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Clinical and Immunomodulating Effects of Ketamine in Horses with Experimental Endotoxemia

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Abstract

Background: Ketamine has immunomodulating effects both in vitro and in vivo during experimental endotoxemia in humans, rodents, and dogs.
Hypothesis: Subanesthetic doses of ketamine will attenuate the clinical and immunologic responses to experimental endotoxemia in horses.

Animals: Nineteen healthy mares of various breeds.

Methods: Experimental study. Horses were randomized into 2 groups: ketamine-treated horses (KET; n = 9) and saline-treated horses (SAL; n = 10). Both groups received 30 ng/kg of lipopolysaccharide (LPS, *Escherichia coli*, O55:B5) 1 hour after the start of a continuous rate infusion (CRI) of racemic ketamine (KET) or physiologic saline (SAL). Clinical and hematological responses were documented and plasma concentrations of tumor necrosis factor-α (TNF-α) and thromboxane B_2 (TXB_2) were quantified.

Results: All horses safely completed the study. The KET group exhibited transient excitation during the ketamine loading infusion ($P < .05$) and 1 hour after discontinuation of administration ($P < .05$). Neutrophilic leukocytosis was greater in the KET group 8 and 24 hours after administration of LPS ($P < .05$). Minor perturbations of plasma biochemistry results were considered clinically insignificant. Plasma TNF-α and TXB_2 production peaked 1.5 and 1 hours, respectively, after administration of LPS in both groups, but a significant difference between treatment groups was not demonstrated.

Conclusions and Clinical Importance: A subanesthetic ketamine CRI is well tolerated by horses. A significant effect on the clinical or immunologic response to LPS administration, as assessed by clinical observation, hematological parameters, and TNF-α and TXB_2production, was not identified in healthy horses with the subanesthetic dose of racemic ketamine utilized in this study.

Keywords
Horses, Immunomodulation, Lipopolysaccharide, Subanesthetic ketamine, Thromboxane, Tumor necrosis factor-α

Disciplines
Large or Food Animal and Equine Medicine | Statistical Methodology | Veterinary Microbiology and Immunobiology | Veterinary Toxicology and Pharmacology

Comments
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Endotoxemia is an important cause of morbidity and death in horses with diseases involving Gram-negative bacteria such as those associated with gastrointestinal dysfunction, pleuropneumonia, metritis, and peritonitis.[1–3] Exposure of host cells to lipopolysaccharide (LPS), or endotoxin, activates multiple inflammatory and immune responses intended to control Gram-negative infection. However, the activation and amplification of these responses can be excessive and result in detrimental effects to the host.[4] Production of tumor necrosis factor-α (TNF-α) is one of the earliest indicators of endotoxemia and contributes directly to the systemic clinical signs observed during periods of endotoxemia in the horse.[4] At a cellular level, TNF-α, activates the arachidonic acid cascade and cyclooxygenase-1 and -2 (COX), resulting in further production of other proinflammatory prostanoids and eicosanoids, such as thromboxanes, prostacyclins, and other prostaglandins.[1,5]

Abbreviations:

COX	cyclooxygenase
f-LPS	time after administration of LPS (time 0)
KET	ketamine-treated horses
LPS	lipopolysaccharide
NFκB	nuclear factor kappa B
SAL	saline-treated horses
TNF-α	tumor necrosis factor-α
TXB_2	thromboxane B_2

LPS-induced platelet and monocyte activation is a source of thromboxane production during periods of shock and endotoxemia in horses, resulting in microcirculatory dysfunction.[1,6–8] Thromboxane also represents COX-1 activity.[9] Therefore thromboxane and its stable metabolite thromboxane B_2 (TXB_2) are indicators of inflammatory changes that occur after LPS-induced endotoxemia in the horse.

Nonsteroidal anti-inflammatory drugs, polymixin B, pentoxifylline, plasma, phospholipid emulsions, pirfenidone, and E5531 have all been used to treat endotoxemia in horses.[10–14] The target of many of these medications is decreasing the production of inflammatory mediators such as TNF-α and thromboxane, which may, in turn, improve clinical status and outcome in endotoxemic horses.

Ketamine is a dissociative anesthetic routinely used in veterinary medicine. Additionally, ketamine has positive immunomodulating and anti-inflammatory effects during periods of experimental endotoxemia and septic shock in rats.[15,16] Specifically, ketamine down-regulates nuclear factor kappa B (NFκB) signaling, decreases

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inflammatory cytokine production (TNF-α, interleukin-6 [IL-6]), and suppresses platelet aggregation in mouse, human, and equine cell lines after exposure to LPS.[17–20] Furthermore, in vitro ketamine inhibits COX-2 expression in rats.[21] In dogs, subanesthetic ketamine demonstrates anti-inflammatory properties during periods of experimental endotoxemia.[22,23]

Immunomodulating properties of subanesthetic ketamine in horses during experimental endotoxemia have not been investigated in vivo. The potential of ketamine to attenuate the inflammatory and immune responses in endotoxemic horses is supported by previous reports.[18,19] Hence, we hypothesized that a subanesthetic ketamine continuous rate infusion (CRI) can safely be administered to conscious horses and attenuate the clinical, inflammatory, and immunologic responses in an experimental model of endotoxemia in healthy horses. Specifically, we investigated proinflammatory cytokine and prostanoid production (TNF-α and TXB_2) and monitored the clinical and hematologic changes after LPS administration.

Materials and Methods

Experimental Animals

Nineteen healthy mares (11 Quarter Horse, 3 American Paint Horse, 2 Thoroughbred, 2 Friesian, 1 Appaloosa) with a mean age of 7.8 years (range 4–11 years) and mean body weight of 563 kg (range 505–664 kg) were randomly selected from the Iowa State University teaching herd. All horses were healthy, had no history of illness or treatments in the last 60 days, were in good body condition (BCS 6–9), and fed free choice grass/alfalfa hay. Health status of all horses was based on a complete physical examination, CBC, and serum biochemistry analysis 24 hours before the study; all parameters were within acceptable reference ranges. Horses were allowed to acclimatize to the study environment for 24 hours and were fed grass/alfalfa hay and water ad libitum during the study period.

Horses were divided into 2 randomized groups: saline-treated horses (SAL) and ketamine-treated horses (KET). The KET group (n = 9) was administered racemic ketamine[a] in a step-wise loading infusion followed by a CRI as stated below. The SAL group (n = 10) received a similar step-wise loading infusion and CRI of 0.9% saline with a matched volume to body weight ratio as KET horses. All horses received LPS (*Escherichia coli* 055:B5)[b] 1 hour after initiation of the loading infusion of ketamine or saline. The investigators were not blinded to treatment groups. All treatments and procedures were performed in accordance with the Iowa State University Institutional Animal Care and Use Committee approved guidelines. All horses were returned to the teaching herd at the end of the study period.

