Locally administered autologous PRP has become standard-of-care practice to augment tissue repair in human medicine. Its clinical applications are diverse, including use for surgical sites, venous ulcers, and orthopedic injuries. In veterinary medicine, use of PRP in horses has become widespread over the past 5 years, most frequently for intralesional treatment of tendon and ligament injuries. Preparation and administration protocols vary, but PRP is most often generated by an automated method and then injected directly, without intentional platelet stimulation. Similar to other aspects of equine practice, clinical use of PRP has outpaced research on the topic, and a best-practice administration protocol has not been determined.

Although the presence of growth factors in platelet α-granules has been established, release of these factors has been minimally investigated in horses and may vary substantially depending on the degree of platelet activation during the preparation or administration process. Indeed, growth factors are released only during the process of platelet activation and subsequent degranulation, not as a result of constitutive secretion. As evidence supporting the clinical use of PRP in horses begins to accumulate, research regarding the actual content of the desired growth factor product is necessary.

The 3 growth factors most often examined in studies of tissue repair are PDGF, TGFβ, and IGF-1, and all are contained within the α-granules of platelets. Platelet-derived growth factor, isoform BB (PDGF-BB); transforming growth factor β, isoform 1 (TGFβ); and insulin-like growth factor, isoform 1 (IGF-1) were quantified by use of ELISAs. Statistical analysis was conducted via repeated-measures ANOVA.

Effects of preparation method, shear force, and exposure to collagen on release of growth factors from equine platelet-rich plasma

Jamie A. Textor, DVM; Jeffrey W. Norris, PhD; Fern Tablin, VMD, PhD

Objective—To test the hypotheses that preparation method, exposure to shear force, and exposure to collagen affect the release of growth factors from equine platelet-rich plasma (PRP).

Sample Population—PRP obtained from 6 horses.

Procedures—PRP was prepared via 2 preparation methods (tube and automated) and subjected to 6 treatment conditions (resting, detergent, exposure to shear via 21- and 25-gauge needles, and exposure to collagen [10 and 20 µg/mL]). Concentrations of platelet-derived growth factor, isoform BB (PDGF-BB); transforming growth factor β, isoform 1 (TGFβ); and insulin-like growth factor, isoform 1 (IGF-1) were quantified by use of ELISAs. Statistical analysis was conducted via repeated-measures ANOVA.

Results—Platelet numbers were significantly higher in tube-prepared PRP than in automated-prepared PRP. Growth factor concentrations did not differ significantly between preparation methods. Mean PDGF-BB concentration ranged from 134 to 7,157 pg/mL, mean TGFβ concentration ranged from 1,153 to 22,677 pg/mL, and mean IGF-1 concentration ranged from 150 to 280 ng/mL. Shear force did not affect growth factor concentrations. Dose-dependent increases in PDGF-BB and TGFβ were detected in response to collagen, but equalled only 10% of the estimated total platelet content. Concentrations of IGF-1 were not significantly different among treatments and negative or positive control treatments. Serum concentrations of PDGF-BB and TGFβ exceeded concentrations in PRP for most treatment conditions.

Conclusions and Clinical Relevance—Release of growth factors from equine PRP was negligible as a result of the injection process alone. Investigation of platelet-activation protocols is warranted to potentially enhance PRP treatment efficacy in horses. (Am J Vet Res 2011;72:271–278)

