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Short Communication

Validation of the Postprandial Interleukin-1β Response in Horses Using Equine-Specific Antibodies

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ABSTRACT

Interleukin (IL)-1β is a commonly studied proinflammatory cytokine, with relevance to arthritis, obesity, aging, and other inflammatory diseases of horses. Evaluating protein concentrations in plasma is a useful measurement for research in these areas of equine health. The objective of this research was to validate a commercially available enzyme-linked immunosorbent assay (ELISA) for equine IL1β and to compare concentrations with those previously published using an ELISA that is no longer available. The ELISA was assessed for linear parallelism and recovery using plasma from four healthy Standardbred mares. The assay was found to have linear parallelism for samples diluted 1:2, when the detection antibody concentration was 3 μg/mL. The average recovery of spiked IL1β was 98.9%. To compare concentrations, plasma was collected from six geldings at −0.5, 1, 2, and 4 hours after consumption of a meal high in starch and sugar (1.2 g/kg bodyweight). Consumption of 1.2 g of starch and sugar per kg of BW increased plasma IL1β concentrations 1 hour after feeding ($P = .053$). In conclusion, the commercially available ELISA is validated, with modifications, for use in equine plasma, and it detects a rise in plasma IL1β concentrations at 1 hour after meal consumption, a finding that is similar to previously reported data.

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1. Introduction

Interleukin (IL)-1β is a proinflammatory cytokine produced by multiple cell types in response to inflammatory stimuli. For instance, white blood cell mRNA concentrations are increased in horses following intravenous lipopolysaccharide infusion [1,2] and also in laminar tissue of horses with carbohydrate-overload induced laminitis [3]. Most measurements of IL1β have involved only mRNA; however, a recent investigation of IL1β protein levels demonstrated that plasma concentrations increase in response to the consumption of a meal high in starch and sugar [4]. This result was found using a canine antibody that cross-reacted with equine. Since then, an equine-specific antibody has been produced. Therefore, the purpose of this research project was to validate the enzyme-linked immunosorbent assay (ELISA) containing equine-specific antibodies, determine plasma concentrations of IL1β observed in horses fed a high starch and sugar meal, and compare these concentrations with previously published data.

2. Materials and Methods

2.1. Horses

The Ohio State University Institutional Animal Care and Use Committee approved this research protocol. Several milliliters of plasma were required for validation, and as such, blood from four healthy, nonfasted Standardbred

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mares (age 10–19 years; body condition score [BCS] 5–6) was collected specifically for the validation procedures. Six mature (6–16 years) geldings of light breeding (one Thoroughbred, one Appaloosa, one Paint, and three Quarter Horses) were used for testing the postprandial IL1β response. Geldings weighed 453 to 491 kg and had a BCS of 5 to 6 [5]. Beginning 24 hours prior to experimentation, horses were housed in individual 12' × 12' box stalls with ad libitum access to water and hay. Hay was removed at 10 PM on the day prior to testing, and horses were fasted overnight. Horses were abruptly switched to the concentrate feed, with no adaptation period, as previous research showed no difference in IL1β response between the first day of feeding and 90 days of feeding [4].

2.2. Treatment Diet and Sampling

At 8 AM on the day of testing, horses were offered a concentrate meal (70% wheat midds, 7% whole oats, 6% molasses, and 3% cracked corn, Table 1) that provided 1.2-g nonstructural carbohydrates (NSCs; where NSC = starch + ethanol soluble carbohydrates)/kg of bodyweight. Blood samples (10 mL into heparinized Vacutainers, BD, Franklin Lakes, NJ) were collected at 7:30, 9:00, 10:00 AM, and 12:00 PM. Blood was centrifuged for collection of plasma, and plasma was frozen at −20°C until analysis.

