of venous blood, 10 mL of PRP and 20 mL of PPP were obtained. Plasma was generated by centrifugation of venous blood (400 *g* for 10 min). Sternal bone marrow samples were aspirated into ACD by use of a bone marrow biopsy needle under sterile conditions following local anesthesia. Complete blood counts, hematocrits, and platelet counts were determined in blood products after collection and again after processing to document the extent of cellular enrichment achieved by centrifugation. Cell counts were not determined in bone marrow aspirates because they were not processed via centrifugation. Samples were separated into aliquots and stored at -80°C until assayed for growth factor concentration or used as culture media for tendon explants. A single freeze–thaw cycle was used to induce platelet activation and release of growth factors.[25]

Growth Factor Quantification

Active IGF-I, TGF-β1, and PDGF-BB concentrations were determined in triplicate aliquots of all samples using the Active IGF-I ELISA (Diagnostic Systems Laboratory Inc., Webster, TX), the Emax TGF-β1 ELISA (Promega Corporation, Madison, WI), and the Quantikine PDGF-BB ELISA kits (R&D Systems, Minneapolis, MN), respectively. Samples were analyzed on a multiple detection plate reader (Tecan SAFIRE, Durham, NC) with wavelength absorption at 450 nm. Standards in each kit were used to generate standard curves. Cross-reaction of antibodies in the IGF-I and TGF-β1 ELISA kits with equine samples have been previously demonstrated.[43–46] There are no previous reports documenting use of the PDGF-BB ELISA kit with equine samples. However, a recent study revealed that the 109-amino-acid human PDGF-B peptide against which the ELISA kit antibody is directed is 89% homologous to equine PDGF-B,[47] and samples tested in the present study all fell within the sample value range provided by the manufacturer of the kit.

Tissue Harvest and Explant Cultures

The tensile region of flexor digitorum superficialis tendons were harvested, the paratenon was removed, and the tendons were chopped into explants ($3 \times 5 \times 5$ mm). Cultures were established with five explants/well of six-well plates with two replicates/treatment group (defined by culture medium)/horse. Culture media were whole blood, plasma, PRP, PPP, or bone marrow aspirate (BMA) at concentrations of 100%, 50%, or 10%, with the remainder of the media consisting of serum-free Delbecco's modified eagle medium (DMEM). For these studies, cultures containing 10% plasma were considered controls, rather than a more typical culture medium containing 10% fetal bovine serum (FBS), because FBS would contain higher concentrations of several growth factors compared to plasma from young adults. Plasma was chosen over serum to minimize variability due to presence/absence of ACD. Cultures were maintained for 3 days at 37°C, 5% CO_2, and 90% humidity. After culture, explants were rinsed repeatedly in phosphate-buffered solution (PBS) to remove residual blood product, snap-frozen in liquid nitrogen, pulverized in a freezer mill, and stored at -80°C until used.

RNA Isolation and Quantitative RT-PCR

Total cellular RNA was isolated using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's directions with slight modifications. Trizol was added to pulverized tendon (1 mL of Trizol/100 mg of tendon) and the mixture was homogenized on ice. A chloroform extraction was performed and RNA was precipitated from the aqueous phase using isopropanol and high-salt solution (1.2*M* $Na_3C_6H_5O_7$, 0.8*M* NaCl). Precipitated RNA was further purified using RNeasy spin columns (Qiagen, Chatsworth, CA) according to the

manufacturer's instructions. The approximate yield was 2 to 8 μg of total RNA/250 mg tendon. RNA was assessed by spectrophotometry and agarose gel electrophoresis, and subsequently utilized for quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) of collagen types I and III (COL1A1, COL3A1),[48,49] cartilage oligomeric matrix protein (COMP),[50–53] and decorin[3,54] as indicators of tendon matrix synthesis, and matrix metalloproteinase-3 (MMP-3)[49,55,56] and matrix metalloproteinase-13 (MMP-13)[48,49] as indicators of tendon catabolism.

Total RNA was reverse transcribed and amplified using the One-Step RT-PCR technique and the ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA). The primers and dual-labeled fluorescent probe [6-FAM as the 5′ label (reporter dye) and TAMRA as the 3′ label (quenching dye)] were designed using Primer Express Software version 2.0b8a (Applied Biosystems). All probes and primers were designed using equine specific sequences either published in Genbank, provided by Dr. Alan Nixon, Cornell University, or sequenced in our laboratory. Total copy number of each gene was obtained from a standard curve and normalized to 18s RNA expression.

