Single-dose nonsteroidal anti-inflammatory drugs in horses have no impact on concentrations of cytokines or growth factors in autologous protein solution and platelet-rich plasma

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OBJECTIVE
To determine the effects of a single dose of the NSAIDs phenylbutazone, firocoxib, flunixin meglumine, and ketoprofen on concentrations of growth factors and cytokines in autologous protein solution (APS) and platelet-rich plasma (PRP).

ANIMALS
6 adult university-owned horses.

METHODS
For the first phase, 6 horses were randomized to receive ketoprofen (1,000 mg) or flunixin meglumine (500 mg) IV. Blood was obtained and processed for APS (Pro-Stride) and PRP (Restigen) before and 6 hours after administration of NSAIDs. Horses underwent a 2-week washout period, after which the protocol was repeated using a crossover design. For the second phase, following at least a 2-week washout period, the study protocol was repeated with phenylbutazone (1 g) or firocoxib (57 mg) administered orally. Plasma was collected 6 hours after administration for evaluation of drug concentrations, and APS and PRP were analyzed for concentrations of drug, platelets, leukocytes, and several growth factors and cytokines (PDGF, fibroblast growth factor, TGF-β1, IL-1β, IL-10, IL-6, IL-8, and tumor necrosis factor-α) before and 6 hours after administration of NSAIDs using immunoassays.

RESULTS
There were no significant differences in concentrations of cytokines or growth factors before or after administration of any NSAID. There were significant differences in concentrations of leukocytes and platelets based on both product and time. NSAID concentrations in plasma were not significantly different from concentrations in APS and PRP.

CLINICAL RELEVANCE
These results help guide clinicians on the appropriate use of these NSAIDs in conjunction with the processing of APS and PRP, which is unlikely to significantly alter the final product after single-dose administration.

Keywords: platelet-rich plasma, nonsteroidal anti-inflammatory drugs, cytokines, autologous protein solution, growth factors

NSAIDs are commonly used in performance horses to ameliorate inflammation and pain due to musculoskeletal disease. These same horses are likely to be managed for musculoskeletal disease with other methods, with a trend toward the use of autologous orthobiologics. Orthobiologics are preferred due to concerns regarding the potential deleterious effects of corticosteroids on articular cartilage and the risk associated with the use of corticosteroids in horses with metabolic disease.¹⁻³ Autologous protein solution (APS) and platelet-rich plasma (PRP) are 2 such autologous blood-based therapies for which there is supporting evidence for their role in reducing pain and improving the quality of healing in musculoskeletal disease (osteoarthritis, tendonitis, and desmitis) in horses, dogs, and humans.⁴⁻⁸ These products are thought to exert their effects by concentrating...
anti-inflammatory proteins in plasma, cytokine-releasing leukocytes, and platelets that release growth factors after activation.9–11

NSAIDs have been shown in both horses and humans in vivo to affect the concentrations of some cytokines and growth factors in blood-derived autologous substances such as PRP.12–14 Based on this, it is possible that the administration of NSAIDs before obtaining blood samples for processing of APS and PRP may result in an inferior or less effective product. Therefore, it is common in both human and veterinary practice to recommend cessation of administration of NSAIDs before and after preparation and administration of blood-based products. There is no peer-reviewed literature evaluating the effects of the administration of NSAIDs in vivo on concentrations of cytokines and growth factors in both APS and PRP, although a previously presented abstract15 at the International Cartilage Repair Society in 2016 suggests that in vitro incubation of blood with phenylbutazone, firocoxib, or flunixin meglumine does not alter concentrations of the IL-1 receptor antagonist protein (IL-1ra) or IL-1B. Therefore, the objective of this study was to determine the acute effects of a single dose of commonly used NSAIDs (phenylbutazone, firocoxib, flunixin meglumine, and ketoprofen) at clinically relevant doses on the concentrations of growth factors and cytokines in APS and PRP. We hypothesized that the single administration of NSAIDs would decrease the concentrations of clinically important anti-inflammatory cytokines and growth factors in APS and PRP. We hypothesized that the single administration of NSAIDs would decrease the concentrations of clinically important anti-inflammatory cytokines and growth factors in APS and PRP when blood was obtained 6 hours after administration of NSAIDs.

Methods

Case selection

Six systemically healthy, adult research horses owned by the University of Pennsylvania (New Bolton Center) were utilized for this prospective, crossover, randomized controlled clinical trial. This study was performed in accordance with the guidelines set forth by IACUC at the University of Pennsylvania (New Bolton Center). Horses were maintained in the field for the duration of the study.

