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Equine bone marrow aspirate and bone marrow aspirate concentrate are enriched with interleukin-1 receptor antagonist protein

Sidney Long, DVM[1]; Grigorios Maleas, DVM[2]; Zarah A. Belacic, BS[1]; Vivian G. Quam, DVM, MS[1]; Sushmitha Durgam, BVSc, MS, PhD[1]*

[1]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, The Ohio State University, Columbus, OH
[2]Equuria GbR Equine Orthopedics, Emstek, Germany

*Corresponding author: Dr. Durgam (durgam.3@osu.edu)

Objective
To analyze the cellular, growth factor, and cytokine composition of equine sternal bone marrow aspirate (BMA) and laboratory-centrifuged BMA concentrate (BMAC).

Methods
This was an in vitro experimental study. Cellular composition, growth factors (IGF-I, VEGF, PDGF, TGF-β1), and cytokines (IL-6, IL-10, tumor necrosis factor-α (TNF-α), and IL-1 receptor antagonist protein [IL-1Ra]) were quantified using a standard automated hematology analyzer and ELISA methods, respectively, in donor-matched BMA, BMAC (in-house centrifugation protocol), whole blood, and platelet-rich plasma (E-PET; Pall Inc) prepared from 25 horses from 2016 through 2020.

Results
Leukocytes, neutrophils, monocytes, lymphocytes, and thrombocytes were increased 4.1-, 3.4-, 4.6-, 4.4-, and 2.5-fold in BMAC compared to BMA, respectively. Interleukin-1 receptor antagonist protein of BMAC was increased 21-fold compared to BMA and increased 117-fold compared to gravity filtration system–based, leukocyte-rich platelet-rich plasma.

Conclusions
Laboratory centrifugation enriches the cellular and IL-1Ra concentrations of equine BMAC compared to BMA, with no significant changes in IGF-I, VEGF, PDGF, TGF-β1, IL-6, IL-10, or TNF-α concentrations.

Clinical Relevance
Equine BMA and BMAC are patient-side biologics enriched with anti-inflammatory IL-1Ra and support further evaluation of equine BMAC for musculoskeletal tissue healing.

Keywords: bone marrow aspirate, BMAC, PRP, horse, Il-1Ra

Bone marrow aspirate (BMA) concentrate (BMAC) is an autologous orthobiologic that can be used IA and intra-/perilesionally for enhancing musculoskeletal tissue healing. Intracystic BMAC injection with or without debridement of cystic lesions of the medial femoral condyle and arthroscopy-guided IA BMAC injection for articular cartilage and subchondral bone restoration are common clinical applications of BMAC in horses.[1] Local BMAC delivery improved the healing of acute, experimental, full-thickness articular cartilage lesions in the equine distal femoral lateral trochlear ridge.[2] Although clinical uses of BMAC for equine soft tissue injuries are less commonly documented, a recent clinical case series of hindlimb proximal suspensory desmopathy treated with intralesional BMAC demonstrated improved lameness scores compared to horses that received intralesional leukocyte-rich platelet-rich plasma (PRP).[3] An advantage with BMAC is that it is a minimally manipulated source of small numbers of mesenchymal stem cells (MSCs) in additional to the cytokine and growth factor cargo with anti-inflammatory and immunomodulatory properties. In general, experimental and clinical studies investigating the efficacy of equine BMAC on musculoskeletal tissue healing are lagging compared

Received December 7, 2024
Accepted February 5, 2025
Published online February 27, 2025

doi.org/10.2460/ajvr.24.12.0380

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to other popular blood-derived orthobiologic solutions, such as PRP, autologous conditioned serum, or autologous protein solution.