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and it is available as the only FDA-approved topically applied growth factor for treatment of complicated wounds in humans.14 Because it is more promiscuous than other isoforms and can bind to either of its corresponding receptor subtypes, PDGF-BB is considered the most potent, although least plentiful, isoform.15 This growth factor is ideally suited for its role in the early stages of healing because it is chemoattractant for neutrophils and macrophages, induces macrophages to produce TGFβ, and serves as a mitogen for fibroblasts and myocytes. It also induces the phenotypic shift of fibroblasts to a contractile myofibroblast, which is important for wound contraction.15 Transforming growth factor β1, isoform 1 induces fibroblast production of extracellular matrix components and also mediates the fibroblast shift to a contractile phenotype.15,16 Insulin-like growth factor, isoform 1 is the main effector protein of growth hormone via its induction of cell proliferation,17 and IGF-1 can augment healing of equine tendon lesions.18 In the study reported here, 2 methods of equine PRP preparation and various methods of handling were performed to mimic the clinical administration process, and resulting concentrations of PDGF-BB, TGFβ, and IGF-1 were measured. The objectives of the study were to compare effects of an automated method and a tube method for preparation of equine samples on concentrations of growth factors; to determine whether treatment conditions encountered during the injection process, such as the shear force created by a needle or exposure to in situ collagen at the site of injection, would maximize release of growth factors into a lesion; and to compare concentrations of growth factors in equine PRP and serum. The hypotheses were that the release of growth factors from platelets would differ between preparation methods, concentrations of growth factors would increase (compared with concentrations for resting control platelets) when exposed to shear forces or collagen, and concentrations of growth factors in PRP would exceed those in serum.

Materials and Methods

Sample population—Blood was obtained from 6 healthy adult horses of various breeds (Arabian, Holsteiner, Quarter Horse, and Thoroughbred). There were 3 mares and 3 geldings; horses ranged in age from 5 to 17 years (mean, 11.3 years). They were owned by and housed at the Center for Equine Health at the University of California-Davis. Animal care and use was approved by the University of California-Davis Institutional Animal Care and Use Committee. Health status of each horse was assessed by a veterinarian each time blood was collected.

PRP processing—Two differential centrifugation methods were used to prepare PRP. A tube method and an automated method10 were used for processing. Blood was kept at 37°C between processing steps. An automated counter10 was used to perform CBCs on whole blood and PRP during each experiment.

For the tube preparation, blood from each horse was collected into 6 standard 8.5-ML evacuated tubes containing ACD-A (total amount of blood collected from each horse, 51 mL). Preparation was performed in accordance with a standard laboratory platelet isolation protocol.19 Briefly, blood was centrifuged1 at 200 X g for 15 minutes, and PRP was harvested and transferred to new tubes. In all centrifugation steps, acceleration and deceleration were set to the lowest rate to minimize shear forces that could activate platelets. Prostaglandin E1, a platelet inhibitor conventionally used in laboratories to prevent platelet activation during centrifugation, was added to the PRP to achieve a concentration of 10 μg of PGE1/mL. The PRP then was centrifuged at 400 X g for 15 minutes to further concentrate platelets. The PPP supernatant was removed. The small residual volume was used to resuspend the platelets, which were then set aside. Prostaglandin E1 was added to the PPP, and the mixture was centrifuged again at 400 X g for 20 minutes to extract as many platelets as possible. The PPP was removed and saved for later use, and the platelets were resuspended and combined with those from the first centrifugation. Resuspension and dilution of platelets were accomplished by the addition of PPP to achieve a target concentration of 1,000 X 103 platelets/μL; when this maximum concentration could not be achieved, all samples were diluted to match the lowest concentration obtained on that experimental day (referred to as the batched platelet count).

For the automated preparation, processing was performed in accordance with the manufacturer’s instructions, except that, in an effort to maximize platelet concentrations, we did not add 2 mL of ACD-A to the PRP compartment prior to centrifugation. The final volume of PRP for the automated method was 7 mL. Each sample was divided into 2 equal volumes, and PGE1 (10 μg/mL) was added to 1 of these volumes to facilitate comparison with the tube preparation method. The addition of PGE1 was considered a third preparation method during data analysis. Dilutions were performed as for the tube method to achieve equal platelet concentrations for all samples in a given experiment.

Treatment conditions—Six treatment conditions were tested: resting (unstimulated [negative control treatment]), 0.5% nonionic detergent2 (saturated membranes [positive control treatment]), shear with a 21-gauge needle, shear with a 25-gauge needle, and collagen2 (10 and 20 μg/mL). Aliquots of equal volumes were subjected to 1 treatment each and then promptly microcentrifuged2 (21,000 X g for 10 minutes). Supernatants were harvested and frozen at –20°C for subsequent analysis of growth factors. Serum samples were also prepared, microcentrifuged, and frozen for comparison with PRP.