DNA Analysis

Pulverized explants were lyophilized and digested in 2.8% papain for 24 h at 65°C. Total DNA content was determined in triplicate aliquots of papain-digested samples. The samples were mixed with bisbenzimide compound for DNA quantification by fluorometric assay[57] using a multiple detection plate reader (Tecan SAFIRE). Calf thymus DNA was used to prepare a standard curve.

Total Collagen Assay

Total soluble collagen content was determined in lyophilized tendon samples using the Sircol Assay (Biocolor Ltd., Newtownabbey, Northern Ireland) according to the manufacturer's directions for pepsin-soluble collagens with modifications. Briefly, 10 to 15 mg of sample (dry weight) was digested for 12 h in pepsin/acetic acid. Collagen standards were prepared using bovine skin collagen provided in the Sircol Assay kit. Sircol dye (sirius red in picric acid) was added to samples and standards, and the assay was completed according to the manufacturer's directions. Total collagen concentration was determined using a multiple detection plate reader (Tecan SAFIRE) with wavelength absorption at 540 nm.

Statistical Analyses

Hematological values, including platelet counts, white blood cell counts, hematocrits, and growth factor concentrations, were analyzed using a one-way analysis of variance (ANOVA) and Tukey's post-hoc test with Statistix 8 software (Analytical Software, Tallahassee, FL). A multivariable approach was used to analyze all other continuous data (gene expression of COL1A1, COL3A1, ratio of COL1A1:COL3A1, COMP, decorin, MMP-3, and MMP-13, DNA concentration, and total collagen concentration) to control for anticipated biological variability between horses and because experiments from each of the horses were performed independently. The general linear model and Duncan's multiple range post-hoc tests were performed with SAS 9.1 software (SAS Institute, Inc., Cary, NC). Significance was set at $p \leq 0.05$.

RESULTS

Hematological Values

Platelet counts, total nucleated cell counts, and hematocrits were compared in whole blood, PRP, and PPP samples to document the extent of platelet enrichment during centrifuge processing. Platelets were significantly concentrated in PRP compared to whole blood and PPP, and in whole blood compared to PPP ($p < 0.0001$). The mean platelet count in PRP was $395 \times 10^3/\mu L$ (range, $180–535 \times 10^3$; SE 44×10^3), compared to $112 \times 10^3/\mu L$ in whole blood (range, $52–195 \times 10^3$; SE 17×10^3) and $35 \times 10^3/\mu L$ in PPP (range, $27–47 \times 10^3$; SE 3×10^3). Platelet counts were an average 3.77-fold greater in PRP compared to whole blood (range, 2.25–4.95-fold increase; SE 0.22).

Total nucleated cell counts were significantly lower in PPP compared to whole blood and PRP ($p < 0.0001$). In contrast, there was no significant difference between total nucleated blood cell counts in PRP compared to whole blood. Mean total nucleated cell count in PPP was $0.02 \times 10^3/\mu L$ (range, $0.00–0.10 \times 10^3$; SE 0.01×10^3), compared to $5.21 \times 10^3/\mu L$ in whole blood (range, $3.70–6.80 \times 10^3$; SE 0.40×10^3) and $4.48 \times 10^3/\mu L$ in PRP (range, $0.60–11.40 \times 10^3$; SE 1.48×10^3).

Hematocrits were significantly greater in whole blood compared to PRP and PPP which were not significantly different from each other ($p < 0.0001$). The average hematocrit was 30.75% in whole blood (range, 24.00%–55.00%; SE 3.67), 2.63% in PRP (range, 0.00%–13.00%; SE 1.50), and 0% in all PPP samples.

Growth Factor Concentration

TGF-β1 and PDGF-BB concentrations were significantly greater in PRP compared to all other blood products (Fig. 1). TGF-β1 concentration was an average of 1.55-fold greater in PRP compared to bone marrow (range, 1.36–1.64-fold increase; SE 0.11) and at least 2-fold greater in PRP compared to plasma, whole blood, or PPP. PDGF-BB