Bone marrow aspirate concentrate is prepared through "patient-side" centrifugation of BMA in laboratory tubes or commercial disposable devices or via gravitational filtration systems.[2–4] The clinical efficacy of bone marrow–derived MSCs for treating equine soft tissue and articular injuries are well known[5,6]; however, MSC preparation from BMAC is expensive and requires prolonged laboratory processing and handling, which poses practical challenges for large-scale clinical use.[7,8] The characterization of human BMAC has shown that in addition to small numbers of MSCs, BMAC is a rich source of the anabolic growth factors and bioactive molecules IL-1 receptor antagonist protein (IL-1Ra), IL-1β, IL-8, PDGF, TGF-β2, and VEGF. Additionally, BMAC contains cellular components of the innate immune system that can mediate paracrine signaling and impact musculoskeletal tissue healing.[9–13] To date, there are no studies describing the cytological, cytokine, and growth factor compositions in equine BMAC. This is a necessary first step for experimental and clinical studies exploring the efficacy of BMAC for treating equine musculoskeletal pathologies. The objectives of this study were to (1) analyze the cellular, growth factor, and cytokine composition of equine BMA and BMAC and (2) conduct comparative analyses of donor-matched BMA, BMAC, whole blood (WB), and gravity filtration system–based, leukocyte-rich PRP. This study tested the hypothesis that laboratory centrifugation of equine BMA during BMAC preparation enriches the constituent cellular, growth factor, and cytokine profiles.

Methods

Patient recruitment

The donor-matched BMA, BMAC, WB, and PRP samples analyzed in this study were prepared from horses presented to Tierklinik in Lusche GmbH Equine Clinic in Germany from 2016 through 2020. Informed consent was obtained from the clients prior to inclusion in the study. Based on preliminary experiments in our laboratory and previous in vitro studies comparing human BMAC and PRP and an expected minimum 20% difference in IL-1Ra concentration between BMAC and PRP with a power calculation at > 0.8, the sample size was estimated at n = 12. All horses were nonracing sport horses diagnosed with unilateral hindlimb lameness due to proximal suspensory desmopathy of > 3 months duration.[3] All laboratory and analytical procedures detailed below were approved by the institutional review board.

Preparation of BMAC

Bone marrow aspirate concentrate was prepared from freshly aspirated sternal BMA via centrifugation in laboratory tubes optimized from a human BMAC protocol and as described previously.[3,14] Briefly, the sternum 5 to 8 cm caudal to the point of the olecranon was clipped, aseptically prepared, and infiltrated with 2% mepivacaine (Zoetis) under standing sedation maintained with 0.008 mg/kg, IV, detomidine HCl (Zoetis). An 11-gauge fenestrated bone marrow biopsy needle (Stryker Spine Inc) attached to a 10-mL syringe was rinsed with heparin before aspiration. Approximately 30 to 40 mL of BMA was harvested from each horse with 1,000 U of heparin/5 mL of BMA and then transferred into sterile 10-mL tubes through a 200-µm filter (Sangofix; B. Braun GmbH). The filtered BMA was centrifuged at 1,000 X *g* for 10 minutes, and the buffy coat and the last one-fourth of the supernatant were subsequently transferred into a separate sterile tube using a 3.5-inch, 18-guage spinal needle. This procedure yielded approximately 4 to 6 mL of BMAC per horse. Cytological analysis was conducted with 0.5 mL each of BMA and BMAC within 30 minutes of harvest and preparation, respectively. About 2 to 4 mL of BMAC was used for intralesional injection, and the remaining 2-mL volume was transferred to 1.5-mL polypropylene microcentrifuge tubes in 500-uL aliquots and stored at −20 °C for growth factor and cytokine analyses.

Preparation of PRP

Platelet-rich plasma was prepared in a fully enclosed gravitational system (E-PET; Pall Inc) as per the manufacturer's recommendations. The jugular vein was aseptically prepared, and 55 mL of WB was collected and mixed with 5 mL of ACD-A to yield 6 to 8 mL of leukocyte-rich PRP in approximately 15 minutes through gravity flow filtration.[3] Cytological analysis was conducted with 0.5 mL each of WB and PRP within 30 minutes of harvest and preparation, respectively, and the remaining volumes were transferred to 1.5-mL polypropylene microcentrifuge tubes in 500-µL aliquots and stored at −20 °C for growth factor and cytokine analyses.