2.3. ELISA Validation

Plasma was analyzed for IL1β using the Equine IL1β Do-It-Yourself ELISA, streptavidin-horseradish peroxidase (HRP), and substrate and stop buffers (Kingfisher Biotech, Inc. St. Paul, MN). For all assays, 96 well plates (Nunc-Immuno MaxiSorp, Sigma Aldrich, St. Louis, MO) were coated with 100 μL/well of the capture antibody provided with the kit and incubated overnight at room temperature. Capture antibody was diluted to a concentration of 3 μg/mL in dulbecco's modified phosphate-buffered saline (DPBS) (BupH Phosphate Buffered Saline Packs, ThermoFisher Scientific, Waltham, MA). Following overnight incubation, plates were blocked for 1 hour at room temperature using reagent diluent (4% bovine serum albumin in DPBS). After blocking, plates were emptied and 100 μL of standard or sample was added to each well and incubated for 1 hour. Plates were then emptied and rinsed four times (200 μL/well) using wash buffer (0.05% Tween 20 in DPBS). Detection antibody (DAB) was diluted in reagent diluent to the concentration being tested (0.5–3 μg/mL), and 100 μL of this dilution was added to each well and incubated for 1 hour. Following this, plates were emptied, rinsed four times, incubated in streptavidin-HRP for 30 minutes, rinsed four times, incubated in substrate buffer for 30 minutes in the dark, and stopped with the addition of stop buffer. Plates were read immediately at a wavelength of 450 nm.

2.4. Linear Parallelism of Assay

During preliminary testing, the suggested concentration of the DAB (0.5 μg/mL) and dilution of plasma (1:4 and 1:8) was used, along with the suggested standard curve of 20, 10, 5, 2.5, 1.25, and 0 ng/mL. However, these conditions provided too little power of detection, and sample optical density values were too low to be of use. The assay was then tested with three concentrations of DAB (0.5, 1, and 1.25 μg/mL), and the same dilutions of plasma. Sample optical density values were above background for the 1.25 μg/mL DAB concentration and calculated an estimated concentration of 40 to 110 pg/mL. Based on these results, the DAB was tested at higher concentrations of 1.25, 2, and 3 μg/mL and the standard curve was adjusted to include values of 1,000, 500, 250, 125, 62.5, 31.25, 15.625, and 0 pg/mL. Plasma was assayed undiluted and at 1:2 and 1:4 dilutions. At the 3 μg/mL concentration of DAB, the 1:2 and 1:4 dilutions showed linear parallelism and had an average coefficient of variation between the back-calculated concentrations of 3%, compared to lower concentrations of DAB that gave an average coefficient of variation of 22%. For these reasons, the 3 μg/mL concentration of DAB was chosen for further testing.

2.5. Spiking and Recovery of Assay

Plasma was diluted 1:2 with either reagent diluent or reagent diluent spiked with a known amount of recombinant equine IL1β (15.6, 31.25, 62.5, 125, 250, and 500 pg). Observed and expected concentrations were compared in order to determine percent recovery (Table 2). Based on the findings of spiking and recovery, a 1:2 dilution of plasma and a DAB concentration of 3 μg/mL were chosen as appropriate assay conditions for the Equine IL1β ELISA. Concentrations of 1:2 diluted plasma ranged from 50 to 280 pg/mL, falling within the linear range of the standard curve (Fig. 1).

2.6. Sample Analysis and Statistical Analysis

Plasma samples from the geldings fed the treatment diet were analyzed for concentrations of IL1β using the above described conditions. Differences in mean IL1β were analyzed for the effect of hour using repeated measures analysis of variance in the mixed models procedure of SAS (SAS v. 9.3, SAS Institute, Cary, NC). Hour within horse was used as the repeated term, and the covariance matrix with the lowest Akaike information criterion corrected was chosen.

3. Results

Mean IL1β concentrations were different by hour ($P = .053$; Fig. 2), with concentrations at 1 hour after feeding

Table 1
Nutrient composition of the pelleted concentrate and hay.

Nutrient, % DM basis	Concentrate	Hay
Crude protein	15.18	7.35
ADF	14.39	41.27
NDF	28.13	70.15
NSC[a]	26.16	12.70
DE, Mcal/kg	3.41	1.83

Abbreviation: ADF, acid detergent fiber; DE, digestible energy; DM, dry matter; NDF, neutral detergent fiber; NSC, nonstructural carbohydrate.
[a] Nonstructural carbohydrates determined as starch plus ethanol-soluble carbohydrates.

Table 2
Concentrations of equine plasma diluted 1:2 with reagent diluent that was spiked with recombinant equine interleukin-1β.

Spike, pg	O, pg/mL	E, pg/mL	Recovery, %
0	182	182	—
15.6	204	198	96.2
31.25	222	213	97.6
62.5	254	244	97.6
125	304	307	103.2
250	429	432	101.7
500	712	682	97.1

Abbreviations: E, expected concentration; O, observed concentration.

higher than pretest values ($P = .061$). The average interassay coefficient of variation was 12%, and the intraassay coefficient of variation was 12%.