 DOI 10.1002/jor

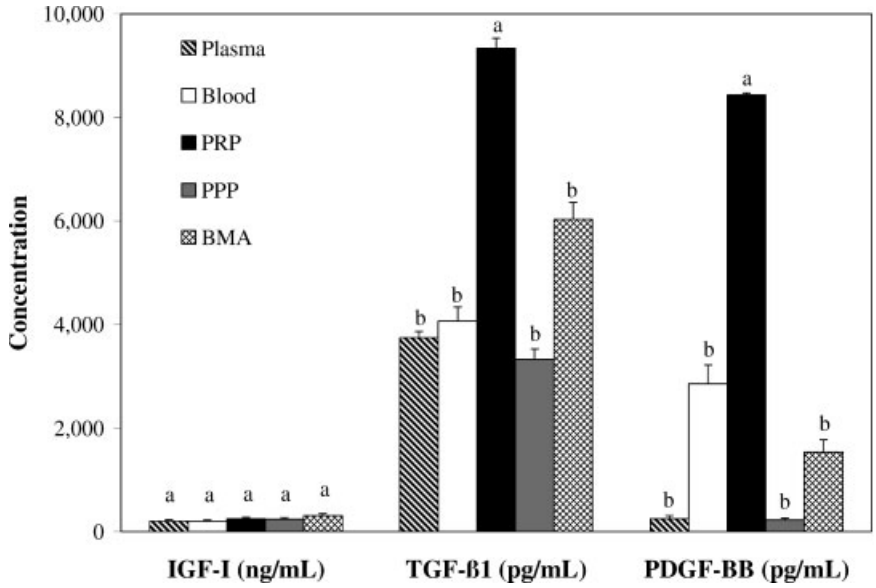

Figure 1. IGF-I (ng/mL), TGF-β1 (pg/mL), and PDGF-BB (pg/mL) concentrations in plasma, whole blood, platelet rich plasma (PRP), platelet poor plasma (PPP), and bone marrow aspirate (BMA). TGF-β1 and PDGF-BB concentrations were significantly greater in PRP compared to all other blood products ($p = 0.0003$ and $p < 0.0001$, respectively). No significant differences in IGF-I concentration were observed between blood products ($p = 0.53$). Bars represent mean $N = 6 \pm$ SE; superscript letters indicate significant differences in a growth factors concentration between blood products (ANOVA with Tukey's post-hoc test).

concentration was an average of 3.12-fold greater in PRP compared to whole blood (range, 2.51–3.93-fold increase; SE 0.52). There were no significant differences in TGF-β1 or PDGF-BB concentrations between any of the other products (blood, plasma, PPP, and BMA) tested. No significant differences in IGF-I concentration between products were detected (Fig. 1).

Gene Expression

COL1A1 expression was greatest in tendons cultured in 100% PRP and this was the only group that was significantly increased compared to control cultures of 10% plasma and compared to its respective 10% concentration group (Fig. 2A). COL1A1 expression in tendons cultured in 100% plasma, 100% blood, 100% PPP, 100% BMA, and 50% BMA was also increased by 2-fold compared to 10% plasma, but the results were not significantly different from either the control or 100% PRP. No treatment resulted in decreased COL1A1 expression compared to 10% plasma control.

COL3A1 expression patterns were similar to that of COL1A1 (Fig. 2B). Tendons cultured in 100% PRP and 100% plasma had significantly increased gene expression of COL3A1 compared to 10% plasma control, but they were not significantly different from each other. In plasma and PRP, the 100% concentration groups were also significantly greater than their respective 10% groups. COL3A1 expression was not decreased by any treatment.

The ratio of COL1A1:COL3A1 expression was significantly greatest in tendons cultured in 100% BMA (mean, 0.28; range, 0.01–0.87; SE 0.16), 100% PRP (mean, 0.13; range, 0.04–0.21; SE 0.03), 100% blood (mean, 0.14; range 0.04–0.22; SE 0.04), and 50% BMA (mean, 0.20; range, 0.01–0.62; SE 0.11), but these groups were not different from each other (general linear model, $p < 0.05$). The ratio of COL1A1:COL3A1 gene expression was not diminished by any treatment.

COMP mRNA was significantly increased in tendons cultured in 100% PRP, 100% PPP, and 100% BMA, although these groups were not significantly different from each other (Fig. 2C). Also, in all three of these groups (PRP, PPP, BMA), the 100% concentrations were significantly greater than their respective 10% concentration treatment groups. No treatment resulted in diminished COMP gene expression.

Decorin gene expression was not significantly increased or decreased in any treatment groups

compared to the 10% plasma control, but treatment groups were significantly different from each other. Decorin gene expression was highest in tendons cultured in 100% BMA, which was significantly different from those cultured in 100% plasma, 50% and 100% blood, 10% and 50% PRP, and 50% PPP (Fig. 2D).