Analysis of growth factors—Concentrations of PDGF-BB, TGF-β1, and IGF-1 were determined by use of ELISAs. Each of the kits, which were designed for use in samples obtained from humans, has been validated for use in horses.20-23 Samples were diluted, prepared, and measured on a microplate reader2 in accordance with the manufacturer’s instructions. Each sample was assayed at least in duplicate. Data were transformed by use of a 4-parameter logistic curve fit (PDGF-BB and TGF-β1) or linear regression (IGF-1), as indicated in each manufacturer’s instructions.
Statistical analysis—The tube and automated methods of PRP preparation were performed on different dates; a paired t test (significance set at \( P < 0.05 \)) was performed to ensure that the hematologic variables of the whole blood samples were comparable between the 2 dates and methods. The PRP indices were similarly analyzed. Because a uniform, target platelet concentration (1,000 \( \times 10^3 \) platelets/\( \mu L \)) was not achieved in every PRP sample, it was important to evaluate whether there was an effect of platelet count on resultant growth factor concentration. Concentrations of growth factors were compared between the measured concentration and the concentration projected on the basis of the target platelet count of 1,000 \( \times 10^3 \) platelets/\( \mu L \). Projected growth factor values were calculated by multiplying the measured growth factor concentration by 1,000 \( \times 10^3 \) platelets/batched platelet count. Data were analyzed by use of a 3-factor (2 repeated within-subject factors [preparation method and treatment] and 1 grouping between-subject factor [sex]), repeated-measures ANOVA. Significance was set at \( P < 0.05 \) for all tests, and Huynh-Feldt adjusted \( P \) values were used to address sphericity violations in the data. When significant differences were detected for the overall ANOVA, post hoc pairwise comparisons were performed by use of the least significant difference test. Additionally, each preparation-treatment combination was compared, including comparison with serum data, by use of a repeated-measures ANOVA.

Results

All horses were systemically healthy throughout the study. Three horses were medicated for minor wounds during the study: all received phenylbutazone, and 2 of these horses also received trimethoprim-sulfadiazine. In each case, phenylbutazone had been discontinued for at least 3 days prior to blood collection. One of the horses that received trimethoprim-sulfadiazine was still being treated during a blood collection date; the platelet count for this horse on that day was consistent with counts measured in this horse on 6 other blood collection days. One horse was entered into another study concurrently and received a vaccination against *Bartonella bovis*; however, this horse remained clinically normal, with CBC and hematologic results within reference ranges. Blood was not collected from this horse for use in the study reported here until 6 weeks after vaccination.

Hematologic variables—Paired t tests of the pooled hematologic data for all horses revealed a significant (\( P = 0.012 \)) difference in WBC counts in whole blood between dates of the 2 preparation methods. However, although WBC counts differed significantly, the WBC counts for whole blood were extremely comparable for all horses (mean ± SD, 5.86 ± 0.94 \( \times 10^3 \) WBCs/\( \mu L \) and 5.3 ± 0.91 \( \times 10^3 \) WBCs/\( \mu L \) in the whole blood used for tube and automated PRP preparations, respectively) and were within the reference range for our laboratory (reference range, 5.0 \( \times 10^3 \) WBCs/\( \mu L \) to 11.6 \( \times 10^3 \) WBCs/\( \mu L \)). There were no other significant differences in hematologic variables of whole blood.