Cytology

Cytological analyses with 0.5 mL of fresh BMA, BMAC, WB, and PRP were performed for all 25 horses on site within 30 minutes of collection or preparation using an automated standard hematology analyzer present in the clinic's laboratory.[3] The analysis included measurements of WBCs, monocytes, lymphocytes, neutrophils, thrombocytes, and PCV.

Growth factor and cytokine analysis

The total storage time for BMA, BMAC, WB, and PRP aliquots intended for growth factor and cytokine quantification did not exceed 18 months, and all samples shipped to the destination arrived frozen. The frozen samples underwent an initial slow thaw process at 2 °C, after which the samples were realiquoted in 50-to-200-µL volumes and frozen at −80 °C until individual growth factor and cytokine analyses. The frozen aliquots underwent 2 additional freeze-thaw cycles with overnight freezing at −80 °C followed by thawing at room temperature for 30 minutes prior to conducting the ELISA.[15] All growth factor and cytokine ELISAs used were equine-specific (Quantikine; R&D Systems) except PDGF-BB and TGF-β1, which were validated for equine use[15] (Quantikine Human PDGF-BB ELISA DBB00 and Quantikine Human TGF-β1 ELISA

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DB100B; R&D Systems), and samples were analyzed in duplicate. Growth factors IGF-I, VEGF, PDGF-BB, and TGF-β1 were quantified in BMA, BMAC, WB, and PRP from 13 horses. Cytokines IL-6, IL-10, tumor necrosis factor-α (TNF-α), and IL-1β were quantified in BMA, BMAC, WB, and PRP from 13 horses. Interleukin-1 receptor antagonist protein was quantified in BMA, BMAC, WB, and PRP from all 25 horses. All procedures were followed in accordance with the manufacturer's instructions.

Statistical analysis

All quantified concentrations were used in the statistical analysis regardless of the minimum detectable concentration for the ELISA. When the concentration measured was 0 or below the lower limit of quantification, a value of 0.1 was assigned prior to statistical analysis. Data were tested for normality using a Shapiro-Wilk test, and since the data were not normally distributed, comparisons between values of BMA, BMAC, WB, and PRP were made using a Friedman test. A P value of < .05 was considered statistically significant. Relationships between growth factors and cytokines with the cellular composition were determined using a Spearman correlation and reported when the R value was in the 0.6 to 0.8 (moderately high) and 0.8 to 1.0 range (high).[16] The statistical analyses and graphs were prepared using Prism, version 9.0.2 (GraphPad Software Inc).

Results

Animals

Twenty-five horses (9 mares, 12 geldings, and 4 stallions) were included. The median (range) age was 11 years (6 to 9 years), and disciplines were grouped as dressage (n = 16), eventing (n = 6), or jumping (n = 3). Owners reported the horses as Warmblood (n = 17), Andalusian (n = 5), Thoroughbred (n = 2), or German Riding Pony (n = 1).

Cellular composition

The cellular concentrations (median, range) of BMA, BMAC, WB, and PRP are summarized in **Table 1**. Leukocytes, neutrophils, monocytes, lymphocytes, and thrombocytes were increased 4.1- (P < .0001), 3.4- (P < .0001), 4.6- (P < .0001), 4.4- (P < .005), and 2.5-fold (P < .01) in BMAC compared to BMA, respectively. The percentages of monocytes, lymphocytes, and neutrophils of total leukocytes in BMAC were not significantly different from BMA. All cellular concentrations, except thrombocytes, in BMAC were significantly higher than gravity filtration system–based, leukocyte-rich PRP prepared from donor-matched horses.

Table 1—Median (range) cellular compositions and the differential cell counts in bone marrow aspirate (BMA), BMA concentrate (BMAC), whole blood (WB), and platelet-rich plasma (PRP) samples from 13 nonracing sport horses diagnosed from 2016 through 2020 with unilateral hindlimb lameness due to proximal suspensory desmopathy of > 3 months duration.

