Protection Against Clinical Endotoxemia in Horses by Using Plasma Containing Antibody to an Rc Mutant E. coli (J5)

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Thirty-two horses with clinicopathologic evidence of endotoxic shock were randomly selected for a double-blind trial of hyperimmune lipopolysaccharide (LPS) core antigen plasma. Horses were suffering from acute toxic enteritis (n=15), 360° volvulus of the large colon (n=9), proximal jejunitis/colitis (n=6), or strangulating obstruction of the small intestine (n=2). Plasma was harvested from suitable equine plasma donors (preimmune plasma) and horses were immunized with a whole-cell bacterin of an Rc mutant E. coli (J5). Plasma was again harvested from these horses when IgG ELISA titers recognizing LPS core antigen were ≥1:32,000. All horses included in the trial received either preimmune or hyperimmune plasma in addition to traditional therapy (fluids, antimicrobials, antiinflammatory agents, etc.) as dictated by the attending clinician. The mortality rate in the group of horses receiving hyperimmune plasma was 13%; in the control group receiving preimmune plasma, the mortality rate was 47% (P=0.045). Horses receiving J5 hyperimmune plasma had a significantly improved clinical appearance 48 hours after plasma administration (P<0.05) and a shorter period to recovery than control horses (P=0.069).

Key words: endotoxin, gram-negative core antigen, passive immunotherapy, equine colic, plasma transfusion

INTRODUCTION

Gram-negative sepsis and endotoxic shock associated with equine diseases such as acute toxic enteritis and strangulating obstructions of the intestine are costly problems. A high mortality rate occurs despite vigorous antimicrobial therapy due to the inability to counteract the lethal effects of bacterial endotoxins once cascades of immunologic mediators are fully initiated [1–6]. The frequent sequela to disease,

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including laminitis, venous thrombosis, or multiple organ failure, add to economic losses [6].

Endotoxin, also referred to as lipopolysaccharide (LPS), is a cell wall component of gram-negative bacteria and present in the intestinal contents of normal animals. Endotoxin is released by bacterial multiplication or bacteriolysis [2] and is absorbed into the circulation when the intestinal mucosal barrier is disrupted by intestinal obstruction, ischemia, or enteritis [3,7–9]. Gram-negative septicemia, secondary to inadequate colostral absorption or immunosuppression, offers another means for endotoxin to access circulation [6]. When a critical amount is absorbed or released, a shock state is produced, which includes tachycardia, reduced pulse pressure, prolonged capillary refill time, injected mucus membranes, cold extremities, and abdominal discomfort. Laboratory data observed with endotoxemia reveal numerous alterations, including hemoconcentration, leukopenia due to neutropenia and lymphopenia, hyperglycemia followed by hypoglycemia, elevated hepatic enzymes, elevated blood lactate, and coagulopathy suggestive of disseminated intravascular coagulation (DIC) [1–3,5]. Treatment for gram-negative sepsis and endotoxemia is costly in large animals and includes intense fluid administration, antimicrobials, corticosteroids, nonsteroidal antiinflammatory drugs, and other therapeutic agents [3,5,6].

A recent approach to therapy of endotoxic shock is the administration of plasma or serum products containing antibody directed toward endotoxins. Passive administration of antisera to a genetically stable rough mutant of E. coli 011:B4 (JS) [10,11] and of polyclonal antisera against LPS [12] has been shown to improve the survival rate of humans suffering from gram-negative bacteremia and endotoxemic shock. JS is a mutant strain of E. coli which lacks specific oligosaccharide side chains, so the core polysaccharide, which is similar among gram-negative bacteria, is exposed. Antibody to these core polysaccharides may thus be cross protective, binding to many gram-negative organisms or endotoxins [13–15]. The host immune reaction to endotoxin is believed to be an important determinant of mortality in diseases such as acute toxic enteritis and strangulating obstructions of the large and small intestine [3,5,6]. Previous studies utilizing antendotoxin antibody in horses with clinical evidence of endotoxemia reported decreased mortality and recovery periods [16,17]. However, some studies in horses failed to show protection of LPS core antigen antibody against endotoxin challenge [18] or neonatal septicemia [19]. While the protective effects of LPS antibody are still debated [18–26], strong evidence exists for their application in therapy for gram-negative disease. The purpose of this study was to perform a controlled clinical trial to determine if hyperimmune plasma containing antibody to gram-negative core antigens would reduce mortality in horses with clinical and laboratory evidence of gram-negative septicemia or endotoxemia.