Sampling

IV jugular catheters[c] were placed aseptically, after SC infiltration of 2% lidocaine, into the right and left external jugular veins. All blood samples were collected via the left jugular catheter while administration of LPS ketamine, saline, or both occurred via the right jugular catheter. Venous blood samples for CBC and TXB_2 analysis were collected in tubes containing EDTA. Samples containing EDTA were processed within 1 hour for CBC or centrifuged at 1,000×*g* for 10 minutes at which point the plasma was harvested and stored at −20°C for future TXB_2 analysis. Venous blood samples for ketamine analysis were collected in tubes containing lithium heparin and centrifuged at 1,000 × *g* for 10 minutes at 20°C for collection of the plasma fraction. Heparinized plasma samples were processed within 1 hour of collection for plasma biochemistry analysis or stored at −20°C for TNF-α quantification.

Treatment Groups

KET Group. Nine healthy mares were administered a loading dose of 0.3% racemic ketamine in 0.9% saline via infusion pump[d] in a step-wise infusion protocol: 4.8, 3.6, 3.0, 2.4 mg/kg/h in 10-minute intervals (Fig 1). At the completion of the 2.4 mg/kg/h loading dose, the ketamine CRI was adjusted to maintain a rate of 1.5 mg/kg/h for 320 minutes, for a total infusion period of 360 minutes (6 hours). The targeted plasma ketamine concentration was 200 ng/mL for 5 hours with an acceptable range of 100–500 ng/mL. This dosing strategy was expected to yield the targeted concentrations.[24] One hour after the initiation of the ketamine loading dose (time 0), 30 ng/kg LPS (*E. coli* O55:B5) in 60 mL of 0.9% saline was administered over 30 minutes via an automated infusion pump.[12] Clinical response to endotoxin administration was assessed by physical examination and clinical scoring at −1, −0.5, 0, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours. Blood samples were collected at −1, 0, 0.5, 1, 1.5, 2, 3, 5, 8, and 24 hours and at 2, 5, and 24 hours for CBC and plasma biochemistry analysis, respectively. Serum ketamine concentrations were determined at −1, 0, 1, 2, 3, 5, and 8 hours. The inflammatory cytokine response to LPS administration was assessed via blood TNF-α (−1, 0, 0.5, 1, 1.5, 2, 3, 5, and 8 hours) and TXB_2 (0, 0.5, 1, 1.5, and 3 hours) concentrations. Collection points were based on previous work[12,25] that indicated peak TNF-α and TXB_2 levels would be achieved 1.5 hours after LPS administration and return to baseline within 3 hours.

SAL Group. Ten healthy mares were administered a loading dose and CRI of 0.9% saline in identical fashion to the KET group horses. Horses in both groups received the same volume (mL/kg body weight) of solution, 0.3% ketamine, or 0.9% saline. Clinical evaluation and blood sample collection were performed as outlined above for the KET group horses.

Physical Examinations

Heart rate (HR), respiratory rate (RR), rectal temperature (RT), and clinical scores were monitored at the time intervals described

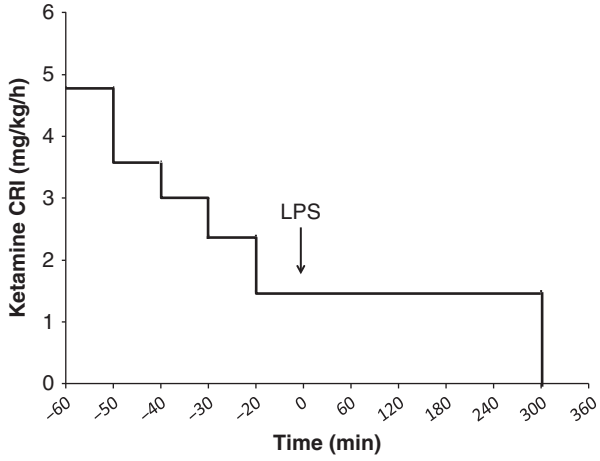

Fig 1. Ketamine step-wise loading infusion starting at time −60 minutes and adjusted every 10 minutes as follows: 4.8, 3.6, 3.0, and 2.4 mg/kg/h. The ketamine continuous rate infusion (CRI) was maintained at 1.5 mg/kg/h from time −20 to 300 minutes. Lipopolysaccharide (*Escherichia coli* O55:B5, 1 ng/kg/min×30 min) was administered to both groups at time 0 minute.

above. Clinical scoring was based on a nonblinded subjective assessment by the investigator (C.A.) utilizing a 4-point severity score (0–3). Horses categorized as: 0—no clinical evidence of abnormal behavior; 1—mild lethargy, restlessness or muscle fasciculations or both; 2—moderate lethargy, restlessness, muscle fasciculations, inappetance or hyperesponsiveness or both, without signs of abdominal discomfort; 3—severe clinical evidence of lethargy, restlessness, pawing, sweating, muscle fasciculations, signs of abdominal discomfort (cramping, looking at sides, rolling), incoordination, hyperesponsiveness or nystagmus or both. Objective data (HR, RR, and RT) were not used to determine clinical scores.

Laboratory Methods

CBC/Plasma Biochemistry Analysis. Analyses were performed by commercial automated analyzers.[e,f]

TNF-α. Quantification was based on previously described ELISA methods.[12] Briefly polyclonal antibody[g] was incubated with heparinized plasma samples in a 96-well microtiter plate.[h] After washing with phosphate-buffered saline with tween,[h] equine recombinant TNF[h] was added in standard dilutions to each well. The optical density was determined at 405 nm after an additional wash and addition of biotin-labeled polyclonal antibody.[h] TNF-α activity was expressed as picograms per milliliter.

TXB_2. One milliliter of plasma was injected into the C18 Sep-Pak column,[i] which had been preconditioned by washing the column with 5 mL of methanol followed by 5 mL of deionized water. After sample loading, the column was washed with 5 mL of deionized water followed by 5 mL of high-pressure liquid chromatography grade hexane. TXB_2 was eluted from the column with 5 mL ethyl acetate containing 1% methanol by gravity. The organic solvent was removed under a stream of nitrogen and the dried sample was stored at −20°C until assayed. For enzyme immunoassay (EIA) of TXB_2, the sample was dissolved with 1 mL of EIA buffer provided by the manufacturer. The TXB_2 concentrations were measured by an EIA kit.[j] The range of the standard curve for TXB_2 EIA was 62.5–1,000 pg/mL. All assays were performed in duplicate with the average value between duplicates being reported. Intra- and interassay variations were <5 and <8%, respectively. The cross-reactivity with 2,3-dino-TXB_2 and 11-dehydro-TXB_2 was 30 and 0.07%, respectively.