	Median (range)			
	BMA	**BMAC**	**WB**	**PRP**
Times 10^3 per microliter				
Leukocytes	18.9 (4.29–45.7)[a]	78.8 (31.1–214)[b]	7.6 (4.68–14.8)[c]	12.8 (2.01–84.8)[a,c]
Monocytes	1.95 (0.7–4.53)[a]	8.98 (2.63–25.9)[b]	0.33 (0.19–1.54)[c]	1.09 (0.16–5.78)[a,c]
Lymphocytes	5.53 (1.59–12.3)[a]	24.2 (8.61–132)[b]	2.68 (1.32–5.9)[c]	8.39 (1.56–29.1)[a]
Neutrophils	9.4 (2.21–16.9)[a]	32 (0.43–108)[b]	4.35 (1.77–10.9)[a,c]	2.25 (0.09–49.7)[c]
Thrombocytes	47.5 (13–75)[a]	125 (45–313)[b]	153 (81–352)[b]	608 (198–1,072)[c]
Percentage of total leukocytes				
Monocytes	9.5%[a,b]	11.9%[b]	5.0%[c]	7.6%[a,c]
Lymphocytes	33.5%[a]	33.5%[a]	33.2%[a]	66.7%[b]
Neutrophils	53.7%[a]	48.4%[a]	57.6%[a]	18.4%[b]

[a–c]Results with different letters differ significantly (P < .05).

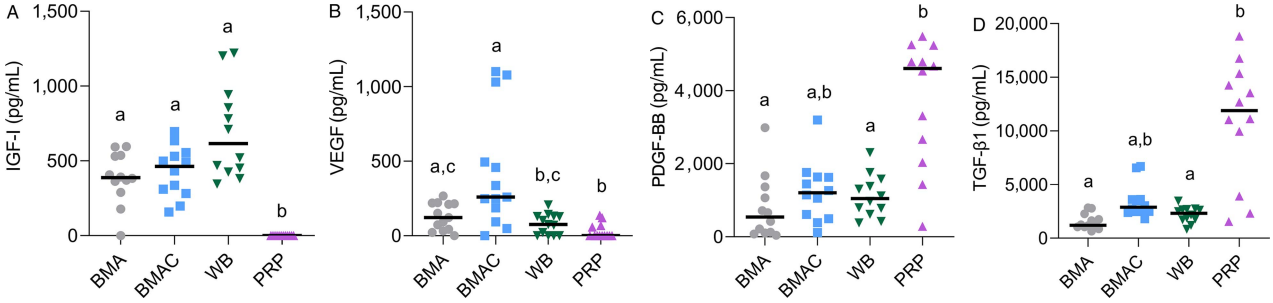

Figure 1—Individual-value plots of IGF-I (A), VEGF (B), PDGF-BB (C), and TGF-β1 (D) concentrations in bone marrow aspirate (BMA), BMA concentrate (BMAC), whole blood (WB), and platelet-rich plasma (PRP) samples from 13 nonracing sport horses diagnosed from 2016 through 2020 with unilateral hindlimb lameness due to proximal suspensory desmopathy of > 3 months duration. For each plot, the horizontal line represents the median. [a–d]Results for plots with different lowercase letters differed significantly (P < .05).

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Growth factor concentrations

The growth factor concentrations (median, range) of BMA, BMAC, WB, and PRP are detailed in **Figure 1 and Table 2**. There were no significant differences in the IGF-I ($P > .9$) and VEGF ($P > .9$) concentrations of BMA and BMAC. There were no significant differences in the PDGF-BB and TGF-β1 concentrations of BMA and BMAC or BMAC and

Table 2—Median (range) BMA, BMAC, WB, and PRP concentrations of growth factors (IGF-I, VEGF, PDGF-BB, and TGF-β1) and cytokines (IL-1Ra, IL-1β, IL-6, IL-10, and TNF-α) for the 13 horses described in Table 1 and of IL-1Ra for 12 donor-matched healthy horses.