Blood collection, drug administration, and product preparation

The left jugular vein was aseptically prepared, and blood was obtained for preparation of APS (Pro-Stride; Zoetis) and PRP (Restigen; Zoetis) in addition to plasma collection for drug concentration evaluation before administration of NSAIDs (T0). APS and PRP products were prepared in accordance with manufacturer instructions. Briefly, 2 sets of 55 mL of blood were drawn from the jugular vein into a 60-mL syringe with 5 mL of anticoagulant (acid citrate dextrose) and placed separately into the APS and PRP separator. The APS and PRP separators were centrifuged at 3,200 X g for 15 minutes. For PRP, platelet-poor plasma was removed from the device, and the remaining PRP was placed into aliquots for characterization. For APS, the PRP was removed from the separator device, transferred to the APS concentrator containing polyacrylamide beads, placed in a centrifuge, and centrifuged at 2,000 X g for 2 minutes. APS was then removed from the device and placed into aliquots for characterization.

For the first phase of the study, horses were randomized to receive ketoprofen (1,000 mg, IV) or flunixin meglumine (500 mg, IV) after blood collection. Doses were chosen to mimic those most commonly utilized in the field. During the first phase of the trial, after blood collection for preparation of APS and PRP in the morning, horses were administered ketoprofen or flunixin meglumine. Six hours after IV administration of ketoprofen or flunixin meglumine, blood was again obtained from the jugular vein after aseptic preparation for processing of APS and PRP (T6). A 6-hour time point was chosen to mimic a common situation encountered in practice, in which a horse has received medication in the morning and has presented for evaluation and/or intra-articular medication in the afternoon. Horses then underwent a 2-week washout period. After 2 weeks, the study protocol was repeated, with horses that received flunixin meglumine during the first phase receiving ketoprofen, and likewise, horses that received ketoprofen during the first phase receiving flunixin meglumine. Blood was again obtained for preparation of APS and PRP before (T0) and 6 hours (T6) after administration of NSAID.

For the second phase of the study, following a minimum 2-week washout period between phases, horses were randomized to receive either phenylbutazone (1 g, orally) or firocoxib (57 mg, orally). Blood was obtained for preparation of APS and PRP before administration of oral NSAID (T0) and again 6 hours (T6) after administration of oral NSAID. Horses underwent a 2-week washout period, after which the study protocol was repeated, with horses that received phenylbutazone receiving firocoxib and horses that received firocoxib receiving phenylbutazone. A 2-week washout period was chosen to account for 10 times the half-life of these substances (4.5 hours for phenylbutazone and 30 hours for firocoxib). At each time point, 60 mL of blood was additionally collected from the jugular vein into a Na-fluoride/K-oxalate tube and centrifuged (2,500 X g for 15 minutes), and 3 aliquots of plasma (2 mL per aliquot) were immediately frozen at −80 °C for further analysis. APS and PRP were separated into aliquots for immediate analysis of platelet and leukocyte concentrations and frozen for evaluation of NSAID concentrations and growth factor and cytokine characterization at a later date. For the evaluation of NSAID concentrations for APS, due to the volume limitations of the product, platelet-poor plasma was utilized from the first centrifuge as an approximation of NSAID concentration in this product. Aliquots for leukocyte and platelet concentrations were analyzed immediately after processing. APS and PRP aliquots for analysis of NSAID concentrations, growth factor, and cytokine concentrations for all drugs and all time points were stored in a −20 °C freezer before analysis.
APS and PRP characterization