Opposite gene expression patterns were present for MMP-13 and MMP-3 in the tendon culture groups. MMP-13 gene expression was significantly lower in tendons cultured in 100% BMA compared to 10% blood, but not compared to the other groups tested, including 10% plasma or BMA at 10% or 50% (Fig. 3A). MMP-3 gene expression was significantly increased in tendons cultured in 100% BMA compared to all other groups which were not significantly different from each other (Fig. 3B). There was a trend toward diminishing MMP-13

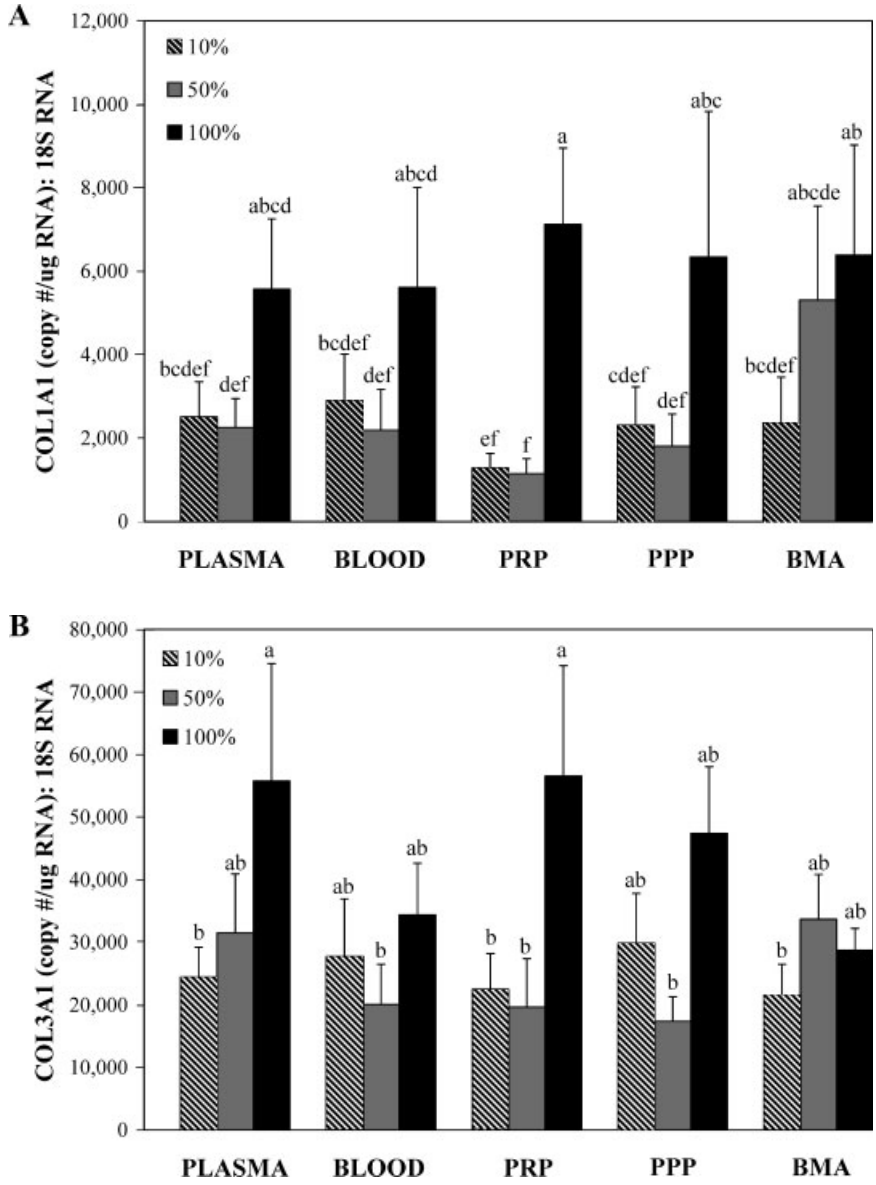

Figure 2. Expression of tendon matrix genes COL1A1 (A), COL3A1 (B), cartilage oligomeric matrix protein (COMP) (C), and decorin (D) in treatment groups consisting of plasma, whole blood, platelet rich plasma (PRP), platelet poor plasma (PPP), and bone marrow aspirate (BMA) at concentrations of 100%, 50%, and 10%. Bars represent mean of $N = 6 \pm$ SE; superscript letters indicate significant differences between treatment groups (general linear model and Duncan's multiple range post-hoc test; $p \leq 0.05$).