	Median (range)			
Picograms per microliter	**BMA**	**BMAC**	**WB**	**PRP**
IL-1Ra	9,082[a]	196,200[b]	6,288[a,c]	1,668[c]
n = 25	(672–58,490)	(23,320–374,900)	(874–10,770)	(2–30,510)
IL-1β	12.5[b,c]	50.9[c]	12.5[b]	12.5[b]
n = 13	(12.5–57.3)	(12.5–245)	(12.5–12.5)	(12.5–12.5)
IL-6	236[b]	295[b]	347[b]	318[b]
n = 13	(12.5–3,779)	(12.5–3,961)	(12.5–8,400)	(12.5–8,400)
IL-10	334[b]	269[b]	313[b]	255[b]
n = 13	(94–1,537)	(53–1,626)	(192–4,189)	(156–6,861)
TNF-α	44.5[b]	63.4[b]	63.4[b]	101[b]
n = 13	(3.13–2,100)	(15.5–1,858)	(15.1–2,100)	(3.13–2,427)
IGF-I	388[b]	463[b]	616[b]	9.38[c]
n = 13	(9.38–596)	(158–695)	(345–1,221)	(9.38–9.38)
VEGF	121[a,b]	259[b]	75.8[a,c]	0.41[c]
n = 13	(0.41–266)	(0.41–1,101)	(0.41–207)	(0.41–137)
PDGF	542[b]	1,209[b,c]	1,047[b]	4,605[c]
n = 13	(42–2,988)	(115–3,198)	(384–2,305)	(287–5,490)
TGF-β1	1,211[b]	2,889[b,c]	2,330[b]	11,898[c]
n = 13	(651–2,847)	(1,814–6,698)	(843–3,468)	(1,550–18,832)

[a–c]Results with different letters differ significantly ($P < .05$).