Both APS and PRP samples were analyzed for concentration of platelets; leukocytes; several growth factors including FGF-2, TGF-β1, and PDGF-BB; and several cytokines including IL-1β, IL-10, IL-6, IL-8, and tumor necrosis factor-α (TNF-α) at each time point (T0 and T6) and for all drugs. Platelet and leukocyte concentrations were quantified with a veterinary hematology analyzer (Element HT5 Veterinary Hematology Analyzer; Heska) with a combination of flow cytometry, impedance, and colorimetry after inverting the product several times. The concentrations of FGF-2, IL-1β, IL-10, IL-6, IL-8, and TNF-α in APS and PRP were quantified with a fluorescent bead-based multiplex assay (MILLIPLEX; Millipore) using the Luminex 200 Instrument (Luminex). Concentrations of TGF-β1 in APS and PRP were quantified with a fluorescent bead-based single-plex immunoassay (MILLIPLEX; MilliporeSigma) using the Luminex 200 Instrument (Luminex). Briefly, 25 μL of standard, control, or sample was added to the appropriate well followed by the addition of 25 μL of antibody-immobilized beads. Plates were incubated overnight at 4°C. The following day, plates were washed 3 times followed by the addition of 25 μL of detection antibody to each well. The plate was incubated at room temperature on a shaker for 1 hour. Then, 25 μL of streptavidin-phycocerythrin was added to each well and incubated for 30 minutes on a shaker at room temperature. The plate was washed 3 times, and then 150 μL of drive fluid was added before analysis. Parameters for analysis on the Luminex 200 with xPONENT software were set at 50 events per bead and a sample size of 100 μL. Concentrations of PDGF-BB were quantified using a commercial ELISA kit (R&D Systems) per the manufacturer’s instructions. All antibodies were equine-specific except for TGF-β1, a human antibody that has been validated for use in horses.

Quantification of flunixin, firocoxib, ketoprofen, and phenylbutazone

Reference standards were purchased as follows: flunixin (CAS No. 42461-84-7), ketoprofen (CAS No. 22071-15-4), phenylbutazone (CAS No. 50-33-9; Frontier BioPharm), firocoxib (CAS No. 189954-96-9), phenylbutazone-d₉ (CAS No. 1189479-75-1; Toronto Research Chemicals), flunixin-d₃ (CAS No. 1015856-60-6; Santa Cruz Biotechnology), and ketoprofen-d₃ (CAS No. 15490-55-8; Fluka Analytical). High-performance liquid chromatography-grade methyl tert-butyl ether and autologous conditioned serum (ACS)-grade phosphoric acid (85%) were purchased from EMD Millipore.

Firocoxib, flunixin, ketoprofen, and phenylbutazone concentrations were quantified in equine plasma, APS, and PRP by liquid chromatography-tandem mass spectrometry. In brief, samples were prepared using liquid-liquid extraction with methyl tert-butyl ether. For flunixin, ketoprofen, and phenylbutazone analysis, samples were acidified with 1 M H₃PO₄ before extraction. The analytes were detected using a Sciex 7500 triple quadrupole mass spectrometer (AB Sciex LLC). The instrument was operated using both positive and negative modes with multiple reaction monitoring (MRM). A reverse-phase ACE C18 column (75 X 2.1-mm ID; 5-μm particle size; MAC-MOD Analytical) was employed to separate the analytes with mobile phases consisting of 5 mM ammonium formate and acetonitrile. The total analysis time was 5 minutes. For firocoxib, the MRM transition used was m/z 337→m/z 283, the internal standard was ketoprofen-d₃, and the linear dynamic range was 1 to 250 ng/mL. For flunixin, the MRM transition used was m/z 295→m/z 231, the internal standard was flunixin-d₃, and the linear dynamic range was 5 to 500 ng/mL. For ketoprofen, the MRM transition used was m/z 255→m/z 209, the internal standard was ketoprofen-d₉, and the linear dynamic range was 1 to 250 ng/mL. For phenylbutazone, the MRM transition used was m/z 255→m/z 209, the internal standard was phenylbutazone-d₉.

Figure 1—Platelet concentrations before and after administration of flunixin meglumine, firocoxib, phenylbutazone, and ketoprofen. APS = Autologous protein solution. PRP = Platelet-rich plasma. *P < .05; n = 6.

Figure 2—Leukocyte concentrations before and after administration of flunixin meglumine, firocoxib, phenylbutazone, and ketoprofen. APS = Autologous protein solution. PRP = Platelet-rich plasma. *P < .05; n = 6.
Figure 3—Cytokine concentrations before and after administration of flunixin meglumine, firocoxib, phenylbutazone, and ketoprofen for IL-10 (A), IL-8 (B), tumor necrosis factor-α (TNF-α; C), IL-1β (D), and IL-6 (E). APS = Autologous protein solution. PRP = Platelet-rich plasma. No significant differences were found; n = 6.
and the linear dynamic range was 10 to 1,000 ng/mL. The coefficient of determination ($r^2$) for all calibration curves was ≥ 0.99. Sample concentrations that were above the linear dynamic range were diluted and reanalyzed.