Ketamine. Ketamine concentrations were determined by ultra-performance liquid chromatography with mass spectrometry (UPLC/MS)[k] by a solid phase extraction technique. A stock solution of ketamine HCl[l] was prepared by dissolving reference standard in 100% methanol at a concentration of 1 mg/mL. Further dilutions were made with 10% methanol in water and those dilutions were used to spike naïve equine plasma for calibration curves. Samples and standards were extracted identically by the following method: phosphoric acid (20 μL) was added to 1 mL of plasma before extraction; extraction was carried out using hydrophilic-lipophilic balance cartridges;[m] the cartridges were conditioned with 1 mL of methanol followed by 1 mL of water; 1 mL of sample or standard was then extracted, followed by 1 mL of 5% methanol in water. Elution was achieved using 1 mL of methanol. The eluent was evaporated to dryness using compressed nitrogen (17 psi) for 12 minutes at 50°C. Standards and samples were reconstituted with 250 μL of mobile phase, placed into filter vials[n] and loaded onto the UPLC for analysis.

An isocratic elution was performed on a C_{18} column[o] using 0.5 μL of sample. The mobile phase consisted of water with 0.2% acetic acid (70%) and methanol (30%) at a flow rate of 0.5 mL/min. The column was maintained at 40°C while samples were kept at 4°C during analysis. Ketamine was monitored in positive electrospray mode, using ion 238 for quantification. The voltages of the MS were as follows: capillary 4.5 kV, cone 30 V, extractor 5 V, and RF lens at 0.1 V. Source and desolvation temperatures were 100°C and 400°C with a nitrogen gas flow of 20 and 600 L/h, respectively. Vacuum was maintained at 1.0×10^{-4} mbar.

The area under the curve of the peak was plotted versus the known standard concentration and linear regression analysis was performed by standard software[p] to obtain a slope, which was used to calculate the unknown sample concentration ($y = mx + b$). Standards for ketamine were quantified from 0.01–0.5 μg/mL and were determined to be linear on each day of analysis with a coefficient of determination (R^2) of >0.99 for each dataset. Limits of detection and quantification of ketamine were determined to be 0.005 and 0.01 μg/mL, respectively, during initial method validation. The mean ± SD coefficients of variation at ketamine concentrations of 0.5, 0.1, and 0.01 μg/mL were 6.87 ± 7.73%. Average recovery of ketamine in plasma was 96 ± 15%.

Statistical Analysis

Data collected 1 hour before ketamine or saline administration (−1 hour) were considered resting values, with the exception of TXB_2 in which time 0 was used. Baseline correction was defined as deducting individual horse-resting values from the corresponding collected data to account for variances in resting values. All data were baseline corrected during statistical analyses with the exception of clinical scores. Log transformation was applied to stabilize variances of the monocyte count and HR, RR, TNF-α, and TXB_2 values. A repeated measures analysis of variance model was used to analyze each of the quantitative responses listed above. Treatment group (KET versus control), time, and their interaction were used as fixed effects, and each horse was the subject of repeated measures. Degrees of freedom were adjusted by the Kenward-Roger method. Treatment groups were compared by Student's *t*-test at each time point. The ordinal response of clinical scoring was assessed between treatment groups via nonparametric Wilcoxon's sum-rank test at each time point. Pearson's correlation coefficients were used to determine correlation among plasma ketamine concentration, WBC, and log converted values for TNF-α and TXB_2. Spearmen's correlation coefficients were used to determine correlation among clinical score and plasma ketamine concentration, WBC, and log converted values for TNF-α and TXB_2. Statistical analyses were performed with SAS[q] software. A P-value ≤ .05 (or smaller) was considered significant in this analysis.

Results

Clinical Assessment

All horses safely completed the study and maintained consciousness. Clinical scores of the SAL group were not significantly changed over time from resting values (time −1 hour), whereas the KET group clinical scores were increased at −0.5 and 0 hours ($P < .05$), coinciding with the ketamine loading infusion period (Fig 2). One horse within the KET group had a consistently increased clinical score during the ketamine CRI (−1 to 5 hours) with a median score of 1 (SD ± 1.33), whereas the remaining horses within the KET group had median scores below 0.5 with 1 horse never exceeding a score of 0. Clinical scores in the KET group were significantly greater than the SAL group at −.5, 0, and 6 hours ($P < .05$). There was not a significant correlation between plasma ketamine concentration and clinical score.

HR increased significantly above resting values (time −1 hour) over time in both groups (Table 1). Peak HR above baseline occurred 2 hours after administration of

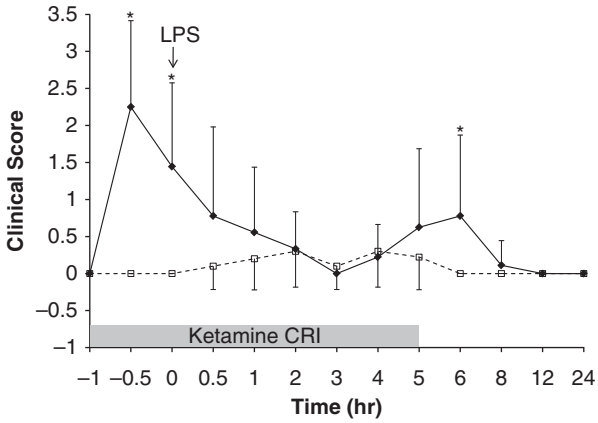

Fig 2. Clinical scores for ketamine-treated horses (KET) group (black diamond) and saline-treated horses (SAL) group (square) horses were based on nonblinded assessment and assigned a severity score 0–3. Horses categorized as 0—showed no clinical evidence of abnormal behavior; 1—had mild lethargy, restlessness or muscle fasciculations or both; 2—had moderate lethargy, restlessness, muscle fasciculations, inappetance or hyperesponsiveness or both, without signs of abdominal discomfort; 3—had severe clinical evidence of lethargy, restlessness, pawing, sweating, muscle fasciculations, abdominal discomfort, incoordination, hyperresponsiveness or nystagmus or both. *Significant difference between groups ($P < .05$). Mean ± SD.

LPS (f-LPS, time 0) in both groups. RR was significantly increased above resting values over time in the KET group at 6 hours ($P < .05$) but not in the SAL group. Peak RR occurred in the SAL group 4 hours and in the KET group 6 hours f-LPS. RT was significantly increased over time above resting values in both groups ($P < .05$) f-LPS. Peak RT occurred 3 hours f-LPS in both treatment groups. Significant differences in HR, RR, or RT were not identified between treatment groups at any time point throughout the study period (Table 1).

