Effect of administration of a phospholipid emulsion on the initial response of horses administered endotoxin

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Objective—To evaluate the effect of a phospholipid emulsion (PLE) on the initial response of horses to administration of endotoxin.

Animals—12 healthy adult horses.

Procedures—Horses were assigned to 2 treatment groups (6 horses/group). The control group was administered 1 L of saline (0.9% NaCl) solution, and the treated group was administered PLE (200 mg/kg, IV); treatments were administered during a period of 120 minutes. An infusion of endotoxin was initiated in both groups starting 1 hour after initiation of the saline or PLE solutions. Physical examination and hemodynamic variables were recorded, and blood samples were analyzed for concentrations of tumor necrosis factor (TNF-α), interleukin-6, thromboxane B2 (TxB2), 6 keto-prostaglandin F (PGF)1α, total leukocyte count, and PLE concentrations. An ANOVA was used to detect significant differences.

Results—Administration of PLE resulted in significantly lower rectal temperature, heart rate, cardiac output, right atrial pressure, and pulmonary artery pressure and higher total leukocyte counts in treated horses, compared with values for control horses. The TNF-α concentration was significantly less in treated horses than in control horses. The TxB2 and 6 keto-PGF1α concentrations were significantly different between treated and control horses at 30 minutes (TxB2) and at 30 and 60 minutes (6 keto-PGF1α).

Conclusions and Clinical Relevance—Prior infusion of PLE in horses administered a low dose of endotoxin decreased rectal temperature, heart rate, pulmonary artery pressure, and TNF-α concentrations. Results of this study support further evaluation of PLE for use in the treatment of horses with endotoxemia. (Am J Vet Res 2002;63:1370–1378)

Gastrointestinal disorders (ie, colic) are the leading cause of mortality in horses, and 27 to 43% of horses with colic reportedly are endotoxemic.1,2 Endotoxemia is a life-threatening condition and is partially responsible for the high mortality of horses with colic.3 Concentrations of endotoxin as high as 80 µg/mL have been measured in the cecal contents of clinically normal horses,4 whereas administration of only a small amount of endotoxin (20 to 100 ng/kg, IV) is needed to cause clinical signs associated with endotoxia in horses.5,6

Endotoxin is a lipopolysaccharide (LPS) that is a normal component of the cell wall of gram-negative bacteria.7 Endotoxin molecules are made up of a hydrophilic and a hydrophobic (lipid-A) portion. The lipid-A domain of LPS is the toxic moiety associated with most of the toxic effects of gram-negative bacteria.6 It is the lipid-A region of LPS that interacts with target cells, such as macrophages and activated neutrophils, initiating the inflammatory cascade. Tumor necrosis factor (TNF)-α is a mediator released from mononuclear cells after exposure to endotoxin. Production of TNF-α requires binding of LPS to CD14, a surface receptor located on mononuclear cells. For LPS to bind to CD14, it must be presented to the cell by LPS-binding protein (LBP).3 This protein has a strong affinity and specificity for the lipid-A portion of LPS and the CD14 receptor on the surface of mononuclear phagocytes.8 When there is a lack of LBP, inhibition of LPS-induced TNF-α production is observed, and a much greater concentration of endotoxin is required to stimulate TNF-α synthesis.4 The CD14 receptor presents the LPS-LBP complex to a transmembrane protein, toll-like receptor 4,9 and the activated mononuclear cells release cytokines, most notably TNF-α, interleukin (IL)-1, and IL-6.10,11 Many of the effects of endotoxin in animals are secondary to the production of these cytokines.1,2 Tumor necrosis factor-α can also stimulate the release of IL-1 and IL-6, cause degranulation, initiate phagocytosis, activate neutrophil adherence factors, and activate phospholipase A2.12 Some of the clinical signs of endotoxemia are increases in rectal temperature, heart rate, and pulmonary artery pressure as well as leukopenia followed by leukocytosis (leukocyte sequestration).12,13

Treatments currently are aimed at providing cardiovascular support, resolving the source of the gram-negative sepsis, and controlling the inflammatory response.4 Administration of fluids,15 antibiotics, non-steroidal anti-inflammatory drugs,16-18 corticosteroids,19 cardiovascular drugs,20 aspirin,21 heparin,22 and pentoxifylline23 have all been used or studied for the treatment of horses with endotoxia.