**Statistical analysis**

Power analysis was calculated using a paired $t$ test using a previous study evaluating concentrations of TGF-$\beta$1 in PRP and assuming means of 15% and 10% (assumed 5% decrease in the means) and an SD of 2.5. Power analysis revealed an estimated required sample size of $n = 6$. A mixed effects model was used to compare continuous variables including cytokine, growth factor, leukocytes, and platelet concentrations in APS and PRP. The horse was considered a random effect. Product and time were considered fixed effects with the interaction of product and time probed. Post hoc pairwise comparisons were made with the Tukey method adjustment for multiple comparisons. A Kruskall-Wallis test was performed to determine if a significant difference existed between concentrations of NSAIDs in APS, PRP, and plasma. All analysis was performed using statistical software (JMP Version 14, JMP). Statistical significance was set at $P < .05$.

**Results**

**Animals**

The median age of horses included in this study was 3.5 years (range, 3 to 12 years). Four horses were geldings, and 2 were mares. Breeds represented included 4 Thoroughbreds, 1 Appaloosa, and 1 Standardbred. All 6 horses completed the study without adverse events.

**Leukocyte and platelet concentrations**

Concentrations of platelets and leukocytes in APS and PRP before and 6 hours following administration of all 4 drugs are shown (Figures 1 and 2). Platelet concentration in APS 6 hours postadministration of flunixin meglumine and ketoprofen was significantly higher than APS before NSAID administration and significantly higher than PRP both before and 6 hours after NSAID administration. For firocoxib, concentrations of platelets were significantly higher in APS than in PRP before administration of firocoxib. For horses receiving phenylbutazone, the concentration of platelets was significantly higher in APS 6 hours after administration of phenylbutazone when compared to both APS and PRP before administration of phenylbutazone. Leukocyte concentration in APS 6 hours postflunixin meglumine administration was significantly higher than before the administration of flunixin meglumine. Concentrations of leukocytes in APS postadministration of flunixin meglumine were also significantly higher than in PRP at both time points. For both firocoxib and ketoprofen, concentrations of leukocytes in APS 6 hours postadministration were higher than concentrations of leukocytes in PRP before either drug administration. For phenylbutazone, concentrations of leukocytes in APS

Figure 4—Growth factor concentrations before and after administration of flunixin meglumine, firocoxib, phenylbutazone, and ketoprofen for FGF-2 (A), TGF-$\beta$1 (B), and PDGF (C). APS = Autologous protein solution. PRP = Platelet-rich plasma. No significant differences were found; $n = 6$.  

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6 hours after administration were significantly higher than concentrations of leukocytes in PRP before administration of phenylbutazone and 6 hours after administration of phenylbutazone. In addition, concentrations of leukocytes in APS before phenylbutazone administration were significantly higher than those in PRP before administration.

**Cytokine and growth factor concentrations**

There were no significant differences in concentrations of cytokines between products or time points (Figure 3). Although concentrations of IL-10, IL-6, TNF-α, and IL-1β appeared to be higher in APS than PRP at all time points, these differences did not reach statistical significance. Likewise, there were no significant differences in the concentration of growth factors between products or time points (Figure 4).

**Plasma, APS, and PRP concentration of NSAIDs**

Concentrations of phenylbutazone, firocoxib, ketoprofen, and flunixin were present in detectable quantities at 6 hours postadministration for plasma, APS, and PRP (Figure 5). No drug was present in any sample before NSAID administration (T0). There were no significant differences in concentrations of NSAIDs between plasma, APS, and PRP.

![Figure 5](image)

**Discussion**

There is a paucity of literature exploring the effect of the administration of NSAIDs in horses on concentrations of cytokines and growth factors in blood-derived products such as APS and PRP. In humans, the use of repeated doses of NSAIDs such as naproxen can alter platelet function and diminish the concentrations of active proteins (specifically PDGF and IL-6) in PRP obtained from these patients. Daily administration of aspirin has also resulted in a reduction in concentrations of PDGF, VEGF, and TGF-β in PRP preparations in humans.

Comparatively less information is available in horses, although surgical stress and reserpine are known to affect the composition of cytokines and growth factors in blood-derived products. The administration of ketoprofen to horses has been shown to increase concentrations of leukocytes and platelets in PRP, which may in fact indicate an increase in desired proteins in these substances with NSAID use, although concentrations of cytokines and growth factors were not directly measured in this study. The current study is the first to evaluate the effects of the administration of commonly used NSAIDs on the cytokine and growth factor profiles in horses in vivo. The results of this study indicate that a single dose of some NSAIDs can significantly increase concentrations of platelets and leukocytes in APS and PRP; however, administration of phenylbutazone, firocoxib, flunixin meglumine, or ketoprofen should not significantly alter the cytokine and growth factor profiles in these products if blood is collected at 6 hours postadministration of these NSAIDs.