Clinical Pathology

Both groups demonstrated a significant leukopenia ($P < .05$), neutropenia ($P < .05$), leukocytosis ($P < .05$), and neutrophilia ($P < .05$) compared with resting values (time −1 hour) over time with a nadir of 1.5 hours (Figs 3 and 4). The KET group had a significantly greater leukocyte response characterized by a neutrophilia (leukocytosis 8 hours, $P < .05$; neutrophilia 8 and 24 hours, $P < .05$) f-LPS compared with the SAL group. Lymphopenia was significant ($P < .05$) over time in both groups relative to resting values with lymphocytosis occurring in both groups 24 hours f-LPS (SAL: $P < .05$, KET: $P < .05$). Monocytopenia was significant from resting values over time in the SAL group ($P < .05$) and not in the KET group, whereas a significant monocytosis occurred in the KET group 8 hours ($P < .05$) f-LPS. There was no significant difference between treatment groups regarding lymphocyte and monocyte counts (Figs 5 and 6). Fibrinogen decreased significantly from resting values over time in the SAL group 5 hours f-LPS ($P = .05$), but was within reported reference intervals. No significant difference in fibrinogen concentration was identified between groups. Hematocrit was not significantly different between groups or from resting values over time. The administration of ketamine did not have a significant effect on the complete blood count before LPS administration (time −1 to 0 hours) in the KET group, nor was there a significant difference between treatment groups during this same time period.

Plasma biochemistry analyses demonstrated minor perturbations from resting values in both treatment groups and are summarized in Table 2. The KET group had a significant increase in plasma chloride (5 hours, $P = .05$) and creatinine (5 hours, $P < .05$) and decrease in phosphorus (24 hours, $P < .05$) relative to the SAL group. Significant differences were not demonstrated between treatment groups at any time point regarding plasma sodium, potassium, bicarbonate, calcium, magnesium, BUN, glucose, total protein, albumin, AST, creatine kinase, ALP, GGT, and total bilirubin.

Immunologic Variables

Plasma TNF-α. TNF-α production was significantly increased above resting values (time −1 hour) over time in the SAL group at 1 and 1.5 hours ($P < .05$), and in the KET group at 1, 1.5, 2, 3, and 8 hours ($P < .05$) f-LPS (Fig 7). TNF-α production was not significantly different between treatment groups, although the SAL group demonstrated a trend of lower production versus the KET group at 1 hour ($P = .06$). Peak TNF-α production occurred at 1.5 hours in both treatment groups (SAL: 2,211 ± 2,162 pg/mL, KET: 3,404 ± 2,633 pg/mL) although these values were not significantly different ($P = .08$).

Plasma TXB_2. Both groups demonstrated significant TXB_2 production from resting values (time 0) over time (SAL: 1 hour, $P < .0001$; KET: 1 hour, $P < .05$) f-LPS (Fig 8). However, no significant difference was observed between treatment groups at any time point during the study period. TXB_2 production was not determined before time 0, which limits interpreting the effects of the ketamine CRI alone; however, values obtained from both treatment groups at time 0 were not significantly different (SAL: 117.5 ± 75.2 pg/mL, KET: 159.4 ± 114 pg/mL, $P = .60$).

Plasma Ketamine

Plasma ketamine concentration obtained throughout the study period of the KET group was 248 ± 95.1 ng/mL (mean, SD, Fig 9). The ketamine loading infusion resulted in a plasma ketamine concentration at time 0 hour of 238 ng/mL (mean, range 137–357 ng/mL). Plasma ketamine concentration peaked 5 hours f-LPS (mean 286, range 114–546 ng/mL) and rapidly declined after discontinuation of the ketamine CRI at 5 hours. Although 1 horse had plasma ketamine levels > 500 ng/mL, no correlation among clinical score, WBC, TNF-α or TXB_2 could be made. Three hours after discontinuation of the ketamine CRI (8 hours f-LPS) mean plasma ketamine concentration was 21.7 ± 16.3 ng/mL.

Table 1. Mean ± SD clinical and CBC parameters of KET group and SAL group horses.

Time (hours)	−1	−0.5	0	0.5	1	1.5	2	3	4	5	6	8	12	24
Heart rate (bpm)														
Saline	42 (5)	42 (0)	41 (5)	47 (10)	47 (5)†		52 (8)†	46 (4)	43 (5)	44 (5)	43 (6)	44 (3)	41 (4)	40 (3)
Ketamine	39 (3)	50 (7)†	50 (7)†	48 (5)†	48 (9)†		62 (20)†	47 (5)†	47 (5)†	47 (5)†	44 (5)†	45 (5)†	40 (3)	41 (2)
Respiratory rate (bpm)														
Saline	21 (8)	21 (6)	20 (6)	20 (5)	20 (5)		21 (9)	20 (8)	34 (14)	29 (14)	24 (6)	23 (10)	20 (7)	17 (5)
Ketamine	21 (4)	30 (9)	31 (11)	27 (7)	29 (7)		27 (11)	27 (10)	29 (8)	45 (18)	46 (28)†	33 (10)	21 (6)	21 (6)
Temperature (°C/°F)														
Saline	37.8 (0.2)/	37.7 (0.2)/	37.7 (0.3)/	37.7 (0.2)/	38.0 (0.2)/		38.5 (0.4)/	38.8 (0.7)/	38.7 (0.6)/	38.4 (0.5)/	38.1 (0.4)/	37.9 (0.3)/	37.8 (0.2)/	37.6 (0.3)/
	100 (0.4)	99.9 (0.3)	99.9 (0.5)	99.9(0.3)	100.3(0.4)		101.2 (0.8)†	101.9 (1.2)†	101.6 (1)†	101.2 (1)†	100.5 (0.7)†	100.2 (0.5)	100 (0.3)	99.7 (0.4)†
Ketamine	37.7 (0.2)/	38.0 (0.3)/	38.0 (0.3)/	38.0 (0.3)/	38.2 (0.3)/		38.6 (0.5)/	39.0 (0.5)/	38.7 (0.5)/	38.5 (0.6)/	37.9 (0.3)/	38.1 (0.3)/	37.9 (0.2)/	37.6 (0.2)/
	99.9 (0.3)	100.3 (0.5)†	100.5 (0.6)†	100.4 (0.5)†	100.7 (0.6)†		101.5 (0.9)†	102.2 (0.9)†	101.6 (1)†	101.3 (1)†	100.3 (0.6)†	100.6 (0.5)	100.2 (0.3)†	99.7 (0.4)
WBC ($\times 10^9$/L)														
Saline	8.0 (1.4)		8.2 (1.4)	7.4 (1.2)	3.8 (1.0)†	3.3 (1.1)†	3.5 (1.0)†	5.3 (1.8)†		9.6 (2.0)		12 (2.5)†		11 (2.3)†
Ketamine	8.5 (1.1)		8.9 (1.3)	8.2 (1.3)	3.8 (0.82)†	3.3 (0.6)†	3.8 (0.92)†	5.9 (2.1)†		12 (3.4)†		18 (2.4)*†		14 (1.5)†
Neutrophil ($\times 10^9$/L)														
Saline	5.1 (1.1)		5.3 (0.97)	5.1 (0.94)	1.9 (1.2)†	1.6 (0.98)†	2.0 (0.79)†	3.8 (1.6)†		8.0 (2.0)†		10 (2.1)†		7.5 (1.7)†
Ketamine	5.7 (1.2)		5.7 (1.1)	5.4 (1.3)	1.6 (1.1)†	1.4 (0.82)†	2.1 (1.3)†	4.0 (2.4)†		10 (4.0)†		15 (2.7)*†		10 (1.7)*†
Lymphocyte ($\times 10^9$/L)														
Saline	2.5 (0.87)		2.5 (0.73)	2.0 (0.60)†	1.7 (0.62)†	1.5 (0.57)†	1.4 (0.59)†	1.3 (0.55)†		1.2 (0.82)†		1.6 (1.0)†		3.3 (1.3)†
Ketamine	2.4 (0.47)		2.7 (0.62)	2.4 (0.83)	1.9 (0.54)	1.7 (0.66)†	1.7 (0.55)†	1.7 (0.67)†		1.3 (0.50)†		1.3 (0.60)†		3.0 (1.3)†
Monocyte ($\times 10^9$/L)														
Saline	0.23 (0.28)		0.24 (0.32)	0.23 (0.25)	0.03 (0.04)†	0.01 (0.02)†	0.02 (0.03)†	0.01 (0.02)†		0.12 (0.14)†		0.35 (0.31)		0.2 (0.15)
Ketamine	0.14 (0.14)		0.24 (0.14)	0.11 (0.12)	0.02 (0.03)	0.06 (0.11)	0.02 (0.07)	0.01 (0.03)		0.12 (0.12)		0.55 (0.34)†		0.2 (0.15)
Fibrinogen (g/L)														
Saline	300 (94)		280 (114)	260 (127)	270 (82)	280 (114)	250 (85)	260 (108)		222 (109)†		260 (84)		320 (79)
Ketamine	267 (50)		244 (88)	267 (71)	244 (53)	322 (97)	267 (50)	300 (54)		238 (74)		325 (212)		289 (60)
Hematocrit (%)														
Saline	35 (4.7)		35 (4.2)	36 (4.3)	35 (2.7)	36 (3.4)	37 (3.4)	38 (2.5)		36 (3.7)		39 (4.4)		34 (4.1)
Ketamine	35 (3.6)		35 (3.7)	34 (3.5)	35 (2.7)	35 (3.4)	37 (3.9)	37 (2.5)		38 (3.8)		37 (2.3)		33 (2.5)