Despite the slight difference for the WBC count in whole blood samples, there was no significant (\( P = 0.816 \)) difference in WBC counts in PRP between the 2 preparation methods. In addition, there were no significant differences in RBC counts or hemoglobin concentration in PRP samples among horses and between the 2 preparation methods. However, although both methods of PRP preparation resulted in a 4- to 10-fold concentration of platelets over whole blood values, the tube method resulted in significantly (\( P = 0.001 \)) greater platelet numbers (mean ± SD, 1,765 ± 399.6 \( \times 10^3 \) platelets/\( \mu L \)) than did the automated method (mean ± SD, 951 ± 243.1 \( \times 10^3 \) platelets/\( \mu L \)). The target platelet count (1,000 \( \times 10^3 \) platelets/\( \mu L \)) was achieved in 6 of 6 tube preparations but in only 3 of 6 automated preparations. Initial platelet counts in PRP were determined for each horse (Figure 1). Mean batched platelet counts were 1,000 \( \times 10^3 \) platelets/\( \mu L \) for PRP prepared via the tube method and 691 \( \times 10^3 \) platelets/\( \mu L \) for PRP prepared via the automated method.

Growth factors—The measured growth factor concentrations were not significantly different from the measured growth factor concentrations (which were in some cases from samples with lower platelet counts) for PDGF (\( P = 0.23 \)) or TGF-\( \beta_1 \) (\( P = 0.06 \)) but were significantly different for IGF-1 (\( P = 0.04 \)). All data reported for growth factors were for growth factor measurements obtained by use of the batched platelet concentrations.

Concentration of PDGF-BB was significantly (\( P = 0.007 \)) greater in PRP prepared by use of the automated method (without the addition of PGE) than in PRP prepared by use of the tube method. However, PDGF-BB concentrations did not differ significantly (\( P = 0.053 \)) between PRP prepared by use of the tube method and PRP prepared by use of the automated method with the addition of PGE.

Concentration of PDGF-BB was significantly (\( P < 0.001 \)) higher for the nonionic detergent than for...
any other treatment. No effect of shear force for the 21- or 25-gauge needles was detected. Compared with results for resting (unstimulated) platelets, exposure to collagen resulted in significantly (10 µg/mL, \(P = 0.007; 20 \mu g/\mu L, P = 0.043\)) higher (3-fold increase) concentrations of PDGF-BB (Figure 2; Table 1).

Mean ± SD PDGF-BB concentration in serum was 1,343 ± 206 pg/mL. This was approximately a 6- to 10-fold increase over the concentrations for resting platelets \((P < 0.001)\), but only 19% to 31% of the concentrations for the nonionic detergent treatment \((P = 0.016)\).

Concentrations of TGF\(_B\) did not differ significantly \((P = 0.201)\) among the 3 preparation methods. No significant effect of shear force for the 21- or 25-gauge needles was detected. Exposure to collagen at 10 µg/mL resulted in a significantly \((P = 0.006)\) higher (2-fold increase) TGF\(_B\) concentration, compared with the concentration for resting platelets, whereas TGF\(_B\) concentration after exposure to collagen at 20 µg/mL did not differ significantly from the concentration for resting platelets. The TGF\(_B\) concentration was significantly \((P < 0.001)\) higher for the nonionic detergent treatment than for any other treatment (Figure 2; Table 2).

**Table 1**—Mean ± SD PDGF-BB concentration (pg/mL) in PRP obtained from 6 horses via 3 preparation methods and subjected to 6 treatment conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tube</th>
<th>Automated + PGE(_I)</th>
<th>Automated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting*</td>
<td>134 ± 104</td>
<td>158 ± 181*</td>
<td>228 ± 143*</td>
</tr>
<tr>
<td>Nonionic detergent†</td>
<td>4,332 ± 2,212</td>
<td>6,561 ± 1,325</td>
<td>7,157 ± 2,229</td>
</tr>
<tr>
<td>Shear force</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-gauge needle</td>
<td>186 ± 111</td>
<td>253 ± 294</td>
<td>251 ± 177</td>
</tr>
<tr>
<td>25-gauge needle</td>
<td>157 ± 272</td>
<td>216 ± 258</td>
<td>207 ± 139</td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 µg/mL</td>
<td>341 ± 249</td>
<td>283 ± 184*</td>
<td>619 ± 270*</td>
</tr>
<tr>
<td>20 µg/mL</td>
<td>337 ± 175</td>
<td>386 ± 218</td>
<td>869 ± 662</td>
</tr>
</tbody>
</table>

*†Concentrations for this treatment differed significantly \((*P < 0.001; †P = 0.016)\) from the mean ± SD PDGF-BB concentration in serum, which was 1,343 ± 206 pg/mL.