There were significant differences in concentrations of leukocytes both between product and time point. Elevations in concentrations of leukocytes in APS when compared to PRP are not unexpected due to a second centrifugation step in the processing of APS, which concentrates leukocytes in the final product. Increases in leukocytes were also noted in APS after the administration of flunixin meglumine. This increase in leukocyte concentration may be due to the systemic effects of NSAIDs, which result in the inhibition of leukocyte chemotaxis, increasing free leukocytes in the vasculature and thereby increasing the concentrations of leukocytes that are present in blood-based products. There is conflicting evidence as to the clinical relevance of elevations in leukocyte concentrations with respect to the effectiveness of blood-derived products. Elevated concentrations of leukocytes are thought to promote a proinflammatory state by the release of proinflammatory cytokines, suggesting that administering a leukocyte-rich product intraleesionally or intra-articularly could result in persistent and deleterious inflammation. Some in vitro studies support this notion, indicating that there is a positive correlation between leukocyte concentration and catabolic gene expression in cocultured tendons and ligaments and that leukocytes increase the concentration of catabolic cytokines in PRP in people. In vitro, leukocyte-rich PRP has also been shown to result in cell necrosis and increased inflammatory markers in cultured synoviocytes. However, the results of these in vitro studies may not be reflective of the in vivo effects of elevated leukocyte concentrations, and some evidence would suggest that increased concentrations of leukocytes may in fact be beneficial in the management of certain musculoskeletal disease processes. For instance, the interaction of platelets with neutrophils has been shown to improve the anti-inflammatory action of PRP, and
positive correlations between leukocyte concentrations and IL-1ra and clinical scores have been demonstrated in people with knee osteoarthritis treated with APS. In addition, intralesional injection of a collagenase-induced Achilles tendinopathy in rabbits with leukocyte rich-PRP was more beneficial than leukocyte poor PRP. Another complicating factor is the recent discovery that processing of APS alters the gene expression of leukocytes and, in particular, monocytes. While in this study there appears to be an effect of NSAID administration on concentrations of leukocytes in some cases, the effects of NSAIDs on gene expression in these cell types are unknown. Further data must be collected before determining if the effects of NSAID administration on concentrations of leukocytes are beneficial or detrimental to their use in treating musculoskeletal disease.

Like leukocytes, there were often significantly higher concentrations of platelets in APS than in PRP, also likely due to the effects of a second centri
trification. The significant elevation in the concentration of platelets in APS 6 hours following NSAID administration is likely due to the inhibition of thromboxane-dependent platelet aggregation secondary to cyclooxygenase-1 (COX-1), resulting in an apparent increase in the total concentration of platelets in these blood-derived products. The absence of this elevation in horses administered a COX-2-selective NSAID (firocoxib) further supports this theory. This finding is in agreement with a previously performed study, demonstrating that horses receiving a 2-mg/kg dose of ketoprofen IV every 24 hours for 5 days results in significant increases in platelet concentrations in equine PRP. Our study would suggest that the effects of NSAIDs on platelet concentration in blood-based products occur more quickly than previously indicated. Given that previously a positive correlation between platelet concentration and growth factor concentration was measured in PRP, the additional increase in platelet concentration in these blood-based products may be of benefit, although a similar correlation between increase in growth factor concentration and platelet concentration was not seen in our study. Studies have also shown a positive clinical effect on time to healing of intralesional injection of PRP with higher concentrations of platelets when compared to lower concentrations. However, some studies suggest that increased concentrations of platelets do not correlate completely with improvements in healing, as increases in platelet concentration resulted in significant reductions in collagen synthesis after culture with superficial digital flexor tendon explants. In addition, standardized concentrations of platelets for PRP have not been determined in horses, and human standards may not be directly applicable given the difference in structure of equine platelets. The clinical relevance of the increase in platelet concentration in APS after NSAID administration is not currently known, and further studies determining the ideal concentrations of platelets are necessary.