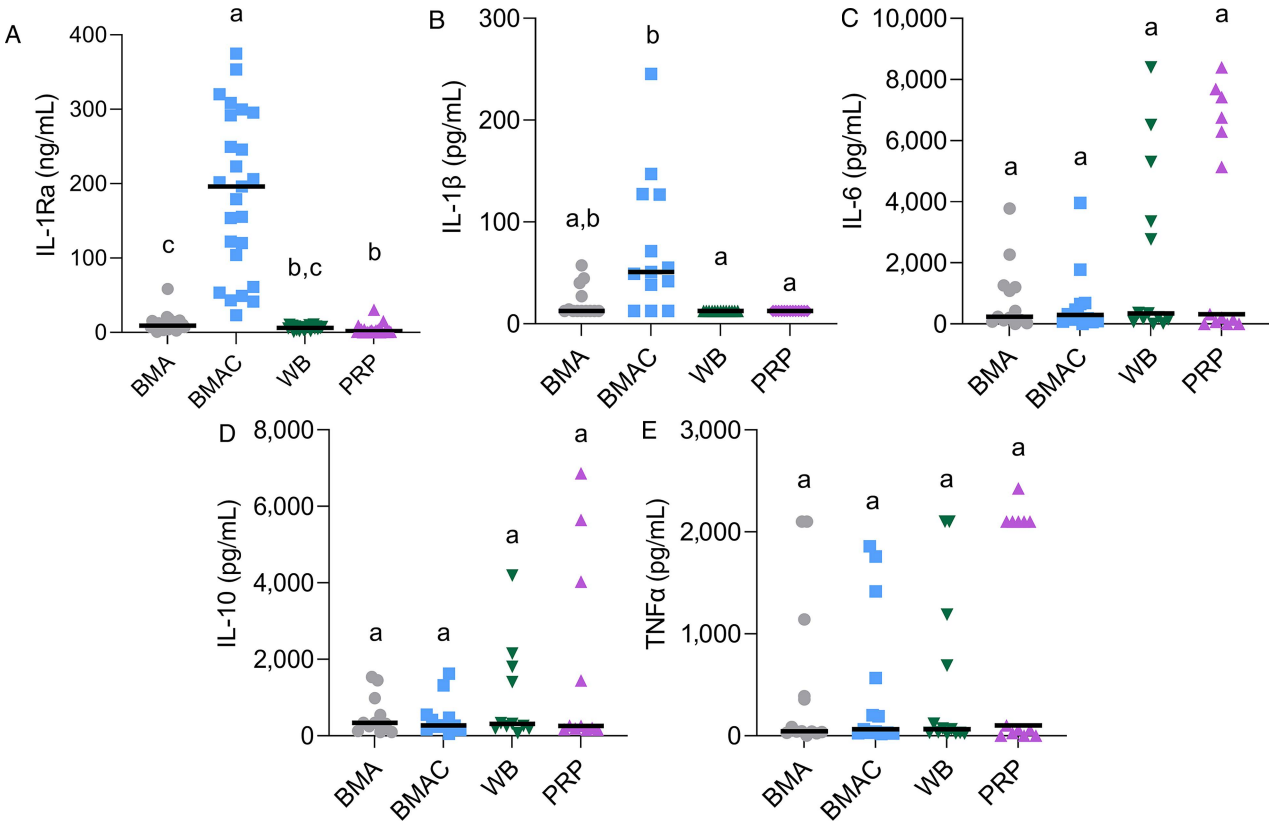

Figure 2—Individual-value plots of IL-1 receptor antagonist protein (IL-1Ra) concentration in BMA, BMAC, WB, and PRP for 12 donor-matched healthy horses and the 13 horses described in Figure 1 (A) and of IL-1β (B), IL-6 (C), IL-10 (D), and tumor necrosis factor-α (TNF-α; E) concentrations in the samples for only the 13 horses. [a–d]Results for plots with different lowercase letters differed significantly ($P < .05$).

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gravity filtration system–based, leukocyte-rich PRP. Transforming growth factor-β1 (R = 0.625; P > .001) and PDGF-BB (R = 0.619; P > .001) exhibited moderately high correlations with thrombocyte concentration. Insulin-like growth factor-1 and VEGF were increased 49-fold (P > .01) and 631-fold (P > .01) in BMAC compared to PRP, respectively.

Cytokine concentrations

Interleukin-1 receptor antagonist protein, IL-1β, IL-6, IL-10, and TNF-α concentrations (median, range) in BMA, BMAC, WB, and PRP are detailed in **Figure 2** and Table 2. Interleukin-1 receptor antagonist protein of BMAC was increased 21-fold (P < .0005) compared to BMA and increased 117-fold (P < .0001) compared to gravity filtration system–based, leukocyte-rich PRP. Interleukin-1 receptor antagonist protein exhibited moderately high correlation with leukocytes (R = 0.673; P < .001), monocytes (R = 0.672; P < .001), and neutrophils (R = 0.746; P < .001). Bone marrow aspirate concentrate IL-1b concentration was 4-fold (P < .01) and significantly higher than WB, BMA, and PRP.

Discussion

This study demonstrates that laboratory centrifugation of BMA significantly enriches leukocyte and IL-1Ra concentrations of BMAC and does not impact the basal IGF-I, VEGF, PDGF, or TGFβ-1 concentrations of BMA. Interleukin-1 receptor antagonist protein, IGF-I, and VEGF were significantly higher in BMAC compared to gravity filtration system–based, leukocyte-rich PRP. Leukocytes, monocytes, and lymphocytes were significantly increased in BMAC compared to PRP, whereas thrombocytes and, therefore, PDGF-BB were significantly higher in PRP. There were no significant differences in the TGF-β1, IL-6, IL-10, or TNF-α concentrations of BMAC and gravity filtration system–based, leukocyte-rich PRP. Interleukin-1 receptor antagonist protein concentration was the highest in BMAC, and IGF-I, VEGF, PDGF, TGF-β1, IL-1β, IL-6, IL-10, or TNF-α concentrations were not significantly different in BMAC and BMA. This is the first study to investigate the cellular, growth factor, and cytokine profiles of equine BMAC, and our results corroborate with studies[10,11] investigating the comparative profiles of human BMAC and PRP.