4. Discussion

Equine IL1β is a proinflammatory cytokine with increased production in response to inflammatory stimuli that also activates inflammation in a variety of tissue types. Specifically, IL1β activates cyclooxygenase-2, which then initiates the synthesis of prostaglandin E_2 [6]. This is an inflammatory process with specific implications in joint degeneration and osteoarthritis of horses [7]. Interleukin-1β has also been studied as a response factor to inflammatory diseases in horses or as an indication of degree of illness. For instance, sepsis increases IL1β white blood cell mRNA concentrations in foals [8]. Further, administration of lipopolysaccharide to healthy horses repeatedly results in increased IL1β mRNA concentrations in white blood cells [1,2] and skeletal muscle [9]. In young horses, IL1β may also inhibit muscle growth and development, as shown through reduced fusion of satellite cells following culture with recombinant IL1β [10]. Interleukin-1β may also be involved in laminitis, an inflammatory condition of the hoof. Horses administered starch at 17.6 g/kg bodyweight developed laminitis and had elevated IL1β concentrations in laminar tissue when they developed obel grade 1 laminitis [3]. Given the wide variety of diseases and conditions that IL1β has been investigated in relation to, an equine-specific ELISA may be helpful to evaluate blood concentrations for comparison to other measurements and clinical diagnostics.

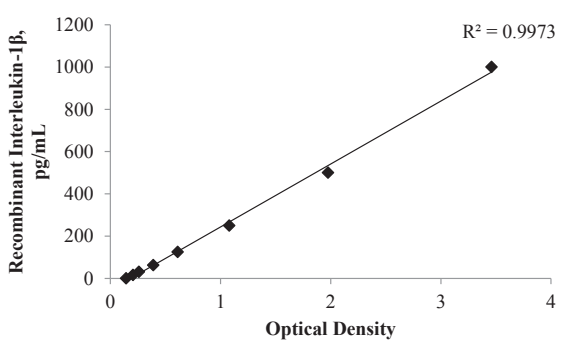

Fig. 1. Representative standard curve for equine IL1β ELISA. ELISA, enzyme-linked immunosorbent assay.

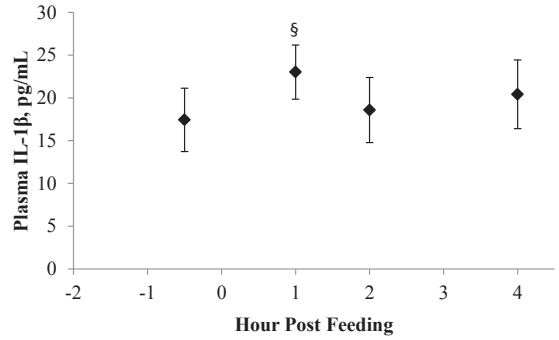

Fig. 2. Mean (±SEM) IL1β concentrations in equine plasma following consumption of 1.2 g of NSC per kg BW. $^{§}P < .1$ as compared to hour −0.5. BW, bodyweight; IL, interleukin; NSC, nonstructural carbohydrate; SEM, standard error of the mean.

An ELISA for detecting equine IL1β was previously validated for use in horses [11]; however, this ELISA utilized a canine antibody (raised in goat) that reacted with recombinant equine IL1β. Updating the production of antibody to an equine-specific antibody, raised in rabbit, resulted in a different concentration range and required the ELISA to be revalidated. Previous concentrations ranged from 1 to 544 ng/mL in healthy nonfasted horses [11] and 100 to 1,400 ng/mL in horses fed a high starch and sugar diet [4]. In the present study, concentrations averaged 18 pg/mL. A few of the samples used for validation ranged as high as 700 pg/mL, and one mare had concentrations that were well above the highest standard on the curve. Thus, significant variation in plasma concentrations exists, a finding that is similar to that discussed for other proinflammatory cytokines in horses.

The final aim of this experiment was to use the newly validated ELISA, with equine-specific antibodies, to confirm that a meal high in starch and sugar would increase plasma concentrations of IL1β. The previously published results utilized a canine antibody [4], and therefore, it became necessary to confirm that a meal high in NSC increases postprandial IL1β; and further, that this increase is detectable by the newly available equine antibody. We felt these confirmations were necessary prior to use of the assay in further studies that measure IL1β as a response metric for postprandial inflammation in horses. We previously reported that IL1β increased in plasma 1 hour after consumption of a meal providing 1.14 g NSC/kg bodyweight and returned to baseline by 3 hours after consumption, whereas horses consuming a meal providing 0.29 g NSC/kg bodyweight showed no postprandial response of IL1β. The present experiment resulted in similar findings of an increase at 1 hour after meal consumption; however, the return to baseline was much quicker and occurred by 2 hours after meal. The rapid rise in circulating IL1β concentrations could be due to gut inflammation; for instance, inflamed gut tissue is a known producer of IL1β [12], and its production is specifically induced by the normal gut microbiota upon damage to epithelial cells [13]. The tissue source of IL1β should be a focus of future study.