**Figure 2**—Effects of treatment condition on concentrations of PDGF-BB (A), TGF\(_B\) (B), and IGF-1 (C) in PRP obtained from 6 horses. Concentrations for each growth factor are reported as the mean ± SD percentage of the concentration achieved for a nonionic detergent treatment (positive control treatment). Treatments were resting (unstimulated) PRP (negative control treatment [R]), shear force via a 21-gauge needle (21), shear force via a 25-gauge needle (25), collagen exposure at 10 µg/mL (C10), and collagen exposure at 20 µg/mL (C20). For IGF-1, the shear force treatments were not performed. *Value differs significantly \((P < 0.05)\) from the value for C10. †Value differs significantly \((P < 0.05)\) from the value for C20.
Mean ± SD TGFβ concentration in serum was 3,510 ± 623 pg/mL. This represented a 1.7- to 3-fold increase over the concentration for resting platelets (P < 0.001) but only 15% to 19% of the concentration for the nonionic detergent treatment (P = 0.013).

**IGF-1**

Because no effect of shear was detected for PDGF-BB and TGFβ, the 21- and 25-gauge needle treatments were not performed for IGF-1. Concentrations of IGF-1 were significantly (P = 0.003) higher for PRP prepared by use of the automated method than by use of the tube method despite lower platelet counts for the automated method. However, IGF-1 concentrations did not differ significantly (P = 0.113) between PRP prepared by use of the tube method and by use of the automated method with the addition of PGE1.

When corresponding treatment conditions were compared, IGF-1 concentrations differed significantly between PRP prepared by use of the tube method and by use of the automated method for resting platelets (P = 0.025), nonionic detergent (P = 0.021), collagen at 10 µg/mL (P = 0.033), and collagen at 20 µg/mL (P = 0.042; Table 2; Figure 2).

Mean ± SD IGF-1 concentration in serum was 201 ± 87 ng/mL. This was comparable with IGF-1 concentrations for resting platelets and the nonionic detergent treatment.

**SEX**

No effect of sex was detected for any of the 3 growth factors. Concentrations did not differ significantly between male and female horses for PDGF-BB (P = 0.169), TGFβ (P = 0.391), or IGF-1 (P = 0.334).

**Discussion**

In the study reported here, we measured the release of 3 major growth factors from equine PRP by use of treatments that mimicked conditions during and after intralesional injection. Horses of various ages and breeds and both sexes were used in the study to reflect a diverse patient population. We compared 2 preparation techniques: a tube method that is designed for benchtop use with standard laboratory centrifuges and an automated system that uses a proprietary collection-container kit and centrifuge with preprogrammed settings. The automated method was originally designed and tested for use in humans, but it is now commonly used in equine practice despite the fact there has been little specific validation. We determined that PRP generated by use of the automated method results in concentrations of growth factors comparable to those obtained by use of manual preparation methods. However, results of this in vitro study suggest that the intralesional injection process causes release of only a small fraction of the growth factor content in PRP, regardless of the preparation method.

During PRP preparation, premature platelet activation may occur, which may result in loss of growth factors into the supernatant (the PPP) rather than retention in the fraction used for treatment (the PRP). Our tube protocol involved the use of PGE1 to prevent premature activation and resulted in an 8-fold greater platelet concentration than has been reported for other tube methods. Use of the protocol in the study reported here was designed to provide a reference standard for comparison with the automated method in that this tube method consistently provides a truly resting, nonactivated platelet population at the desired concentra-