Bone marrow aspirate concentrate was prepared via in-house centrifugation of heparinized BMA at 1,000 X *g* for 10 minutes in laboratory tubes optimized from a human BMAC protocol.[14] This protocol yielded significantly higher concentrations of leukocytes, lymphocytes, neutrophils, and monocytes in BMAC compared to BMA. On the other hand, only the lymphocyte and thrombocyte concentrations in the gravity filtration system–based, leukocyte-rich PRP were significantly increased from WB. Although the percentage of neutrophils of total leukocytes in BMAC was higher than PRP, this was not significantly different from BMA or WB. We are not aware of previous studies comparing lymphocyte and neutrophil percentages in equine BMAC and PRP in relation to BMA and WB, respectively. While E-PET is a leukocyte-rich PRP preparation method,[3,17] our results show that 66.7% and 18.4% of the leukocytes consisted of lymphocytes and neutrophils, respectively. In contrast, Hessel et al[18] did not show lymphocyte enrichment, and the lymphocyte and neutrophil percentages of total leukocytes were equivocal and similar to WB.[18] The utility and suitability of leukocyte-rich versus leukocyte-poor PRP for musculoskeletal applications have been under debate; however, many studies[19,20] do not report leukocyte subtypes and assume that increased leukocytes represents increased neutrophils. The clinical significance of leukocyte subtype concentration, specifically the percentage of neutrophils versus lymphocytes, has yet to be elucidated for equine BMAC or PRP. Overall, ascertaining the role of innate immune cells in musculoskeletal tissue healing will serve as a strong foundation for future research determining BMAC indications for enhancing equine musculoskeletal tissue healing.

Insulin-like growth factor-I and VEGF concentrations were significantly higher in BMAC compared to PRP. This finding is consistent with an increased VEGF concentration in human BMAC compared to PRP.[10] Insulin-like growth factor-I was not detected in any PRP sample, and VEGF was detected in low levels in only 4 PRP samples. We are not aware of previous studies that have quantified IGF-I and VEGF concentrations in E-PET PRP, and it is likely a reflection of the E-PET system. Platelet-derived growth factor and TGF-β1 concentrations of this study's E-PET PRP are consistent with studies[17,18] using fresh and frozen E-PET PRP. Both PDGF and TGF-β1 positively correlated with thrombocyte concentration, as expected, since thrombocytes produce PDGF and contain the highest cellular concentration of TGF-β1.[21,22] Among a multitude of biological functions executed by PDGF and TGF-β1, recruiting MSCs, osteogenic cells, and tenocytes to promote tissue regeneration[23–25]; converting fibroblasts to myofibroblasts[26–28]; matrix formation and tendon cell proliferation[29,30]; and collagen content increase in healing tissues[27,31,32] are a few relevant effects attractive for musculoskeletal tissue regeneration. While the specific effects of individual growth factors in PRP and BMAC is an ongoing area of research, manipulating thrombocyte content in BMAC may allow for titrating PDGF and TGF-β1 concentrations of BMAC.

The median concentration of IL-1Ra in equine BMAC was 196,200 (range, 23,320 to 374,900) pg/mL and was 21-fold higher than BMA (9,082 pg/mL). To our knowledge, this is the first report documenting the concentrations of IL-1Ra in equine BMAC and BMA. Our result of increased IL-1Ra in BMAC compared to donor-matched, leukocyte-rich PRP samples are consistent with human studies.[10,11] Human studies[10,11] report, on average, a 4,500-to-5,200-pg/mL IL-1Ra concentration in BMA with a 3- to 5-fold increase in BMAC. It is important to note that the large intersample variability present in human BMA and BMAC samples is also present in equine BMA and BMAC; however, the higher IL-1Ra concentration and IL-1Ra:IL-1β ratio of equine BMA and BMAC relative to human studies warrants further

