ENDOTOXEMIA

Endotoxemia occurs when gram negative bacteria or their toxins gain access to the systemic circulation. Endotoxin is a structural component of the outer cell membrane of gram negative enteric bacteria. It is composed of three parts, each of which has important biologic characteristics. The innermost portion is termed the lipid A, which is unique because it is well conserved among different species of gram negative bacteria and it imparts the toxic qualities to the endotoxin molecule. The middle region of endotoxin is the core oligosaccharide which links the lipid A with the outer polysaccharide portion. The core region is well conserved in gram negative bacteria. The outermost portion is comprised of repeating polysaccharides, which is quite diverse and accounts for the serologic differentiation among bacterial species.

Because endotoxin is an integral component of the outer cell wall of gram negative bacteria it is liberated when the bacterium dies or undergoes periods of rapid proliferation. The gastrointestinal tract lumen harbors large quantities of gram negative bacteria and free endotoxin. To prevent the development of endotoxemia, the horse has evolved several efficient mechanisms to restrict transmural movement of endotoxin across the bowel wall and to remove endotoxin from the portal blood. The mucosal epithelial cells of the intestine function as a physical barrier against transmural movement. These mucosal epithelial cells also secrete substances such as lysozyme, enzymes, and antibodies which limit the ability of enteric bacteria to invade the mucosal lining. If a small quantity of endotoxin traverses the intestinal mucosal barrier and gains access into the portal circulation, Kupffer cells (hepatic macrophages) become effective scavengers of endotoxin. Additionally, many horses have small quantities of circulating anti-endotoxin antibodies directed against the core region which can bind endotoxin and permit its removal from the circulation.

If the integrity of the intestinal mucosal barrier is disrupted, the quantity of endotoxin traversing the barrier may exceed the ability of these protective mechanisms from removing endotoxin from the circulation. Additionally, endotoxin can also traverse full-thickness bowel, enter the peritoneal cavity, and reach the systemic circulation via the thoracic duct. Once endotoxin gains access to the systemic circulation, it may associate with high density lipoproteins or lipopolysaccharide-binding protein, which has a strong avidity for the lipid A region of endotoxin. This protein seems to play a crucial role in the binding of endotoxin to specific sites on the surface of mononuclear phagocytes and other cells. The endotoxin-lipopolysaccharide-binding protein complex recognizes the CD14 which is present as a surface
antigen on inflammatory cells and present as a soluble for allowing for cells without the surface antigen to enhance their response to LPS. Since CD14 lacks a transmembrane and cytoplasmic domain has no intrinsic signaling capabilities; therefore, the CD14-LBP-LPS complex must interact with another receptor which is known to be toll-like receptor 4/MD-2 complex. Binding to the TLR4/MD-2 complex activates a signal transduction cascade that leads to the production of the transcription factors such as NF-κB. This results in inflammatory gene expression and subsequent synthesis of inflammatory mediators which result in the characteristic clinical signs and clinicopathologic findings characteristic of endotoxemia.

Approximately 25% of horses admitted to university teaching hospitals for acute gastrointestinal tract disease have detectable endotoxin in the plasma. Because the half-life of circulating endotoxin in plasma is less than 2 minutes, these results suggest that there is constant movement of endotoxin from the intestinal lumen into the circulation. Most horses with gastrointestinal tract disease with endotoxemia have an ischemic or inflammatory bowel disease. Therefore, the prevalence of endotoxemia is greatest for horses with intestinal strangulation obstruction (small intestinal or large colon volvulus, incarceration, etc), enteritis (colitis, proximal enteritis), and septic peritonitis.

The interaction of gram negative endotoxin and the horse’s inflammatory cells results in the synthesis and release of numerous inflammatory mediators, including tumor necrosis factor, interleukin-1 and -6, thromboxane A2, prostaglandin I2, platelet activating factor, and procoagulant activity. Entrance of endotoxin into the systemic circulation results in a complex pathophysiologic cascade of events that frequently leads to morbidity and mortality despite aggressive treatment.