While differences in leukocyte and platelet concentrations were noted, there were no significant differences in cytokine or growth factor concentrations that were dependent on product or time point. Interestingly, there were significant differences in TGF-β1 concentrations depending on drug administration, with those horses in the phenylbutazone and firocoxib groups having higher concentrations than those in the ketoprofen and flunixin meglumine groups, both before and after drug administration. Given that these 2 phases of the study (firocoxib/phenylbutazone and ketoprofen/flunixin meglumine) were performed 2 weeks apart, this difference may be due to temporal variables. The reason why this difference was not reflected in other evaluated growth factors and cytokines is unclear.

When evaluating cytokine and growth factor profiles, a single horse was noted to have elevations in most cytokines across time points in both APS and PRP. No obvious differences in this horse’s clinical state or significance were identified. Whether this horse is reflective of an inherent proinflammatory state, or if there are other, unknown factors (such as stress) at play, is unclear. Surgical stress, dehydration, and exercise have been shown to alter cytokine profiles and cellular concentrations of PRP in horses, and therefore, it is possible that an unidentified factor played a role in the elevations of cytokines in blood-based products in this horse. However, this degree of variability may indicate that evaluation of cytokine and growth factor profiles may be helpful in determining the suitability of the product for intralesional or intra-articular injection, given the potential deleterious effects of elevations in proinflammatory proteins when administered in these locations. However, it is important to note that the ratio between pro- and anti-inflammatory cytokines is considered by some to be critical when evaluating these autologous blood-based products, and therefore, absolute concentrations may not affect the overall quality of the product.

Detectable concentrations of all NSAIDs evaluated in this study were present in APS, PRP, and plasma at 6 hours postadministration. Concentrations of NSAIDs in these blood-based products were not significantly different from those in plasma. This is consistent with previously performed studies evaluating the concentration of firocoxib in ACS 4 hours after administration of firocoxib orally, although this study was performed after 2 doses of the medication. Although some concerns may arise from the potential for prolonging the risk of a positive drug test with the use of blood-based autologous substances with detectable quantities of the drug, the previously referenced study additionally indicated that no horses medicated intra-articularly with ACS prepared from horses administered firocoxib resulted in subsequent increases in plasma concentrations of firocoxib, making this an unlikely scenario as long as appropriate withdrawal guidelines are followed for the NSAID administered.

Limitations of this study included the use of primarily young Thoroughbreds, which may not be reflective of the wider sport horse population, and
been previously demonstrated in APS.24 Between leukocyte concentrations and IL-1ra has in IL-1ra in these products, as a positive correlation leukocyte concentrations in APS after NSAID admin-

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the unknown systemic status of the horses used in this study, although all horses used were apparently healthy by visual inspection. By performing this study in 2 phases rather than as a 4-way crossover study, it is possible that the administration of NSAIDs in the first phase of the study influenced the second phase. However, given that plasma levels of NSAIDs were zero before the administration of all NSAIDs, the washout period between phases was likely appropriate and unlikely to affect the findings in the second phase of the study. A single, 57-mg dose of firocoxib was also administered, rather than a loading dose of 3X, as is sometimes utilized to reach therapeutic concentrations of the drug more quickly in horses. As labeled instructions for this medication do not include the use of a loading dose, and a loading dose is not ubiquitously used, the decision was made to maintain dosage as labeled instructions dictate. In addition, only selected cytokines and growth factors were investigated during this study, which may not reflect the acute effect of NSAIDs on other such proteins. Due to the limited volume of substance obtained for APS, and the high-volume requirement for investigation of leukocyte and plate-

In conclusion, our hypothesis was rejected as there were no significant differences in concentra-
tions of cytokines or growth factors in APS or PRP 6 hours after administration of any of the evaluated NSAIDs. Significant alterations in cytokine and platelet concentrations were present both by prod-
and platelet concentrations were present both by product and time; however, as the cytokine and growth factor profiles of these products were not concurre-
antly significantly altered, the clinical relevance of this finding is unknown. Based on these results, single-dose administration of ketoprofen, flunixin meglumine, phenylbutazone, or firocoxib at clinically relevant doses should not significantly alter the cyto-
kine and growth factor profile of APS or PRP when blood is obtained for the production of this sub-
stance 6 hours postadministration. These results will help inform clinicians on the appropriate use of these NSAIDs in conjunction with obtaining blood for pro-
cessing of APS, which is unlikely to significantly alter the final product. The cumulative effect of prolonged administration of NSAIDs on cytokine and growth factor profiles in APS and PRP is an avenue of future exploration and warrants investigation.