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**Table 2**—Mean ± SD TGFβ concentration (pg/mL) in PRP obtained from 6 horses via 3 preparation methods and subjected to 6 treatment conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tube</th>
<th>Automated + PGE1</th>
<th>Automated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting*</td>
<td>1,153 ± 197</td>
<td>1,653 ± 685*</td>
<td>2,076 ± 439*</td>
</tr>
<tr>
<td>Nonionic detergent</td>
<td>22,677 ± 12,125</td>
<td>13,622 ± 2,988</td>
<td>18,183 ± 2,396</td>
</tr>
<tr>
<td>Shear force</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-gauge needle</td>
<td>1,278 ± 577</td>
<td>1,363 ± 810</td>
<td>1,886 ± 474</td>
</tr>
<tr>
<td>25-gauge needle</td>
<td>1,358 ± 680</td>
<td>1,440 ± 560</td>
<td>1,667 ± 399</td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 µg/mL</td>
<td>1,932 ± 838</td>
<td>2,144 ± 1,177</td>
<td>3,031 ± 705</td>
</tr>
<tr>
<td>20 µg/mL</td>
<td>2,219 ± 1,028</td>
<td>2,164 ± 1,194</td>
<td>3,707 ± 2,509</td>
</tr>
</tbody>
</table>

*Concentrations for this treatment differed significantly (*P < 0.001; †P = 0.013) from the mean ± SD TGFβ concentration in serum, which was 3,510 ± 623 pg/mL.

See Table 1 for remainder of key.

**Table 3**—Mean ± SD IGF-1 concentration (ng/mL) in PRP obtained from 6 horses via 3 preparation methods and subjected to 4 treatment conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tube</th>
<th>Automated + PGE1</th>
<th>Automated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting*</td>
<td>180 ± 87†</td>
<td>215 ± 93*</td>
<td>280 ± 139*</td>
</tr>
<tr>
<td>Nonionic detergent</td>
<td>173 ± 81</td>
<td>232 ± 97</td>
<td>253 ± 96</td>
</tr>
<tr>
<td>Collagen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 µg/mL</td>
<td>153 ± 75†</td>
<td>143 ± 89</td>
<td>238 ± 128*</td>
</tr>
<tr>
<td>20 µg/mL</td>
<td>152 ± 60†</td>
<td>174 ± 81†</td>
<td>217 ± 101†</td>
</tr>
</tbody>
</table>

Shear force treatments via a 21- or 25-gauge needle were not used for IGF-1. Mean ± SD IGF-1 concentration in serum was 201 ± 87 ng/mL, which did not differ significantly (P = 0.05) from the concentration for any treatment.

See Table 1 for remainder of key.
tion specified in the human literature. We used a target platelet concentration of 1,000 × 10^3 platelets/μL because this concentration has been suggested as the therapeutic dose in humans.\textsuperscript{26} In comparison, our results indicated that the automated method can yield platelet concentrations greater than those previously reported for this system\textsuperscript{22,23} (likely because we did not add 2 mL of ACD-A to the PRP reservoir), although the counts were still substantially lower than those obtained by use of laboratory methods. Although the automated method still resulted in comparable concentrations of growth factors, several reports\textsuperscript{22–26} have indicated that maximizing the absolute number of platelets in PRP is important. A dose-dependent proliferative response to platelet numbers in media has been determined for fibroblasts\textsuperscript{3} and several other cell lines, including tenocytes,\textsuperscript{27} mesenchymal stem cells,\textsuperscript{28} endothelial cells,\textsuperscript{29} and osteoblasts.\textsuperscript{30,31} Nonetheless, although the automated PRP preparation resulted in lower platelet numbers than did the tube method, it is clinically convenient and does not appear to result in premature loss of growth factors. On the contrary, PRP obtained by use of the automated method actually yielded higher absolute concentrations of PDGF-BB and TGF\textsubscript{β} despite lower platelet numbers, compared with results for the tube method, and this was likely attributable to some platelet activation during the automated preparation process. This remained true even when PGE\textsubscript{2} was used because it was added fairly late in the process (ie, after centrifugation, rather than before centrifugation as for the tube method).