DIAGNOSIS

The diagnostic approach to endotoxemia in horses includes performing a thorough physical examination, complete blood count, and arterial blood gas analysis. Horses typically have tachycardia, tachypnea, fever, discolored mucous membranes, prolonged capillary refill time, dehydration, and decreased gastrointestinal borborygmi. Horses may be sweating profusely and have a weak pulse. Complete blood count often reveals leukopenia, neutropenia and a left shift. An arterial blood gas usually reveals arterial hypoxemia and evidence of a metabolic acidosis. Horses often develop an early hyperdynamic (systemic arterial hypertension) followed by a more prolonged hypodynamic (hypotension) phase. Horses often develop pulmonary arterial hypertension.

TREATMENT

The cornerstones for treatment of endotoxemia involve several approaches including prevention of endotoxin from entering the circulation, neutralization of endotoxin, eliminate or decrease the production of inflammatory mediators, and providing supportive care. Controlling the primary disease is initially one of the most important aspects of treatment or prevention of endotoxemia in horses. In regard to gastrointestinal tract disease, this often involves administering mineral oil to horses with grain overload, surgically removing ischemic bowel, or providing supportive care to horses with inflammatory bowel disease. Administering broad spectrum antibiotics is important in helping to treat horses with septic processes; however, rapid death of gram negative bacteria could theoretically lead to increased release of endotoxin from their cell wall.

Attempts to neutralize endotoxins can be accomplished by two different treatments. The first is the use of hyperimmune serum or plasma which can be administered intravenously to horses with endotoxemia or those predisposed to developing endotoxemia. These products are probably most likely to be beneficial if administered prior to endotoxin gaining access to the
circulation because these anti-endotoxin antibodies presumably must complex with endotoxin before it interacts with inflammatory cells to exert a protective effect. Although there is controversial and contradictory results using these hyperimmune plasma or serum products in experimental and naturally acquired endotoxemia, there may be a place for them in the therapeutic regimen of horses with or predisposed to develop endotoxemia. Anecdotally, administration of 0.5 to 1.0 liters of hyperimmune serum or plasma raised against a rough mutant of E. coli (J5) or Salmonella typhimurium can have fairly profound protective effects in individual horses depending upon the timing of administration and the magnitude of endotoxemia. In a double-blind clinical study performed on horses with clinicopathologic evidence of endotoxemia, J5 hyperimmune plasma treatment was associated with an increased survival rate (87% vs. 53%) and improved clinical appearance and a shorter hospitalization period compared with horses treated with preimmune plasma. In another study of sublethal experimental endotoxemia, J5 hyperimmune serum administration did not improve clinical or clinicopathologic variables. Pretreatment with 1.5 ml/kg of Salmonella typhimurium antiserum administered IV before 0.25 μg/kg E. coli lipopolysaccharide to 3-5 month old foals had no positive protective effect; it was suggested that under certain circumstances that it could exacerbate the actions of endotoxin. The variable effects of administration of hyperimmune plasma or serum to horses with endotoxemia explains the diverse opinions of clinicians regarding the clinical efficacy of these solutions.