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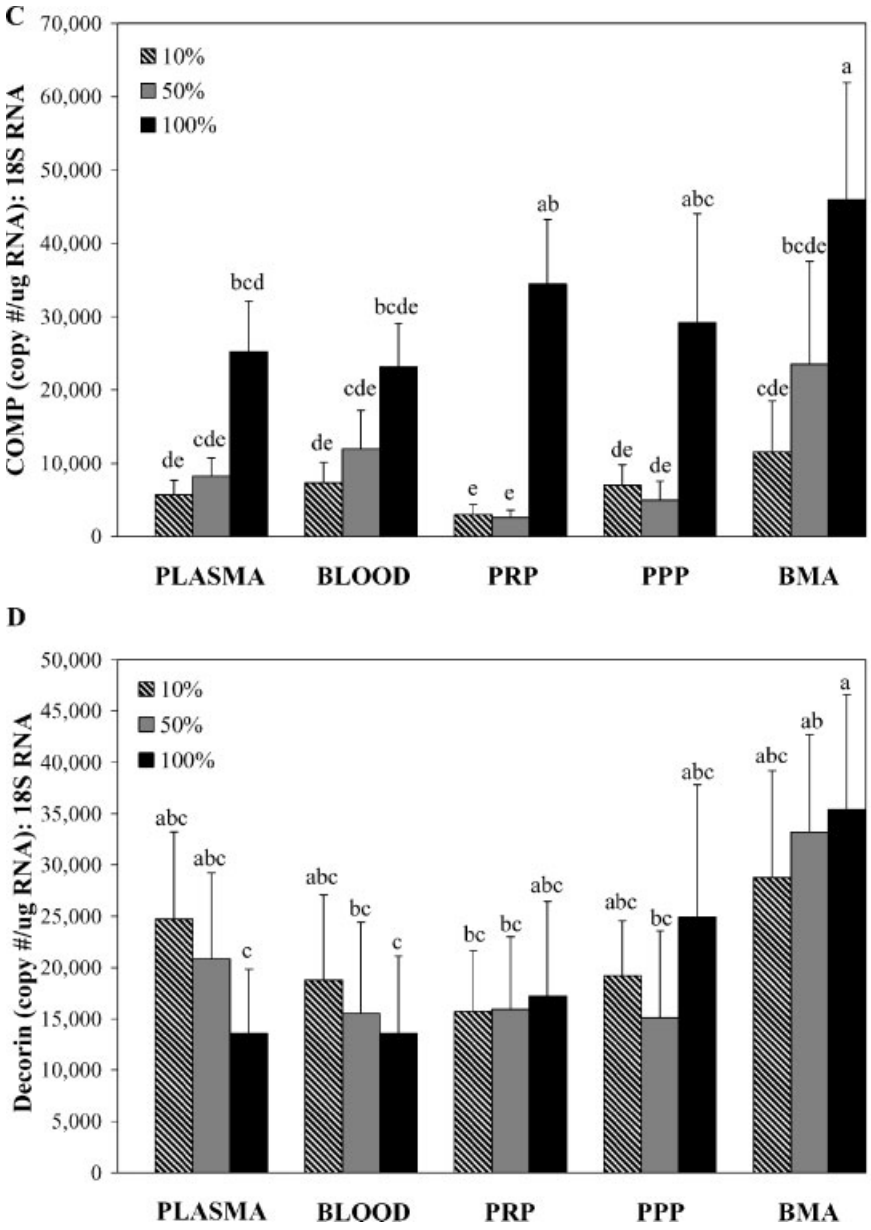

Figure 2. (*Continued*)

expression with increasing blood product concentration in all treatment groups, and an opposite trend toward increased MMP-3 expression with increasing blood group concentrations; both patterns were most pronounced in the BMA group.

DNA Concentration

DNA concentration was significantly higher in tendons cultured in 100% plasma compared to all groups except 50% and 100% PRP, which were not significantly different from each other (Fig. 4). No other treatment groups were significantly different from the 10% plasma control group, and no treatment resulted in decreased DNA content.

Total Soluble Collagen (Sircol Assay)

There were no significant differences in total soluble collagen concentration in tendons between any of the treatment groups ($p = 0.47$; data not shown). The average total soluble collagen concentration was lowest in tendons cultured in 100% blood (mean, 585.45 μg/mg; range, 603.47–707.85; SE 76.40) and highest in tendons cultured in 10% blood (mean, 744.16 μg/mg; range, 743.76–825.75; SE 42.59).