Ketamine and saline CRIs were initiated at 1 hour and continued to 5 hours. LPS (055:B5, 30 ng/kg) was administered over 30 minutes beginning at time 0 hour to both groups.
CRI, continuous rate infusion; KET, ketamine-treated horses; LPS, lipopolysaccharide; SAL, saline-treated horses.
*Significant ($P < .05$) difference from the saline group.
†Significant change from resting values.

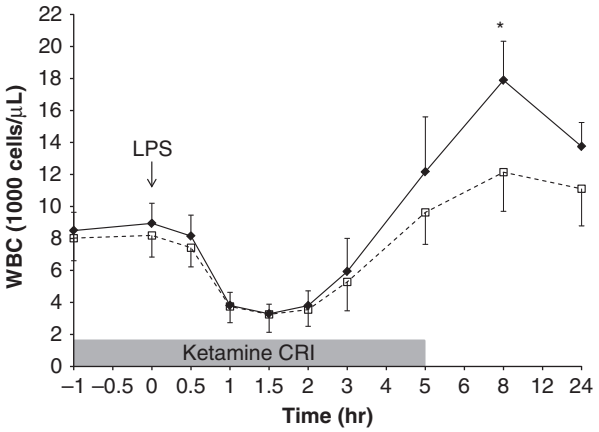

Fig 3. White blood cell count of ketamine-treated horses (KET) group (black diamond) and saline-treated horses (SAL) group (square) horses. Ketamine and saline continuous rate infusion (CRIs) were initiated at −1 hour and continued to 5 hours. Lipopolysaccharide (LPS) (055:B5, 30 ng/kg) was administered over 30 minutes at time 0 hour to both group. *Significant ($P < .05$) difference between groups after LPS administration. Mean ± SD

Discussion

This study documents the immunologic response to LPS in healthy horses receiving a subanesthetic dose of ketamine as a CRI. All horses completed the study, and those in the KET group were safely administered a subanesthetic ketamine CRI of 1.5 mg/kg/h during experimental endotoxemia. Both KET and SAL treatment groups demonstrated mild lethargy, tachycardia, tachypnea, fever, and a neutropenic leukopenia consistent with previous reports of experimental endotoxemia induction by administration of 30 ng/kg LPS (*E. coli* O55:B5).[13,26] Horses in the KET group had plasma ketamine concentrations above 100 ng/mL throughout the 5-hour study period consistent with our target concentration. The subanesthetic dose of ketamine utilized in this study did not appreciably attenuate the clinical and immunologic response to endotoxin administration in healthy horses when compared with those receiving a solution of 0.9% saline (SAL group).

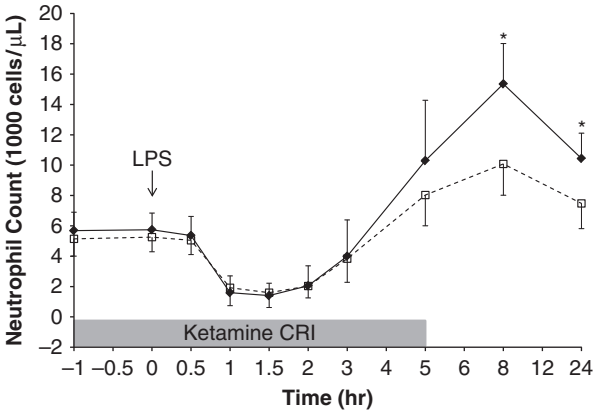

Fig 4. Neutrophil count of ketamine-treated horses (KET) group (black diamond) and saline-treated horses (SAL) group (square) horses. Ketamine and saline continuous rate infusion (CRIs) were initiated at −1 hour and continued to 5 hours. Lipopolysaccharide (LPS) (055:B5, 30 ng/kg) was administered over 30 minutes at time 0 hour to both group. *Significant ($P < .05$) difference between groups after LPS administration. Mean ± SD.