The second way to neutralize endotoxin is through the administration of Polymyxin B, which is a cationic polypeptide antibiotic that has been shown to bind lipid A and to neutralize the actions of endotoxin in vitro. Polymyxin B is a broad-spectrum antibiotic, however, because of its high potential for nephrotoxicity and neurotoxicity it is not administered to horses. Because polymyxin B exerts antiendotoxic activity at serum concentrations substantially lower than that required for its antimicrobial effects, it has been used in clinical trials for prevention and treatment of endotoxemia in human patients. In some studies, patients given polymyxin B have been shown to have improved immunologic function, decreased plasma endotoxin concentrations, and decreased mortality, compared to patients not given polymyxin B. There were also no adverse effects of polymyxin B observed. Pretreatment with 6,000 U/kg polymyxin B administered IV before 0.25 μg/kg E. coli lipopolysaccharide to 3-5 month old foals caused significantly lower maximal TNF and IL-6 activities and significantly lower rectal temperature and respiratory rate, compared with foals given endotoxin but no polymyxin B. Although, better if administered before endotoxin release, treatment 30 minutes after endotoxin infusion in horses also resulted in significantly reduced fever, tachycardia and serum TNF concentrations demonstrating a benefit of administration even after the onset of endotoxemia. Clinically, polymyxin B is administered to horses at a dose of 2,000-6,000 U/kg every 12 hours diluted in approximately 1 liter of polyionic fluid. This therapy is typically continued for approximately 3 days or until the signs of endotoxemia subside. Although polymyxin B administered at 6000 U IV q8 hours did not have an accumulating affect in the vasculature and appeared safe, caution should be used in horses that are obviously dehydrated, hypovolemic, or azotemic until these abnormalities are corrected. If possible, it would seem that polymyxin B would have the best chance of providing protection to horses as a preventative measure; administration should probably be considered in horses predisposed to endotoxin absorption such as horses with ischemic or inflammatory bowel disease.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the mainstay of treatment of horses with endotoxemia. Flunixin meglumine and phenylbutazone are the two most commonly used NSAIDs in these horses. In general, flunixin meglumine seems to be more effective at attenuating the cardiovascular effects of endotoxin whereas phenylbutazone appears to offset the inhibitory effects of endotoxin on bowel motility. Administration of flunixin meglumine at
0.25 mg/kg IV every 8 hours has been shown to decrease eicosanoid concentrations, attenuate hemodynamic effects, and reduce lactic acidemia associated with experimental endotoxemia if administered before endotoxin infusion. In another report, flunixin meglumine administered at 1.1 mg/kg IV 15 minutes before and 8 hours after endotoxin also caused a decreased in rectal temperature, total leukocyte count, and thromboxane B₂ concentration. Phenylbutazone can be administered at a dose of 2.2 mg/kg IV every 12 hours to inhibit the effects of endotoxin on intestinal motility. Flunixin meglumine and phenylbutazone can be used together to minimize the effects of endotoxin on hemodynamics and intestinal motility provided the horse is well hydrated. The COX-2 specific inhibitors, melaxocam and firocoxib, purportedly do not have the detrimental effects on mucosal barrier restitution in an ischemic-injured jejunum as reported for flunixin meglumine; therefore the use of these medications may be beneficial in inhibiting or limiting the amount of endotoxin that can enter the circulation. Other NSAIDs have limited use in horses with endotoxemia.

Dimethylsulfoxide (DMSO) is often administered to horses for its putative anti-inflammatory effects, which are related to its ability to scavenge oxygen free radicals. Experimental evidence for the use of DMSO in horses with endotoxemia has been shown to have a mild effect when administered prior to endotoxin challenge (0.2 ug/kg IV). DMSO ameliorated endotoxin induced fever when administered at a high dose of 1 g/kg. No other significant changes in heart rate, respiratory rate, WBC count, blood glucose concentration, or blood lactate concentration were reported after DMSO administration at either the high (1g/kg IV) or low (20 mg/kg IV) dose. There was a trend to have less of a TNFa response after endotoxin administration in horses pre-treated with high-dose DMSO compared to saline and low dose DMSO treated horses. If used, DMSO should be administered at a dose of 0.02 g/kg to 1 g/kg; it should be administered at a concentration no greater than a 10-20% solution. Other antioxidants such as allopurinol, a competitive antagonist of xanthine oxidase, and 21-aminosteroids (inhibit lipid peroxidation) have been shown to be protective against endotoxemia in other species, but there is no experimental evidence to support their use in horses.