Conservation of the lipid-A region among species and families of gram-negative bacteria has led to the development of specific treatments targeted at inacti-
...ing endotoxin. These include hyperimmune plasma and polymyxin B. However, for maximal efficacy, endotoxin-specific binding agents must have greater affinity for endotoxin than the affinity of LBP for endotoxin, thus preventing interaction of the LPS-LBP complex on cell receptors. Because the first step in the host inflammatory response is binding of endotoxin to mononuclear cells, which is followed by the release of TNF-α, prevention of endotoxin binding would negate the subsequent release of inflammatory mediators. Conflicting results on the usefulness of antiserum and the association between nephrotokicosis and polymyxin B in dehydrated patients have limited the usefulness of these treatments. A conjugate of polymyxin B and dextran 70 has been investigated, but the horses in that study had mild discomfort associated with infusion of the conjugated product, although those signs were easily corrected by administration of ketoprofen prior to administration of the conjugated product. In other studies, investigators have evaluated a monoclonal antibody against equine TNF-α that can reduce hematogetic and clinical responses in horses administered LPS and also can significantly decrease peak IL-6, lactate, and 6-keto-prostaglandin F (PGF) concentrations. A lipid-A analog (ie, E5531) has been evaluated in a low-dose endotoxin study in humans. Results of that study support the importance of blocking TNF-α release. Despite such scientific advances, endotoxemia continues to be a problem that is faced by many veterinarians and physicians.

Lipoproteins are agents that bind lipid-A. They are produced naturally by the body and are composed of apolipoproteins, cholesterol, triglycerides, and phospholipids. Lipoproteins are important for transport of cholesterol from tissues to the liver and can bind and neutralize endotoxins. Importance of the lipid fractions of plasma for host defense in septic people has been documented by the observation that mean concentrations of high-density lipoprotein (HDL) cholesterol are significantly lower in patients with sepsis, compared with concentrations in patients without sepsis. The mechanism responsible for this phenomenon is unknown, but HDLs play a major role in reverse cholesterol transport and may also be involved in LPS elimination. Other studies have revealed significant decreases in TNF-α release in LPS-stimulated human blood samples exposed to reconstituted high-density lipoproteins (R-HDLs) in vitro. The phospholipid component of R-HDLs is the active portion of the molecule involved in binding LPS. It is believed that the lipid-A domain of LPS is masked by insertion into the phospholipid bilayer of discoidal R-HDLs or the phospholipid monolayer on the surface of the spherical plasma HDLs. There is also evidence that R-HDLs may have an effect by reducing CD14 expression on monocytes. In 1 study, the phospholipid content of R-HDLs accounted for 94% of the activity of lipoproteins as measured by changes in TNF-α concentrations. On the basis of those initial studies, a protein-free phospholipid-rich emulsion was developed to address the potential concerns of the use of R-HDLs, a plasma-derived product. The objective of the study reported here was to evaluate the effects of a reformulated phospholipid emulsion (PLE) on the initial response of horses to administration of endotoxin.

Materials and Methods

Animals—Fifteen adult horses of various breeds that were 5 to 22 years old and weighed between 468 and 564 kg were used in the study. Horses included in the study were free of disease as determined by evaluation of results of physical examination, a CBC, and serum biochemical analysis. None of the horses had obvious signs of chronic laminitis; which may be an indicator of prior exposure to endotoxin. Foals were not used in the study, because they can be extremely susceptible to the effects of endotoxin. Furthermore, differences in transfer of passive immunity can affect the response of foals to endotoxin. This study was approved by The Ohio State University Animal Care and Use Committee.

Insertion of instruments—Horses were restrained in stocks where they remained until the end of data acquisition. Horses were provided ad libitum access to hay throughout the restraint period.