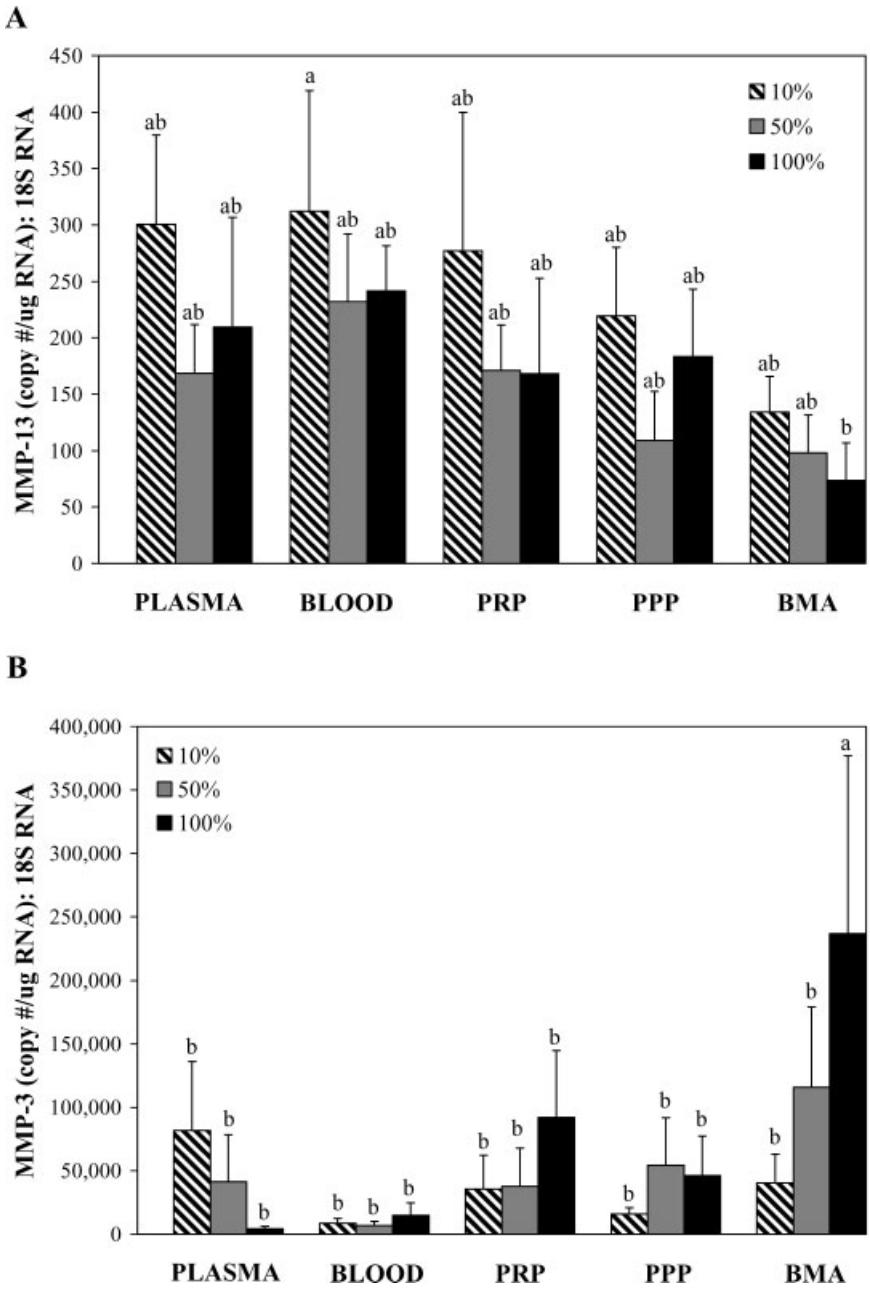

Figure 3. Matrix metalloproteinase-13 (MMP-13) (A) and matrix metalloproteinase-3 (MMP-3) gene expression in treatment groups consisting of plasma, whole blood, platelet rich plasma (PRP), platelet poor plasma (PPP), and bone marrow aspirate (BMA) at concentrations of 100%, 50%, and 10%. Bars represent mean of $N = 6 \pm$ SE; superscript letters indicate significant differences between treatment groups (general linear model and Duncan's multiple range post-hoc test; $p \leq 0.05$).