Pentoxifylline (PTX) is a methylxanthine derivative that has been used for several years for treatment of intermittent claudication in people. PTX is a rheologic agent that improves capillary blood flow by reducing blood viscosity and increased RBC deformability. More recently, PTX has been shown to exert pharmacologic effects in vivo and in vitro that may be beneficial in the treatment of endotoxemia such as inhibition of TNF production, decreased thromboxane B₂ concentrations and tissue thromboplastin activity and increased prostacyclin concentrations. However, PTX has not been shown to attenuate the clinical signs of endotoxemia in human patients and it does not exert any antipyretic or analgesic effects. Intravenous administration of 8 mg/kg of PTX 15 minutes before and 8 hours after IV administration of 30 ng/kg E. coli lipopolysaccharide resulted in a significantly higher prostacyclin concentration at 1.5 hours and lower PAI-1 activity at 12 hours, but TNF and IL-6 activities were not different compared with non-treated horses. Administration of a combination of flunixin meglumine (1.1 mg/kg IV) and PTX (8 mg/kg IV) did not cause an appreciable difference in the measured variables when compared with administration of flunixin meglumine alone. However, a bolus administration of 7.5 mg/kg PTX immediately after IV administration of 20 ng/kg E. coli lipopolysaccharide and followed by an infusion of PTX 3 mg/kg/hr over 3 hours resulted in significant differences in some measured variables compared with horses receiving only endotoxin. Although heart rate, rectal temperature, mean blood pressure, total leukocyte count, whole blood recalcification time, plasminogen activator inhibitor activity, TNF and IL-6 activities and plasma TXB₂ concentrations were significantly changed across time in horses receiving endotoxin and PTX and endotoxin alone, those receiving PTX had lower
rectal temperature and respiratory rate and longer whole blood recalcification time, compared to those not receiving PTX. Although it appeared that administration of PTX to horses as a bolus followed by a constant infusion caused significant changes in some measured variables, there appears to be minimal beneficial effects of PTX when administered IV using this regimen in this nonlethal model of endotoxemia in horses.

The last approach for managing horses with endotoxemia is supportive care, which involves administration of IV fluids to replace dehydration and volume depletion and to keep up with ongoing losses. Endotoxin has a profound effect on the cardiovascular system evidenced by the clinical signs of poor peripheral pulses, tachycardia, hemoconcentration, and lactic acidosis. In severe endotoxemia, hypertonic saline (2-4 mg/kg IV) followed by crystalloids may be necessary to rehydrate and improve vascular tone. Administration of plasma is useful to help replenish plasma proteins, especially albumin, which helps maintain the necessary oncotic pressure to keep fluids in the intravascular compartment. Hydroxyethyl starch (hetastarch; 10 ml/kg/day) can also be used to help maintain colloid oncotic pressure and its effects may last longer than plasma having a COP of 30 mm vs 20-25 mm in plasma. Endotoxemia has also been reported to result in numerous electrolyte abnormalities including hypocalcemia, hypomagnesemia, hypokalemia, and hypophosphatemia. Supplementation of these electrolytes, if abnormal, can be essential for normal cellular function.

**PROGNOSIS**

The prognosis for horses with endotoxemia should be considered guarded because of the severity of the primary disease and the rapidly progressing pathophysiologic processes that are initiated when appreciable quantities of endotoxin gain access to the systemic circulation. Horses that develop endotoxemia secondary to gastrointestinal tract ischemia or inflammation (enteritis, colitis) are often so severely ill that they either succumb to the effects of the primary disease or the inflammatory cascade initiated by the interaction of endotoxin with the host's inflammatory cells. Thrombophlebitis disseminated intravascular coagulopathy, ileus, laminitis, and multiorgan failure are reported sequelae to endotoxemia and treatments and preventions for these complications should be addressed as indicated. Overall, early aggressive treatment of the primary disease process along with a combination of the above mentioned medications can improve the outcome in some horses if the quantity of endotoxin reaching the systemic circulation is not overwhelming.

**REFERENCES**

References are available from author upon request.