All catheters were placed percutaneously. The skin was prepared aseptically in a routine manner, and anesthetic was infused locally into the subcutaneous tissues. A 14-gauge, 13.3-cm fluoride-coated catheter was inserted into each of the jugular veins. The catheter in the left jugular vein was used for administration of PLE or saline (0.9% NaCl) solution, and the catheter in the right jugular vein was used for administration of endotoxin. A balloon-tipped, flow-directed thermolumination catheter was inserted through a cannula placed in the right jugular vein distal to the 14-gauge catheter used for endotoxin administration. The thermolumination catheter was advanced until the distal port was positioned in the pulmonary artery; this catheter was used to record pulmonary artery pressures. Polyethylene tubing was inserted through a cannula inserted in the left jugular vein distal to the 14-gauge catheter used for administration of PLE or saline solution. The tubing was advanced until the distal tip was positioned within the right atrium; it was used to infuse ice-cold dextrose for measurement of cardiac output (CO) and recording of right atrial pressures. Correct placement of the catheters was verified by observation of characteristic waveforms. The zero-pressure reference point for all pressure transducers was the level of the scapulohumeral joint.

Experimental design—Three horses were administered PLE (200 mg/kg, IV, throughout a period of 120 minutes) for determination of pharmacokinetics of PLE. The PLE was a proprietary drug with a composition of 7.5% triglycerides and 92.5% phospholipid in 18 mmol cholate plus 2.6% glycerc. Serum samples were obtained for determination of phospholipid concentrations at the start of the infusion (time 0 [baseline]) and 1, 2, 3, 4, 12, and 24 hours after initiation of the PLE infusion.

Effects of administration of PLE and endotoxin to horses were investigated by use of a single-factor design study. A crossover design was not used because of the possibility that an immune response could have been mounted after the first dose of endotoxin (referred to as endotoxin tolerance). On the day of the experiment, each horse was assigned to 1 of 2 treatment groups (6 horses/group). Horses in the control group were administered 1 L of saline solution during a period of 2 hours and an infusion of endotoxin (Escherichia coli O11:B4 LPS) at the rate of 30 ng/kg, IV, during a period of 1 hour. Horses in the treated group were administered PLE (200 mg/kg, IV, in a volume of 1 L infused during a period of 2 hours) and infused with endotoxin (30 ng/kg, IV, dur-
ing a period of 1 hour). Infusions of saline and PLE solutions were started 1 hour prior to the start of the endotoxin infusions. The start of endotoxin infusions was designated as time 0.

All infusions were delivered continuously via a mechanical infusion pump. Baseline blood samples were collected after catheter placement and immediately prior to initiation of the infusions. Hemodynamic variables and physical variables were recorded at –60 and 0 minutes and then at 15-minute intervals for 4 hours. Blood samples were collected for cytokine, eicosanoid, and hematologic analyses via the polyethylene tubing in the left jugular vein at –60, 0, 30, 60, 90, 120, 180, 240, 300, and 360 minutes after the onset of endotoxin administration for both groups of horses. Cytokines and eicosanoids that were evaluated were TNF-\(\alpha\), IL-6, thromboxane B\(_2\) (TxB\(_2\)), and 6 keto-PGF\(_{1\alpha}\).

Blood samples were collected into evacuated tubes that contained EDTA for leukocyte analysis, tubes that did not contain anticoagulant for phospholipid analysis, tubes that contained sodium heparin for cytokine analysis, and tubes that contained 100 mM EDTA and 10 mM meclofenamic acid (1 part EDTA-meclofenamic acid:20 parts blood) for analysis of eicosanoids. Samples for cytokine, eicosanoid, and phospholipid analyses were immediately placed on ice. Samples then were centrifuged for 10 minutes, and serum or plasma was harvested and stored at –80°C until assayed.

Physical examination and hemodynamic evaluation—Heart rate, respiratory rate, and rectal temperature were recorded at –60 and 0 minutes, at 15-minute intervals for 4 hours, and at 5 and 6 hours after onset of endotoxin administration. Recorded hemodynamic variables included mean arterial pressure, pulmonary artery pressure (PAP), right atrial pressure (RAP), and CO. Cardiac output was measured by use of a thermodilution method and recorded as the mean value of 3 measurements obtained by use of a CO computer.