DISCUSSION

The findings of this study did not support our hypothesis that PRP and other blood products tested would have a dose-related effect on mRNA synthesis of tendon matrix molecules and DNA content of tendon explants. We proposed that the blood products would stimulate tendon matrix gene expression at concentrations less than or equal to 50%, but would be nonstimulatory or suppressive at 100%. In contrast to our hypothesis, most blood products stimulated tendon matrix molecule expression to the greatest extent at 100% concentration, and no concentration (10%, 50%, or

 DOI 10.1002/jor

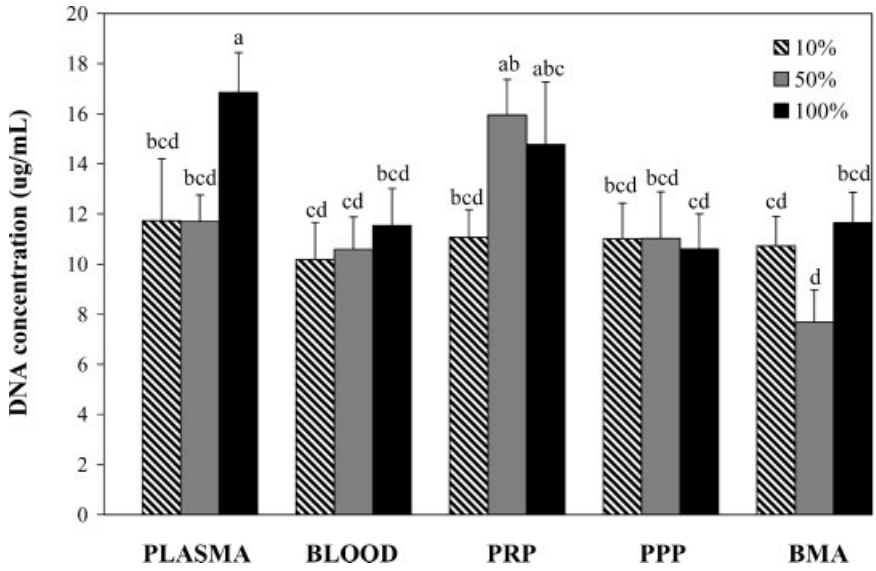

Figure 4. DNA concentration of tendon explants cultured in plasma, whole blood, platelet rich plasma (PRP), platelet poor plasma (PPP), and bone marrow aspirate (BMA) at concentrations of 100%, 50%, and 10%. Bars represent the mean of $N = 6 \pm \text{SE}$; superscript letters indicate significant differences between treatment groups (general linear model and Duncan's multiple range post-hoc test; $p \leq 0.05$).

100%) significantly decreased matrix molecule gene expression, DNA content, or total soluble collagen concentration. While the majority of genes analyzed were stimulated to the greatest extent by blood groups at 100% concentrations, there were differences between the blood products, and overall PRP at 100% concentration stimulated the greatest number of matrix molecule genes without increasing expression of the pro-inflammatory mediators MMP-3 or MMP-13.

When compared to the control group of 10% plasma, 100% PRP significantly increased mRNA expression of COL1A1, COL3A1, ratio of COL1A1:COL3A1, and COMP. Two other blood products, 100% plasma and 100% BMA, also significantly increased several, but not all of these genes. For example, neither 100% plasma nor 100% BMA increased COL1A1, and 100% plasma did not increase the ratio of COL1A1:COL3A1 or COMP expression. In addition, 100% PRP did not stimulate MMP-13 or MMP-3 expression, whereas 100% BMA significantly increased MMP-3 expression. Together, this suggests that the anabolic growth factors released from platelets concentrated during the centrifugation of PRP prevail over the potentially catabolic or pro-inflammatory mediators released from white blood cells and platelets. We did not see a dose response to increasing blood product concentration for any molecule tested. This study is the first to report the effects of 100% PRP on tendon explant or isolated tenocyte cultures, and our findings suggest that the beneficial effects of 100% PRP could have been overlooked in previous in vitro studies due to dilution.[16–18]

Additional significant differences between treatment groups might have been observed if a culture duration of greater than 3 days was chosen for this project. A 3-day culture duration was used because the blood products clotted around the tendon explant within 12 h of application. The clots that formed were variable in consistency between and within groups, with some being more solid than others. However, there were no observable differences in clot consistency due to treatment group. Exchange of clotted medium was not considered feasible for continued in vitro culture, and it precluded the addition of radioisotopes for protein or DNA synthesis studies. The formation of a clot upon application in a clinical situation should provide a reservoir for sustained growth factor release during tendon regeneration and suggests that the addition of thrombin is not necessary to form a clot when blood products are exposed to tendon matrix.