The treatment conditions used in the present study were designed to reflect factors encountered during the injection process into a tendon. At our institution, the administration of PRP into a lesion in a tendon or ligament has historically been performed with ultrasonographic guidance by use of a 21-gauge, 1.5-inch needle and a 3-mL syringe, without any additional platelet stimulation. Although a 23-gauge needle would not be practical for clinical use, it was used here to evaluate the effect of a more extreme shear force on platelets. No effect of shear was detected when compared with concentrations for resting platelets, and even high-pressure injection through a small-bore (25-gauge) needle did not increase concentrations of growth factors released by the platelets in PRP.

In vivo platelet activation by interactions with subendothelial collagen has been characterized.\textsuperscript{32} For that reason, it was our expectation that in situ collagen as exists in a tendon lesion would be adequate for full platelet activation and release of growth factors. The collagen used in this study was soluble type I fibrillar collagen from equine tendon. Although we observed a significant effect of collagen on growth factor release from PRP, these amounts were < 1% of the total platelet content of PDGF-BB and TGF\textsubscript{β}. Lack of a significant collagen effect was reported in a study\textsuperscript{33} on explant cultures of equine suspensory ligaments.

We used a nonionic detergent as a positive control treatment because it solubilizes plasma and organelle membranes, such as those of the platelet α-granule. It should be mentioned that this substance would not be safe for use in tissues, but it does provide a useful index for comparison in laboratory studies. On that basis, it was interesting that resting PRP released only 3% and 5% to 11% of its PDGF-BB and TGF\textsubscript{β} content, respectively.

The concentration of PDGF-BB known to induce proliferative and migratory responses in human fibroblasts in vitro is approximately 5 ng/mL, with the effect reaching saturation at 20 ng/mL.\textsuperscript{12} In experiments with equine tendon explants, however, it was determined that concentrations of 100 ng of recombinant-human PDGF-BB/mL were required before an effect on collagen mRNA expression was detected\textsuperscript{35} and that, surprisingly, endogenous mRNA expression for PDGF-BB monomers was not upregulated after tendon injury.\textsuperscript{34} These findings suggest that differential dose responses to PDGF-BB may exist, such that fibroblast infiltration into a lesion could possibly be improved by use of low doses of PDGF-BB but that improvements in collagen quantity or type may require substantially higher concentrations of PDGF-BB. In PRP obtained by use of the automated method, we detected PDGF-BB concentrations ranging from 0.207 ng/mL for resting PRP up to 0.869 ng/mL for collagen-treated PRP, with use of the nonionic detergent providing concentrations of approximately 7 ng/mL. Considered together, results reported in the literature and results for the study reported here indicate that a window of opportunity exists for administration of supplemental exogenous PDGF-BB in equine tendon lesions but that effective doses may not be achieved with current strategies for PRP use (ie, in its resting, nonactivated form).

In general, TGF\textsubscript{β} concentrations increase later during tissue repair and are sustained longer than are concentrations of PDGF-BB. In equine tendon, TGF\textsubscript{β} concentrations peak 4 weeks after injury at values \(15\) times as high as concentrations in uninjured tendon.\textsuperscript{35} In a recent study\textsuperscript{20} conducted by our laboratory group, fibroblast migration, alignment, and contraction were significantly improved by the addition of freeze-dried platelets, which delivered TGF\textsubscript{β} concentrations of approximately 1,700 pg/mL. These concentrations were attained in the present study by use of the automated method of preparation with shear and collagen treatments, for which mean values ranged from 1,667 to 3,707 pg/mL. Although the authors are not aware of any published studies conducted to evaluate purified TGF\textsubscript{β}, treatment of equine tendon lesions, our results indicated that injection of 0.5 mL of PRP would provide 10 to 16 times the amount of TGF\textsubscript{β} that would be endogenously produced by 1 mg of injured tendon tissue.\textsuperscript{35} Furthermore, data for the nonionic detergent treatment indicated that the quantity of TGF\textsubscript{β} released from resting PRP represents only a small fraction of the total amount available. Enhancement of the growth factor content of PRP via specific platelet activation may allow optimal growth factor doses to be achieved, which could drive the injured tissue into a truly regenerative response rather than one of repair, with scar formation and reduced function.