Hematologic and serum biochemical analyses—Venous blood samples were collected in tubes that contained EDTA, and hematologic values were determined by use of an automated cell counter. Blood samples were collected from a jugular vein in all horses for serum biochemical analysis (hepatic enzymes and bile acids). Blood samples were collected at baseline, 24 and 72 hours, and 7 and 14 days.

Analysis of phospholipid, cytokine, and eicosanoid concentrations—Analysis of phospholipid concentrations was accomplished by using serum samples. Concentrations were determined by use of a clinical chemistry analyzer.

Analysis of TNF-\(\alpha\) concentrations was performed by use of a human TNF-\(\alpha\) assay conducted in accordance with the protocol provided by the manufacturer. The assay was a solid-phase, 2-site chemiluminescent immunometric assay. Limit of detection of the assay was 4 pg/mL. Validation of samples was performed against samples containing known concentrations of TNF-\(\alpha\).

Analysis of IL-6 concentrations was performed by use of a commercially available ELISA kit. The assay involved the use of the quantitative sandwich enzyme immunoassay technique, and monoclonal antibodies raised against human cytokines were used as standards. Assays were performed on unextracted plasma samples in accordance with protocols provided by the manufacturers. After incubation of a mixture of conjugate, antibody, and sample at 24°C, excess reagents were removed by washing, and substrate was added. After incubation for 45 minutes, the enzyme reaction was stopped, and color that had been generated was measured at a wavelength of 405 nm. Limit of detection of the assays was 14 and 3 pg/mL for TxB\(_2\) and 6 keto-PGF\(_{1\alpha}\) respectively. Samples that had concentrations greater than the upper limits of the reference ranges (10,000 and 50,000 pg/mL for TxB2 and 6 keto-PGF\(_{1\alpha}\) respectively) were diluted and analyzed again.

Data analysis—Data were reported as mean ± SEM. A 2-way ANOVA with repeated measures was used to determine differences among treatments and over time for all normally distributed variables. Appropriate transformations were made on all data that did not meet assumptions for the ANOVA (ie, data for TNF-\(\alpha\), IL-6, 6 keto-PGF\(_{1\alpha}\), and TxB\(_2\) concentrations). When main effects were detected, a post hoc Student Newman-Keuls test was performed to determine differences among individual means. Significance of all tests was set at a value of P < 0.05. Half-life (t\(_{1/2}\)) for phospholipid in clinically normal horses was determined by use of a nonlinear regression of plasma concentration of phospholipid versus time to a single exponential decay model.

Results
Pharmacokinetics of phospholipid—Mean ± SEM baseline phospholipid concentration in 3 clinically normal horses was 102 ± 9.3 mg/dL. Peak serum phospholipid concentration after PLE administration...
was 401 ± 19 mg/dL, which was detected at the end of PLE infusion. The \( t_{1/2} \) was 6.61 ± 1.9 hours. Hemolysis was observed starting 2 hours after the end of infusion, but it did not result in significant changes in PCV or serum total protein concentration.

Effects of PLE and endotoxin on results of physical examination—In both groups, there was a significant increase in rectal temperature over time. In addition, there was a significant \( (P = 0.03) \) difference in rectal temperature between control and treated horses (Fig 1). Mean peak rectal temperature in control horses was 39.3 ± 0.1°C and was reached at 195 minutes, compared with 38.7 ± 0.2°C that was reached at 195 minutes in the treated horses. Heart rate was significantly \( (P < 0.001) \) increased in control horses from 60 to 120 minutes, compared with values for treated horses during that same period (Fig 2). We did not detect a significant difference in respiratory rate between groups or over time (data not shown).