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evaluation. As expected, IL-1Ra concentration correlated with the leukocyte concentration. Interleukin-1 receptor antagonist protein is a monocyte-produced IL-1β competitive antagonist for IL-1 receptors and a clinically significant inhibitor for the downstream catabolic pathways.[33,34] From a clinical standpoint, IL-1Ra–enriched equine BMAC can be beneficial to inhibit IL-1β in inflamed and osteoarthritic joints since equine experimental studies investigating IA IL-1Ra and IL-1Ra gene therapy improved lameness scores and articular cartilage histology.[35,36] Interleukin-1β, the master inflammatory cytokine for osteoarthritis, was also significantly increased in BMAC compared to BMA. Research investigating the level of IL-1Ra needed to inhibit IL-1β in vitro and in vivo are lacking and will help determine the clinical and biological significance of IL-1Ra and IL-1β in equine BMAC. Investigating the comparative IL-1Ra concentrations of BMAC, autologous conditioned serum, and autologous protein solution is also warranted as the latter blood-derived biologics are commonly used for IA IL-1Ra delivery in practice.

There are limitations of this study to consider. The gravity filtration–based system used to prepare the leukocyte-rich PRP samples investigated in this study has been discontinued for clinical use. Additionally, Hauschild et al[17] showed that platelet and PDGF-BB concentrations of gravity filtration system–based, leukocyte PRP were 1.6- and 3-fold higher than centrifuge-based PRP, respectively, and comparing the profiles of centrifuge-based equine PRP and BMAC preparations are warranted as these systems represent the current clinical standard. Equine BMAC and PRP prepared using only a single method were compared in this study. These differences may not represent findings with other commercial and in-house methods used for preparing equine PRP or BMAC and warrants further investigation. Erythrocyte and MSC quantification in PRP and BMAC, respectively, were not included. According to PRP classification studies,[37,38] the minimal data reported for PRP should include erythrocyte concentration. Although the standard growth factors and cytokines investigated in equine orthobiologic solutions were quantified in this study, detection assays specific, as well as those that crossreact, to equine samples are limited, and incorporating multiplex or mass spectroscopy approaches would allow for comprehensive comparative assessments between equine BMAC and PRP. The WB and BMA used to prepare PRP and BMAC, respectively, in this study were obtained from horses diagnosed with chronic hindlimb proximal suspensory desmopathy.[3] While a clinically relevant study group was used, including fresh BMAC and PRP from a healthy control population would ensure a robust study design. The large intersample variability in all growth factor and cytokine concentrations in BMAC and PRP, although consistent with the results of similar equine studies, may impact the detection of subtle differences among BMA, BMAC, and PRP groups. Finally, 3 freeze-thaw cycles were used for platelet activation as previously described,[15] and consistent protocols were followed for PRP and BMAC. Although platelet activation is unlikely to impact IL-1Ra concentrations in BMAC and PRP, further investigation with alternative platelet activation protocols via 10% CaCl2 commonly used in in vitro and clinical PRP studies may be beneficial.

In addition to the mononuclear cells critical for innate and adaptive immune responses, BMA and BMAC contain 0.001% to 0.01% of MSCs.[39,40] While the number of MSCs required for equine musculoskeletal clinical applications are yet to be specified, studies have reported 4.62 CFUs/10^6 nucleated cells in BMAC. The clinical use of equine BMAC for enhancing musculoskeletal tissue healing is not as common as PRP. The sternal bone marrow aspiration procedure is more invasive and technically involved than jugular venipuncture and will need to be factored prior to BMAC clinical applications. Although there is concern that BMAC stimulates dystrophic mineralization within soft tissues, there are no published studies documenting this in equine or human patients. In vitro equine suspensory ligament tissue culture studies[41,42] demonstrate that acellular BMAC stimulated extracellular matrix synthesis to a greater extent than PRP. Equine BMAC provides a patient-side orthobiologic that contains anti-inflammatory IL-1Ra and MSCs. The cellular, cytokine, and growth factor characteristics of equine BMAC detailed in this study along with the existing in vitro and in vivo BMAC studies support further evaluation of equine BMA and BMAC as "off-the-shelf" autologous orthobiologics for enhancing equine musculoskeletal healing.

Acknowledgments

None reported.

Disclosures

The authors have nothing to disclose. No AI-assisted technologies were used in the composition of this manuscript.

Funding

Funded by The Ohio State University College of Veterinary Medicine Start-Up Funds.

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