Despite the presence of IGF-1 in platelet α-granules, there appears to be minimal contribution of the platelets relative to the overall plasma content of IGF-1.\textsuperscript{9,21,29,38} The greater proportional content of
plasma, relative to platelets, in PRP with lower platelet concentrations may explain the apparent paradox of higher IGF-1 concentrations in those samples from our study. Because solubilization with the nonionic detergent did not change IGF-1 concentrations, platelet activation is not expected to result in physiologically relevant increases in IGF-1 quantities. Interestingly, PRP treatment of human fibroblasts results in upregulation of IGF-1 transcription,6 which suggests that PRP treatment may also induce endogenous intraleisonal production of IGF-1. Concentrations of IGF-1 are low in early equine tendon lesions,35 and intraleisonal treatment with IGF-1 can increase cellularity and collagen production and decrease lesion size, compared with untreated control lesions.14 Treatment with PRP should be reasonably expected to induce these same effects, with the added benefit that it represents a treatment that contains multiple growth factors at physiologic ratios. Another pattern in our study was that IGF-1 concentrations actually decreased slightly from those of resting samples when the PRP was treated with the nonionic detergent or collagen, similar to results reported by other investigators.37 This could suggest IGF-1 utilization by platelets during activation because platelets are known to express the IGF-1 receptor. Recently, IGF-1 has been reported as a likely autocrine mediator of platelet aggregation.38,39

A comparison of our findings with those of another recent report6 indicates that both PRP preparation and experimental methods matter with regard to the resulting growth factor content. Investigators in that study produced PRP by use of a commercially available system and reported PDGF-BB and TGFβ1 concentrations from unstimulated PRP that were approximately 4 times as high as the serum concentrations, which is in contrast to our findings. However, their PRP samples were frozen without separation of the platelets from plasma prior to the analysis of growth factor concentrations, which would result in activation and lysis of the platelets and release of growth factors. Freezing of platelets and other cell types in the absence of cryoprotectants is known to result in membrane damage and leakage of cellular contents.40 As a result, a freeze-thaw cycle has been a common experimental method used to induce maximal platelet activation and release of growth factors from PRP, but the delay created by freezing and thawing makes this method somewhat impractical for clinical use. The PRP growth factor concentrations reported in the aforementioned study6, which were measured after freezing, are therefore unlikely to represent those actually liberated into the tissue after injection with resting PRP. Regardless, the authors of that study6 were the first to report a controlled in vivo PRP experiment in horses and evaluated endpoints at 6 months in subjects with induced tendon lesions. On the basis of multiple variables, including DNA content, tissue architecture, and stress to failure, the authors of that study6 detected a significant positive effect of PRP in the healing of equine tendon lesions.

Our in vitro results suggest that the PRP administration techniques in common clinical use may not achieve a clinician’s intended goal of achieving substantial local augmentation of growth factor concentrations within a lesion. The methods assessed in the study reported here were representative of a typical nonactivated PRP injection and resulted in the release of < 1.5% of the total TGFβ1 and PDGF-BB content; these concentrations typically were exceeded by those in serum. The in situ events after PRP injection remain to be characterized, as does the complex issue of the optimal time course and concentrations of growth factors required to induce restoration of tissue function. Whether intraleionally injected resting platelets remain static and are then phagocytized as a largely untapped reservoir of growth factors or whether they slowly release the remaining growth factors over time are key questions that must be addressed when developing effective strategies for PRP treatment. Further research is indicated to determine an activation protocol for PRP use in horses to ensure that the intended delivery of growth factors to a lesion is actually achieved. The use of thrombin to activate PRP is routine in human medicine but has been only variably and infrequently used in horses.43,44 Although the aforementioned study revealed positive effects of PRP without an exogenous activator, maximal benefit as provided by the total growth factor content of PRP may not yet have been realized in clinical settings. To that end, investigation of a standardized PRP activation protocol for practical, clinical use in horses is needed.

References

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