Effects of PLE and endotoxin on hemodynamic variables—Mean arterial blood pressure did not differ significantly between groups or over time (data not shown). Significant \( (P = 0.04) \) differences in PAP were detected between the control and treated groups at 15 (33 ± 2 vs 27 ± 2 mm Hg, respectively) and 30 (43 ± 2 vs 29 ± 2 mm Hg, respectively) minutes. The PAP began to increase within 15 minutes after initiation of endotoxin infusion in control horses and reached a mean peak of 44 ± 3 mm Hg at 45 minutes (Fig 3). Mean peak PAP in treated horses was 39 ± 5 mm Hg, which was reached at 60 minutes. Cardiac output was significantly \( (P = 0.03) \) greater in the control group at 30 and 45 minutes, compared with CO in treated horses at those same time points (Fig 4). The RAP was significantly \( (P = 0.049) \) higher in the control group from

![Figure 2](image1.png)

**Figure 2**—Mean ± SEM heart rate in control horses (solid circle) administered saline solution and endotoxin and treated horses (open square) administered PLE and endotoxin. Endotoxin was administered during a period of 60 minutes. Approximately 1 L of PLE was administered during a period of 120 minutes. Infusions of saline solution and PLE were initiated 60 minutes prior to start of the endotoxin infusion. Time 0 = Onset of endotoxin infusion. See Figure 1 for key.

![Figure 3](image2.png)

**Figure 3**—Mean ± SEM pulmonary arterial pressure in control horses (solid circle) administered saline solution and endotoxin and treated horses (open square) administered PLE and endotoxin. Endotoxin was administered during a period of 60 minutes. Approximately 1 L of PLE was administered during a period of 120 minutes. Infusions of saline solution and PLE were initiated 60 minutes prior to start of the endotoxin infusion. Time 0 = Onset of endotoxin infusion. See Figure 1 for key.

![Figure 4](image3.png)

**Figure 4**—Mean ± SEM cardiac output in control horses (solid circle) administered saline solution and endotoxin and treated horses (open square) administered PLE and endotoxin. Endotoxin was administered during a period of 60 minutes. Approximately 1 L of PLE was administered during a period of 120 minutes. Infusions of saline solution and PLE were initiated 60 minutes prior to start of the endotoxin infusion. Time 0 = Onset of endotoxin infusion. See Figure 1 for key.

![Figure 5](image4.png)

**Figure 5**—Mean ± SEM right atrial pressure in control horses (solid circle) administered saline solution and endotoxin and treated horses (open square) administered PLE and endotoxin. Endotoxin was administered during a period of 60 minutes. Approximately 1 L of PLE was administered during a period of 120 minutes. Infusions of saline solution and PLE were initiated 60 minutes prior to start of the endotoxin infusion. Time 0 = Onset of endotoxin infusion. See Figure 1 for key.
Effects of PLE and endotoxin on results of hematologic and serum biochemical analyses—Horses in the control group had significantly (P < 0.004) lower total leukocyte counts, compared with values for treated horses (Fig 6). There was a significantly lower total leukocyte count in the control group within 30 minutes after initiation of endotoxin infusion, with a nadir of 3.3 ± 0.5 × 10^6 cells/L achieved at 90 minutes. Total leukocyte count for the treated group remained within the reference range for our facility until 5 hours; at that point, the treated horses had leukocytosis.

We did not detect a significant change from baseline values for results of serum biochemical analyses for control or treated horses at all time periods (data not shown). Results of all serum biochemical analyses were within the respective reference ranges of our facility.

Effects of PLE and endotoxin on concentrations of phospholipid, cytokines, and eicosanoids—Peak serum phospholipid concentration in the treated group was 517 ± 10 mg/dL at the end of PLE infusion, and it was > 400 mg/dL for 4 hours after the end of infusion. Hemolysis was observed starting 2 hours after the end of PLE infusion, but it did not result in significant changes in PCV or serum total protein concentration.

Mean plasma TNF-α concentrations differed significantly (P < 0.001) between control and treated groups at all time periods after endotoxin infusion (Fig 7). Peak TNF-α concentration was 55.3 ± 18.9 pg/mL in control horses, compared with 8.1 ± 2.2 pg/mL in treated horses. At 240 minutes, there was an approximately 7-fold difference in TNF-α concentrations between the control and treated groups.

Concentrations of IL-6 did not differ significantly between groups. Concentrations of TXB_2 were significantly (P = 0.01) higher at 30 minutes and concentrations of 6 keto-PGF_1α were significantly (P = 0.02) higher at 30 and 60 minutes in control horses, compared with concentrations of those eicosanoids in treated horses (Table 1).