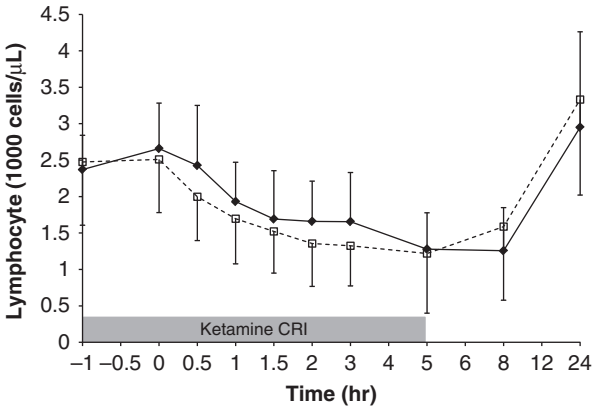

Fig 5. Lymphocyte count. Mean ± SD See Figure 4 description.

The exact immunomodulating dose of subanesthetic ketamine in the horse is unknown. In vitro work indicates that ketamine concentrations >100 ng/mL significantly reduced the production of LPS-induced TNF-α and IL-6 in equine macrophages and ketamine's dose-dependent inhibition of LPS-induced proinflammatory cytokines is well documented in other species.[16,17,27] The typical ketamine anesthetic induction dose used in horses (2.2 mg/kg) can result in peak plasma levels >5,000 ng/mL, with CNS dissociative effects occurring at 800 ng/mL and anesthetic recovery beginning when plasma levels decline below 1,000 ng/mL.[19,28,29] In the this study, the KET group attained plasma ketamine concentrations >200 ng/mL throughout the study period with 1 horse exceeding 500 ng/mL at 5 hours f-LPS. The staged loading infusion protocol (Fig 1) resulted in plasma ketamine concentrations above the targeted 100 ng/mL (mean 238 ± 67 ng/mL) within 60 minutes and through the subsequent 4 hours within the hypothesized therapeutic range.

Clinical assessment of horses in both treatment groups was based on nonblinded clinical scoring and its interpretation should be viewed with caution. The clinical

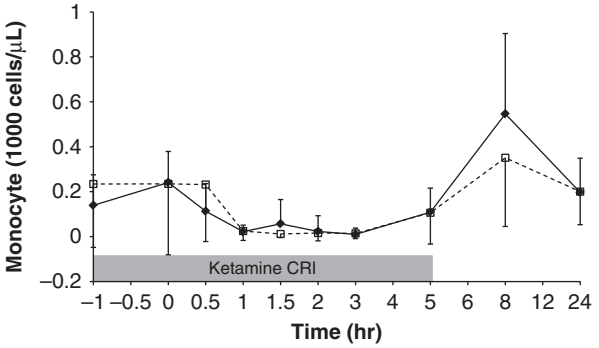

Fig 6. Monocyte count. Mean ± SD. See Figure 4 description.

Table 2. Mean±SD serum biochemistry analyses of KET group and SAL group horses.

Time (hours)	−1	2	5	24
Sodium (mEq/L)				
Saline	138 (2)	139 (2)	139 (2)	139 (2)
Ketamine	139 (1)	141 (2)†	143 (3)†	139 (3)
Potassium (mEq/L)				
Saline	3.4 (0.3)	3.3 (0.4)	3.1 (0.4)	3.04 (0.4)
Ketamine	3.2 (0.6)	3.1 (0.3)	3 (0.4)	3.03 (0.7)
Chloride (mEq/L)				
Saline	99 (2)	102 (1)†	102 (2)†	100 (2)
Ketamine	100 (2)	104 (1)†	105 (1)*†	101 (2)
Bicarbonate (mEq/L)				
Saline	29 (2)	27 (2)†	26 (2)†	28 (2)
Ketamine	29 (2)	26 (2)†	26 (1)†	27 (2)†
Calcium (mg/dL)				
Saline	12 (0.6)	12 (0.6)	12 (0.7)	12 (0.4)
Ketamine	12 (0.5)	12 (0.5)	12 (0.7)	12 (0.4)
Phosphorus (mg/dL)				
Saline	6.2 (11)	3.1 (0.8)	2.4 (1)	2.2 (0.6)†
Ketamine	3.2 (0.8)	3.3 (0.8)	2.1 (0.7)†	1.8 (0.5)*†
Magnesium (mg/dL)				
Saline	1.8 (0.2)	1.5 (0.2)†	1.4 (0.2)†	1.7 (0.2)
Ketamine	1.7 (0.2)	1.5 (0.1)†	1.5 (0.1)†	1.6 (0.2)
BUN (mg/dL)				
Saline	21 (2)	19 (3)	18 (3)†	17 (2)†
Ketamine	21 (2)	20 (3)	19 (3)†	18 (3)†
Creatinine (mg/dL)				
Saline	1.03 (0.1)	1.2 (0.2)†	1.1 (0.2)	1.1 (0.1)†
Ketamine	1.09 (0.1)	1.3 (0.2)†	1.4 (0.3)*†	1.1 (0.2)
Glucose (mg/dL)				
Saline	108 (11)	107 (5)	110 (8)	121 (21)†
Ketamine	107 (11)	112 (20)	105 (19)	123 (27)†
Total protein (gm/dL)				
Saline	6.9 (0.4)	7.3 (0.7)†	7.1 (0.6)†	7.1 (0.5)
Ketamine	6.9 (0.4)	7.4 (0.4)†	7.2 (0.3)†	7.2 (0.5)†
Albumin (gm/dL)				
Saline	3.2 (0.2)	3.4 (0.3)†	3.3 (0.2)†	3.2 (0.2)
Ketamine	3.1 (0.2)	3.3 (0.2)†	3.2 (0.2)†	3.1 (0.2)
AST (IU/L)				
Saline	360 (40)	404 (61)	403 (45)	367 (45)
Ketamine	361 (49)	446 (111)†	426 (84)†	378 (71)
Creatine kinase (IU/L)				
Saline	349 (145)	349 (97)	317 (88)†	395 (146)
Ketamine	315 (57)	310 (41)	267 (42)	287 (66)
ALP (IU/L)				
Saline	162 (30)	232 (41)†	240 (31)†	232 (43)†
Ketamine	181 (31)	274 (68)†	305 (55)†	253 (40)†
GGT (IU/L)				
Saline	19 (5)	22 (8)†	21 (9)†	21 (7)†
Ketamine	19 (4)	22 (5)†	22 (4)†	21 (3)†
Total bilirubin (mg/dL)				
Saline	0.9 (0.3)	1.2 (0.2)†	1.3 (0.3)†	1.5 (0.6)†
Ketamine	0.8 (0.2)	1.2 (0.3)†	1.3 (0.4)†	1.4 (0.5)†

Ketamine and saline CRIs were initiated at 1 hour and continued to 5 hours. LPS (055:B5, 30 ng/kg) was administered over 30 minutes beginning at time 0 hour to both groups.