We also noted that the concentration range was different from previous research, a finding that is likely due to a different antibody being used. However, the overall pattern appears to be similar, showing that IL1β increases in plasma in response to the consumption of a grain-based meal. In the present study, this meal consisted of 1.2 g of NSC/kg bodyweight. Further research should evaluate the effects of different starch and sugar sources and concentrations on postprandial inflammation.

5. Conclusions

An equine-specific ELISA designed to detect IL1β has been validated, with modifications, for use in equine plasma. Concentrations of IL1β are lower than previously reported using a canine antibody; however, the pattern of response to a high NSC meal is similar. Further research should be conducted to elucidate the mechanism behind this response.

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References

[1] Nieto JE, MacDonald MH, Braim AEP, Aleman M. Effect of lipopolysaccharide infusion on gene expression of inflammatory cytokines in normal horses in vivo. Equine Vet J 2009;41:717–9.
[2] Tadros EM, Frank N. Effects of continuous or intermittent lipopolysaccharide administration for 48 hours on the systemic inflammatory response in horses. Am J Vet Res 2012;73:1394–402.
[3] Leise BS, Faleiros RR, Watts M, Johnson PJ, Black SJ, Belknap JK. Hindlimb laminar inflammatory response is similar to that present in forelimbs after carbohydrate overload in horses. Equine Vet J 2012;44:633–9.
[4] Suagee JK, Splan RK, Swyers KL, Geor RJ, Corl BA. Effects of high-sugar and high-starch diets on postprandial inflammatory protein concentrations in horses. J Equine Vet Sci 2015;35:191–7.
[5] Henneke DR, Potter GD, Kreider JL, Yeates BF. Relationship between condition score, physical measurements and body fat percentage in mares. Equine Vet J 1983;15:371–2.
[6] Farley J, Sirois J, MacFarlane PH, Kombe A, Laverty S. Evaluation of coexpression of microsomal prostaglandin E synthase-1 and cyclooxygenase-2 in interleukin-1- stimulated equine articular chondrocytes. Am J Vet Res 2005;66:1985–91.
[7] Mangal D, Uboh CE, Jiang Z, Soma LR. Interleukin-1β inhibits synthesis of 5-lipooxygenase in lipopolysaccharide-stimulated equine whole blood. Prostaglandins Other Lipid Mediat 2014;108:9–22.
[8] Castagnetti C, Mariella J, Pirrone A, Cinotti S, Mari G, Peli A. Expression of interleukin-1β, interleukin-8, and interferon-γ in blood samples obtained from healthy and sick neonatal foals. Am J Vet Res 2012;73:1418–27.
[9] Wearn JG, Suagee JK, Crisman MV, Corl BA, Hulver MW, Hodgson DR, et al. Effects of the insulin sensitizing drug, pioglitazone, and lipopolysaccharide administration on markers of systemic inflammation and clinical parameters in horses. Vet Immunol Immunopathol 2012;145:42–9.
[10] LaVigne EK, Jones AK, Londoño AS, Schauer AS, Patterson DF, Nadeau JA, et al. Muscle growth in young horses: effects of age, cytokines, and growth factors. J Anim Sci 2015;93:5672–80.
[11] Suagee JK, Corl BA, Crisman MV, Pleasant RS, Thatcher CD, Geor RJ. Relationships between body condition score and plasma inflammatory cytokines, insulin, and lipids in a mixed population of light-breed horses. J Vet Intern Med 2013;27:157–63.
[12] Reimund JM, Wittersheim C, Dumont S, Muller CD, Kenney JS, Baumann R, et al. Increased production of tumour necrosis factor-alpha interleukin-1 beta, and interleukin-6 by morphologically normal intestinal biopsies from patients with Crohn's disease. Gut 1996;39:684–9.
[13] Seo SU, Kamada N, Muñoz-Planillo R, Kim YG, Kim D, Koizumi Y, et al. Distinct commensals induce interleukin-1β via NLRP3 inflammasome in inflammatory monocytes to promote intestinal inflammation in response to injury. Immunity 2015;42:744–55.