Figure 6—Mean ± SEM total leukocyte count in control horses (solid circle) administered saline solution and endotoxin and treated horses (open square) administered PLE and endotoxin. Endotoxin was administered during a period of 60 minutes. Approximately 1 L of PLE was administered during a period of 120 minutes. Infusions of saline solution and PLE were initiated 60 minutes prior to the end of the endotoxin infusion. Time 0 = Onset of endotoxin infusion. See Figure 1 for key.

Figure 7—Mean ± SEM concentration of tumor necrosis factor-α (TNF) in control horses (solid circle) administered saline solution and endotoxin and treated horses (open square) administered PLE and endotoxin. Endotoxin was administered during a period of 60 minutes. Approximately 1 L of PLE was administered during a period of 120 minutes. Infusions of saline solution and PLE were initiated 60 minutes prior to the end of the endotoxin infusion. Time 0 = Onset of endotoxin infusion. See Figure 1 for key.

Table 1—Effect of administration of saline (0.9% NaCl) solution and endotoxin (control) and phospholipid emulsion (PLE) and endotoxin (treated) on concentrations of interleukin (IL)-6, thromboxane B2 (TXB_2), and 6 keto-prostaglandin (PGF)_1α in horses

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<th>TXB_2 (pg/mL)</th>
<th>6 keto-PGF_1α (pg/mL)</th>
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Values reported are mean ± SEM.
*Control horses were administered saline solution and endotoxin (30 ng/kg; Escherichia coli 011:B4 lipopolysaccharide), and treated horses were administered PLE (200 mg/kg) and endotoxin. Endotoxin was administered during a period of 60 minutes. Approximately 1 L of PLE was administered during a period of 120 minutes. Infusions of saline solution and PLE were initiated 60 minutes prior to the start of the endotoxin infusion. Time 0 = Onset of endotoxin infusion.
**Within a row within each variable, values with different superscript letters differ significantly (P < 0.05).
Discussion

The low-dose endotoxin model (dosage of 30 mg/kg infused during a period of 60 minutes) that was used for the study reported here provided a predictable change in hematologic, hemodynamic, and physical variables. Our data are comparable to those in other studies in other species and from our own pharmacokinetic data. In other species, doubling the plasma concentration of HDL resulted in a 3- to 4-fold increase in survival in mice, and further increases in HDL concentrations provided additional protection. The dosage selected for use in the study reported here resulted in a 3- to 4-fold increase over the serum phospholipid concentrations we had detected in clinically normal horses. Pharmacokinetics were not determined in the treated group. The effect of endotoxin administration on endogenous phospholipid concentration has not been determined in horses and may affect determination of the t1/2 of the emulsion.

Peak rectal temperature in control horses was achieved at 195 minutes (39.3 ± 0.1°C). Mediators of the inflammatory cascade that are initiated by TNF-α are responsible for influencing the hypothalamic thermoregulatory center to cause fever. Other factors other than TNF-α are responsible for the increase in temperature. Interleukin-1 is a major contributor during up-regulation of the thermoregulatory centers of the hypothalamus. In the study reported here, the treated group had a delayed and diminished febrile response, and the 2 groups differed significantly for > 3 hours. Heart rate in the control group began to increase within 15 minutes after endotoxin administration and peaked at 90 minutes, which would be expected with the early increase in TNF-α concentrations. The CO values in the treated and control groups were increased, compared with baseline values. The CO values in this study were similar to those found in clinically normal horses in another study, however, there was a significant increase for our control horses, compared with the treated horses, at 30 and 45 minutes. The early increase in PAP was a response to administration of endotoxin. The PAP in the control group peaked earlier and reached a higher value than that detected in the treated group. The greatest difference in PAP was evident at 30 minutes, which coincided temporally with increased concentrations of TXB2. Thromboxane is a potent vasoconstrictor, and response to this eicosanoid is evident early in experimentally induced endotoxemia.