CRI, continuous rate infusion; KET, ketamine-treated horses; LPS, lipopolysaccharide; SAL, saline-treated horses.

*Significant ($P < .05$) difference from the saline group.

†Significant change from resting values.

response to a subanesthetic ketamine CRI is well documented, so the objective of this study was to monitor for unanticipated clinical changes warranting immediate discontinuation of treatment.[30,31] None were observed. The limited sensitivity of the clinical scoring scheme likely underrepresented some clinical features as most horses in the SAL group demonstrated varying degrees of mild lethargy consistent with the previously mentioned models of experimental endotoxemia.[12,13,15] The increased clinical scores in the KET group are consistent with administration of the ketamine loading infusion (time −1 to 0 hours) and were expected given the rapid nature of the infusion. As clinical scores in the KET group at time 0 were increased above baseline, it is difficult to interpret the effects of LPS administration alone in this treatment group. Unexpectedly, clinical scores were increased in the KET group 6 hours f-LPS and were characterized by transient excitation and hyperresponsiveness. Rapid accumulation of ketamine and its metabolites, norketa-

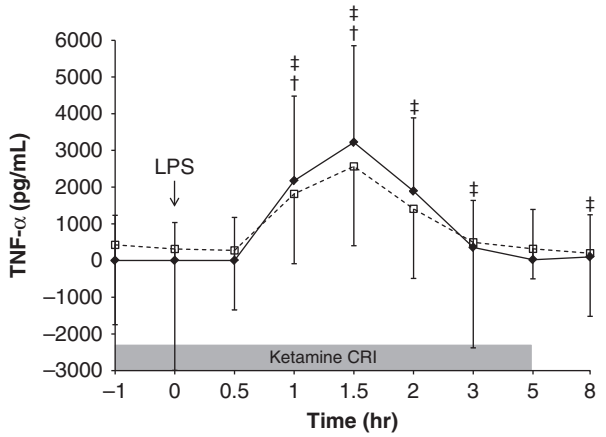

Fig 7. Plasma tumor necrosis factor-α (TNF-α) production in saline-treated horses (SAL) (square) and ketamine-treated horses (KET) (black diamond) groups. See Figure 3 for further details. No significant ($P < .05$) difference was determined between treatment groups. †Significant ($P < .05$) difference from baseline in the SAL group. ‡Significant ($P < .05$) difference from baseline in the KET group. Mean ± SD.

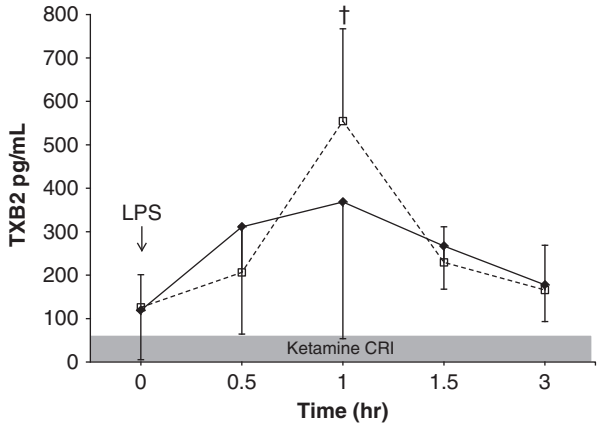

Fig 8. Plasma thromboxane B_2 production in saline-treated horses (SAL) (square) and ketamine-treated horses (KET) (black diamond) treatment groups. See description in Figure 3. No significant difference was demonstrated between treatment groups. †Significant ($P < .0001$) increase above baseline in both treatment groups. Mean ± SD.

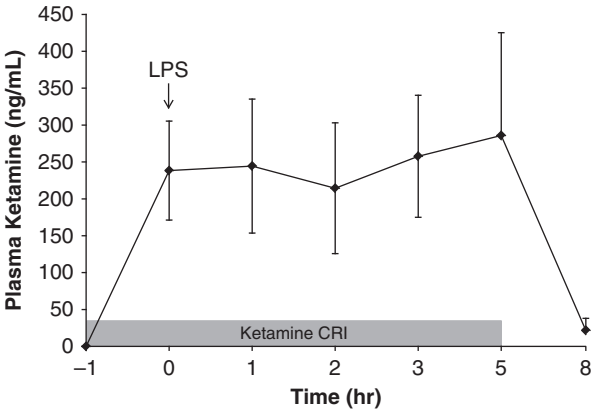

Fig 9. Plasma ketamine concentration of the ketamine-treated horses (KET) group. The KET group received a ketamine loading infusion as descried in Figure 1. Mean plasma ketamine concentration from 0 to 5 hours was 248, 95.1 ng/mL. Mean ± SD.

mine and hydroxynorketamine, occurs after loading boluses/infusions, of which the influence on CNS stimulation, immune modulation, or analgesia in the conscious horse is still unclear.[24,32] It is unknown if ketamine's CNS stimulatory effects potentially masked any attenuation of the clinical response in this study as a study group receiving a ketamine CRI and not challenged with LPS was not included.

Previous reports evaluating the pharmacokinetic and pharmacodynamic properties of subanesthetic ketamine infusions indicated that plasma ketamine concentration can be maintained in the conscious horse with minimal behavioral responses.[24,31] Administration of an α-2 adrenoceptor agonist, synthetic opioid, or both before the ketamine loading phase would likely have negated the significantly higher clinical scores in the KET group, given long-standing evidence that ketamine's use without prior sedation results in significant CNS stimulation in horses, but was not utilized in this study to avoid sedation and potentially slowing the distribution and hepatic metabolism of ketamine. Alteration of the loading phase to allow more time to acclimatize to abrupt changes in ketamine may have lessened the observed CNS stimulation in the KET group horses, but it is unclear what implications this may have on the metabolism or immunomodulating properties of ketamine.

The tachycardia and fever documented in the SAL group is consistent with previous reports using similar LPS dosing protocols that demonstrated peak tachycardia, tachypnea, and RT occurring 2–3 hours after LPS administration.[12,25] In the present study, tachycardia and tachypnea after LPS infusion were not significantly different between treatment groups, although the KET group had an increased HR above baseline for 6 hours longer than the SAL group and an increased RT 1.5 hours earlier than the SAL group. Peak tachypnea was not significantly above resting values in the SAL group and delayed 1 hour compared with previous reports.[26] The reason for this is uncertain. A subanesthetic ketamine CRI of 0.8 mg/kg/h had little effect on baseline physiologic parameters beyond the initial IV bolus.[31] Proposed reasons for this, in our study relative to other studies, include variances in LPS concentration or content, concurrent IV fluid administration, and LPS administration technique. This study suggests that ketamine had little influence attenuating the cardiorespiratory response to experimental endotoxemia.