The anabolic effects of PRP on tendon metabolism are likely the result of growth factors released from platelet α-granules. Platelet counts in PRP were an average of 3.77-fold greater than those in whole blood. This result is consistent with previously published results using the Harvest

Technologies SmartPReP2 system and is greater than that reported with other devices used to prepare PRP, where 1.3- to 1.9-fold platelet concentrations were achieved.[12] There are numerous commercially available machines, all of which yield slightly different increases in platelet and mononuclear cell counts.[25] Greater fold concentrations of platelets compared to whole blood have been obtained using laboratory buffy coat and apheresis methods, with up to a 13.1-fold increase reported in the concentration of platelets,[22] but these methods are not widely available for clinical use. Interestingly, while both the laboratory methods and some of the commercially available devices have been shown to concentrate mononuclear cells in PRP compared to whole blood,[22,58] our results revealed variable degrees of enrichment using the SmartPReP2 system. In this study, PRP preparations had a mean 1.85-fold decrease in mononuclear cell counts compared to whole blood. This finding is of clinical importance when considering the pro-inflammatory cytokines contained in mononuclear white blood cells,[25,40] and their potential for inciting an undesirable inflammatory reaction in an environment such as a tendon or joint.

The most significant differences observed between PRP and the other blood products tested, and what makes PRP a more attractive product for clinical use than plasma, BMA, or PPP, was the increased concentration of TGF-β1 and PDGF-BB. The concentration of TGF-β1 in PRP was an average of 1.55-fold greater than in BMA and at least 2-fold greater than in PPP, plasma, or whole blood. TGF-β plays a major role in wound healing through numerous activities, including recruitment of fibroblasts and macrophages and stimulation of collagen production.[26,59] In addition, TGF-β inhibits matrix metalloproteinases and downregulates proteinase activity.[26,59] A recent in vivo study using a rabbit zone II flexor tendon wound-healing model showed that TGF-β receptors are upregulated after flexor tendon injury and repair.[60] In the rabbits, the equivalent of the middle digit flexor digitorum profundus tendon was sharply transected and then repaired. Immunohistochemistry on harvested tendons revealed that all three TGF-β receptor isoforms were present by day 1 postoperatively, peaked at day 14 postoperatively, and remained elevated until day 56 postoperatively, suggesting that TGF-β is involved in tendon healing. TGF-β1, β2, and β3 have also been shown to increase production of collagen types I and III by rabbit tenocytes in vitro, with the most dramatic effects in collagen production occurring after addition of TGF-β1.[26] PDGF also plays an important role in tendon healing,[23,61] and it has been shown to promote collagen synthesis by rat tenocytes in vitro.[32] IGF-I also increases tenocyte metabolism and improves tendon healing in animal models.[29,38] In the present study, the concentration of IGF-I was similar in all blood products tested, and it did not reach a concentration equivalent or greater than the 2 to 25 μg utilized in the previously mentioned animal studies. In addition to the growth factors that were measured in the present study, platelets contain and release numerous other growth factors, such as VEGF, which also improves tendon healing in animal models.[28] The growth factors that were quantified in the present study were chosen based on their known anabolic effects on tendon metabolism and accessibility of ELISAs which cross react with equine peptides.

Cartilage oligomeric matrix protein (COMP) is a noncollagenous pentameric glycoprotein that is abundant in tendon. It is proposed to have an organizational role in the formation of collagenous matrices by binding to collagen types I and II and promoting collagen fibrillogenesis.[50,51,53] Studies have shown an age-dependent increase in COMP production, with immature flexor tenocytes synthesizing more COMP than mature flexor tenocytes, suggesting an important role in tendon growth and potentially repair.[51,52,62] Another study showed a dose-dependent increase in COMP protein in equine neonatal digital flexor tendons treated with TGF-β1 at concentrations ranging from 1 to 200 ng/mL of medium.[51] In our study, COMP gene expression was significantly higher for tendons cultured in 100% PRP, 100% PPP, 100% BMA, and 50% BMA. PRP and BMA were determined to have the highest concentrations of TGF-β1, which is consistent with the previous study in which a dose-dependent increase in COMP production was observed in tenocytes treated with TGF-β1. Similar results were observed for gene expression of COL1A1, COL3A1, and for the ratio of COL1A1:COL3A1.

In conclusion, results of this study support the application of platelet rich plasma for treatment of tendonitis, and suggest that it would be most beneficial at 100% concentration. Taken together, the findings indicate that in comparison to the other blood products tested, 100% PRP contains the highest concentration of growth factors with known anabolic effects on tendon matrix synthesis and it enhances tendon matrix gene expression patterns without simultaneous stimulation of catabolic cytokines. Although no deleterious side effects of high concentrations of PRP were detected,

 DOI 10.1002/jor

in vivo studies are warranted prior to implementing PRP in the routine clinical management of tendonitis.

ACKNOWLEDGMENTS

This work was supported by a grant from the Harry M. Zweig Memorial Fund for Equine Research Program.

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