The low-dose infusion of endotoxin did not cause significant changes in mean arterial pressure in our study. In another study, in which investigators examined the effects of a slow infusion of low-dose endotoxin (30 ng/kg, IV, during a period of 1 hour), a significant increase in mean carotid arterial pressure was not detected until 270 minutes after initiation of the endotoxin infusion. When higher dosages of endotoxin are administered as a bolus (125 μg/kg, IV, during a period of 1 minute), there is a significant decrease in mean arterial pressure. This difference is likely attributable to the fact that there is not an overwhelming release of inflammatory mediators with slow infusion of a low dose of endotoxin, which allows the body to compensate for these effects without causing dramatic changes in CO and mean arterial pressure.

Effects of PLE on total leukocyte count were notable. The control group had a marked decrease in total leukocyte count within 30 minutes after onset of endotoxin administration. The treated group maintained values that were within the reference range until 300 minutes, when there was a mild rebound leukocytosis. In other studies, the WBC nadir was also detected 1 to 1.5 hours after endotoxin administration. The ability of PLE to abate the effects of endotoxin on the margination of neutrophils was substantial. There was not a rapid decrease in total leukocyte count in our treated group of horses, compared with values for the control horses. Endotoxin (ie, LPS) causes increased adhesiveness of neutrophils and margination as a result of formation of a LPS-LBP complex that allows for interaction with CD14 on the surface of neutrophils and monocytes. Tumor necrosis factor-α and other cytokines are also responsible for inducing expression of neutrophil surface glycoproteins and promoting adherence of neutrophils to the vascular endothelium. Down-regulation of CD14 expression on monocytes by R-HDLs, which has been documented in vitro in human blood, could be another factor for preventing margination of neutrophils.

The increase in TNF-α production was first detected at 60 minutes, and the maximal concentration in the study reported here was detected 4 hours after initiation of endotoxin, compared with peaks at 1 to 2 hours after initiation in other studies. The ability of the PLE to completely abolish release of TNF-α, compared with release in the control group, is similar to findings in another study in which investigators examined the efficacy of R-HDLs for the treatment of endotoxia. Treatment with a monoclonal antibody against equine TNF-α also almost completely neutralizes LPS-induced TNF-α activity in miniature horses given endotoxin. The effect on TNF-α release in the study reported here agrees with that of other studies, because prior treatment with PLE caused a decrease in the release of TNF-α in response to administration of LPS.

Studies, in which investigators examined IL-6 concentrations have revealed significant increases within 2 hours after administration of endotoxin, and these values remained significantly increased for 6 to 12 hours. Interleukin-6 causes induction of hepatic synthesis of acute-phase proteins and can act as an endogenous pyrogen. In the study reported here, there was a peak increase in IL-6 concentration in the
treated horses at 60 minutes and the control horses at 90 minutes, but this change did not differ significantly between groups. We expected to detect an increase in IL-6 concentrations that closely followed the increase in rectal temperature. The increase in the treated group was detected earlier than has been reported in other studies, and was unexpected. Possible reasons for the findings in our study could have been related to an effect of the PLE on IL-6 release or problems related to IL-6 analysis. The ELISA used to measure cytokine concentrations in this study contained antibodies raised against human recombinant molecules. Cross-reactivity of human and equine cytokines to these antibodies has not been documented, although others have reported a high degree of homology between human and equine cytokines, specifically IL-1 and IL-6. Other investigators have suggested a targeted epitope (IL-6) with commercial ELISA kits because of the finding that there were appreciable concentrations of IL-6 in the control groups. Molecular cloning of equine IL-6 has revealed nucleotide identity of 81.7% and amino acid similarity of 77.6% between human and equine IL-6.

Another complicating factor with the cytokine assay used in the study reported here is the possibility of alterations in the assay attributable to the effects of hemolysis. Because of the fact that this assay has not been validated for use in horses, the specific concentrations may not be accurate, but these values should serve as a good comparison between our groups of horses.