Administration of LPS to horses consistently produces a leukopenia followed by a rebound leukocytosis, typically characterized by neutropenia followed by neutrophilia, along with proinflammatory cytokine production.[1,12,25] This study demonstrated a similar neutropenic leukopenia with a nadir 1.5 hours after LPS-infusion. However, the subsequent neutrophilic leukocytosis was earlier (5 versus 8 hours) and significantly greater in the KET group (leukocytosis at 8 hours and neutrophilia at 8 and 24 hours) compared with the SAL group. The effect of subanesthetic ketamine on equine leukocyte function, specifically neutrophils, has not been studied in the horse but has been explored in humans where ketamine demonstrated anti-inflammatory effects via inhibition of neutrophil adhesion molecule expression in vitro.[26,33] Interestingly in this study, the anticipated leukocytosis and neutrophilia that commonly occur in healthy horses after LPS administration were significantly amplified in the KET group, which may be a consequence of attenuated adhesion molecule expression. Although lymphocyte and monocyte counts were not significantly different between groups, changes from baseline within the SAL group demonstrated an earlier lymphopenia and monocytopenia compared with the KET group. A recent report in dogs indicates that subanesthetic ketamine did not alter leukocyte response to LPS.[23] The clinical impact of the leukocyte response observed in this study during periods of endotoxemia is unclear.

In this study, a significant effect of subanesthetic ketamine on CBC findings before LPS administration (time −1 to 0 hours) was not observed, suggesting that ketamine did not significantly influence the CBC until after the LPS infusion. This should be interpreted with caution as plasma ketamine levels were not determined until 1 hour after beginning the ketamine loading infusion (Fig 9), and although the loading infusion attained a rapid plasma ketamine concentration, it is unknown if this was within the hypothesized therapeutic range. Further in vivo studies evaluating ketamine's impact on leukocyte function at subanesthetic concentrations may provide insight for these findings. Minor perturbations of the plasma biochemistry results were observed from baseline in both treatment groups as summarized in Table 2, but were considered clinically insignificant.

Subanesthetic ketamine did not attenuate the LPS-induced production of TNF-α compared with control horses in this study. Average resting plasma TNF-α concentration varied widely in both treatment groups, suggesting variability in methods used to quantitate TNF-α (ELISA), a problem in study design or subclinical inflammation in the study subjects (Fig 7). When resting values were deducted from subsequent collection times, no significant difference between treatment groups was identified, although TNF-α production in the KET group remained increased above resting values for a longer duration than the SAL group. Peak TNF-α production occurred 1.5 hours after LPS administration, consistent with previous

reports and suggesting the dose of LPS utilized was adequate.[12,13,26] TNF-α is considered one of the earliest markers of inflammatory cytokine response during periods of endotoxemia and is a common parameter used to objectively assess endotoxemia in the horse.[34] In vitro evidence has supported ketamine's role in attenuating cellular proinflammatory cytokine gene induction, via NFκB inhibition, after LPS challenge at various doses (0.05–70 mg/kg) and infusions (5–20 mg/kg/h).[16,19,21,27,35–37] Rodent and canine in vivo models have documented the anti-inflammatory effects of subanesthetic ketamine via inhibition of proinflammatory cytokine production during experimental endotoxemia.[15,23,36,38] In contrast to previous findings in species other than the horse, subanesthetic ketamine did not diminish the inflammatory response to LPS as assessed by TNF-α production in this study.

Several factors may have been responsible for the observed difference between studies. The higher ketamine CRI used in this study is greater than that previously reported and some reports did not determine plasma ketamine concentrations, nor did they explore experimental models of endotoxemia in the horse.[28,31] Ketamine's immunomodulation is speculated to be dose related with moderate doses correlating with greater proinflammatory cytokine reduction when compared with higher doses.[16,17,26,39] It is possible that a lower ketamine CRI might have improved immunomodulation than the one chosen for this study. Pharmacokinetic principles of an IV bolus versus a stepwise CRI loading infusion strategy are additional variables that might account for the difference observed between studies. Individual horse variation as related to genetics, metabolism of ketamine, and sensitivity to LPS might have influenced our results as well. A cross-over study design would ideally diminish the influence of these variables, with LPS tolerance considered a further confounding factor.[40]

Plasma TXB_2 concentrations were not altered by the administration of subanesthetic ketamine in this study. Plasma TXB_2 samples were not determined before ketamine administration (time −1), so the effects of ketamine alone are unknown, although no significant difference was determined between treatment groups before LPS administration. What role subanesthetic ketamine plays in modulating COX activity and thrombocyte activation during experimental endotoxemia has not been studied in the horse. Ketamine has shown significant gastrointestinal protective effects through inhibition of COX-2 in rats which we hypothesized would be evidenced in this study by a reduction in TXB_2 production.[21,27] The addition of a COX-inhibitor to EDTA blood collection tubes has been used in previous reports to limit further platelet COX activity and was not performed in this study. This potentially influenced our TXB_2 results, but given that both treatment group's sample collection and processing techniques were similar, the implications were likely minimized.

In conclusion, the subanesthetic CRI of ketamine used in this study had minimal adverse effects and negligible beneficial effects regarding ketamine's ability to attenuate the immune response to endotoxin administration in healthy horses.

Footnotes

[a] Ketamine HCl Inj., Ketaset, Fort Dodge Animal Health, Fort Dodge, IA
[b] Sigma-Aldrich, St Louis, MO
[c] Angiocath 14 G, 5.25 in., Becton Dickinson Infusion Therapy Systems Inc, Sandy, UT
[d] Micro-Macro XL Fluid Pump, Abbott Laboratories, North Chicago, IL
[e] Siemens (Bayer) Advia 120, Seimens Healthcare Diagnostics, Deerfield, IL
[f] Roche Hitachi 912, Roche Diagnostics Corporations, Indianapolis, IN
[g] Thermo Fisher Scientific (Endogen), Rockford, IL
[h] Mediatech Inc, Manassas, VA
[i] C18 Sep-Pak, Waters Corporation, Milford, MA
[j] Oxford Biomedical Research, Oxford, MI
[k] Waters Acquity UPLC and EMD 1000 MS with Empower 2 software, Waters Corporation
[l] United States Pharmacopeia, Rockville, MD
[m] Oasis-HLB (1 mL), Waters Corporation
[n] Mini-UniPrep 0.45 μm PTFE, Whatman Inc, Clifton, NJ
[o] Acquity UPLC BEH, 1.0×50 mm, 1.7 μm pore size, Waters Corporation
[p] Microsoft Excel, Microsoft Corp, Seattle, WA
[q] SAS Institute Inc, SAS Version 9.1.3, Cary, NC

Acknowledgment

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