Thromboxane and prostacyclin increase in response to endotoxemia. Thromboxane causes vasoconstriction and platelet aggregation, whereas prostacyclin is a potent vasodilator and an inhibitor of vasoconstriction and platelet aggregation, whereas prostacyclin is a potent vasodilator and an inhibitor of platelet aggregation. There was an early increase in both of these metabolites of the arachidonic acid cascade in the study reported here. The commercial immunoassays have been questioned for use on fluids originating from domestic animals, because the antibodies are raised against recombinant human mediators. Commercial immunoassays for the quantitative determination of the eicosanoids in biological fluids can be used accurately in any species, because there are no chemical differences among species for these low-molecular-weight carbon structures. The 6 keto-PGF₁α had an early increase (0 minutes) in the control group, which was before the administration of endotoxin. In the treated group, there was a delay in this increase in 6 keto-PGF₁α at 0 minutes. In another study, a single peak of 6 keto-PGF₁α production was detected 2 hours after administration of endotoxin. The initial increase in the study reported here was not associated with the administration of endotoxin; thus, a likely cause is eicosanoid release in response to placement of monitoring equipment. Acute-phase proteins released in response to the microtrauma of catheter placement could have initiated the early release of inflammatory mediators. The 6 keto-PGF₁α concentrations in the control group decreased steadily from 30 to 120 minutes. This early increase made it difficult to evaluate the effect of endotoxin on 6 keto-PGF₁α concentrations in the control group. We did not detect any increase from baseline concentrations in the treated group. The increase in TxB₂ concentrations at 30 minutes is similar to that reported in other studies. This significant increase in TxB₂ concentration corresponds with the increase in PAP that would be expected secondary to the vasoconstrictive effects of TxB₂.

A mild to moderate amount of hemolysis was observed in the treated group, which occurred within 1 to 2 hours after initiation of the PLE infusion and remained until the end of sample collection (360 minutes). The amount of hemolysis did not result in a significant decrease in the PCV, but it was grossly visible in the serum and plasma samples. Cause of the hemolysis is unknown. A possible causative factor may have been related to incorporation of the PLE into the RBC membrane. Certain lipid emulsions are rapidly incorporated into the phospholipid membrane of monocytes of horses. This may also occur in RBCs of horses, causing increased fragility of the RBCs and accounting for the delayed onset of gross hemolysis. More studies will be needed to formulate an emulsion that does not cause hemolysis in horses.

The PLE may have had several mechanisms of action on the initial response to endotoxin in horses. It is believed that the lipid-A portion of LPS is inserted into the phospholipid bilayer of discoidal R-HDLs or the phospholipid monolayer of HDLs, blocking the toxic effects of LPS and subsequent release of TNF-α. Another mechanism of action may be an effect of the lipid emulsion on the membrane composition of the monocytes. A short infusion of omega-3 fatty acids alters the composition of equine monocytes and modifies the inflammatory response in vitro. This could have accounted for some of the changes seen in the eicosanoid concentrations. The PLE may also have had an effect on the uptake of LPS by CD14 receptors. High-density lipoprotein compounds can cause down-regulation of these receptors on the cell surface. Additional studies to examine the effect of these emulsions will be needed to define the exact mechanism of action in horses.

In the study reported here, prior treatment of horses with PLE before IV administration of endotoxin ameliorated the deleterious effects of endotoxin. Beneficial effects on heart rate, rectal temperature, PAP, RAP, CO, total leukocyte count, and TNF-α concentration were observed with prior PLE treatment. However, we only examined the effect of PLE on the initial effects of endotoxin when the PLE was administered prior to endotoxin treatment. Further studies will be needed to evaluate administration of PLE in animals with other methods of induced endotoxemia and finally in clinical settings for horses with naturally developing endotoxemia.

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1Angiocath, Deseret Medical Co, Sandy, Utah.
2Swan-Ganz, Edwards Laboratories, Santa Ana, Calif.
3Rogosin emulsion, Fresenius Kabi, Clayton, NC.
4Escherichia coli O11:B4 LPS, Sigma Chemical Co, St Louis, Mo.
5Cardiowax II, Columbus Instruments, Columbus, Ohio.
7Roche COBAS FARA II, Roche Diagnostic Systems, Branchburg, NJ.
8Immulite TNF-α, Diagnostic Products Corp, Los Angeles, Calif.
References