MATERIALS AND METHODS

Preparation of Plasma

Three healthy horses were selected as plasma donors based on blood typing and absence of alloantibody. All donors were negative for A, C, and Q red blood cell

[1]"
Protection Against Clinical Endotoxemia in Horses

antigens, antierythrocyte antibodies, and equine infectious anemia. Horses were dewormed every 3 months and were vaccinated every 6 months against eastern and western encephalomyelitis, tetanus, and equine influenza A1 and A2. Donors were monitored for anemia by hematocrit determination prior to collection, and volume was replaced with lactated Ringer’s solution. Eight liters of blood was collected aseptically at approximately 30-day intervals. Sterile acid citrate dextrose (10%) was used as the anticoagulant and plasma was harvested after centrifugation (221G for 40 minutes). All plasma was collected in 1 L plastic bags, coded by number, and stored at −20°C. Once sufficient plasma was banked to serve as preimmune (control) plasma, the horses were immunized with a heat-killed E. coli J5 bacterin with incomplete Freund’s as adjuvant. The E. coli J5 bacterin was prepared as described by Ziegler et al. [10]. Horses were immunized intramuscularly with 3.5 ml of J5 (5 × 10⁹ CFU/ml) mixed with 1 ml of Freund’s incomplete adjuvant. Plasma was harvested when the anti-J5 IgG ELISA titer was ≥1:32,000. Horses were boosted every 30 days with bacterin to maintain elevated titers. J5 antibody titers were measured prior to all collections. The number of immunizations required to reach a titer of ≥1:32,000 was variable between donors, usually requiring at least four injections at 10-day intervals and two injections at monthly intervals. All preimmune plasma had an anti-J5 IgG titer of <1:500; the geometric mean IgG titer of hyperimmune plasma units was 1:41,499. All plasma batches (approx. 5 L plasma/harvest) were coded with a three-digit number picked at random and recorded by a research assistant. Horses were administered plasma from one code number. The plasma was stored in a freezer accessible to the large-animal clinicians. Horses were not preassigned to treatment groups; the selection was arbitrary based upon the coded plasma selected for the patient. Neither the clinicians or the investigators knew if the plasma was hyperimmune or control until the end of the trial. Equal amounts of hyperimmune and control plasma were harvested. The trial was terminated when the plasma stores were exhausted. No attempts were made to achieve equal division of hyperimmune and control plasma between disease groups.

Serology

An indirect enzyme-linked immunosorbent assay (ELISA) was used to determine anti-J5 IgG antibody activity [38]. In brief, 96-well flat-bottom microtiter plates were coated with heat-killed, washed whole-cell E. coli strain J5 (100 µl/well) that had been adjusted spectrophotometrically with 0.9% sterile saline to an optical density of 0.854 at 610 nm. Plates were incubated overnight at 37°C, washed, and sequentially incubated with 100 µl of diluted test plasma, conjugate (peroxidase-labeled antiequine IgG⁺), and substrate (40 mM ABTS, 0.05 M citrate buffer, 0.5 M H₂O₂). Stopping reagent (5 mM Na azide) was added and plates were read immediately with an automated plate reader. All samples were processed in triplicate with titers defined as the reciprocal of the highest dilution in which a

²Transfer pack unit with coupler, Fenwal Laboratories, Deerfield, IL.
³Falcon Probind 3916, Becton Dickenson Labware, Oxnard, CA.
⁴Kirkegaard and Perry Laboratories Inc., Gaithersburg, MD.
⁵Dynatech Laboratories, Alexandria, VA.
Day 3 after the infection of the animals with the virus of interest, the animals were housed in individual cages in the animal facility. The cages were equipped with food and water, and the animals were observed for signs of illness. The experiment was performed in a controlled environment to minimize external factors. The results were analyzed using statistical software to determine the efficacy of the vaccine candidate.

**Horses chosen for study:**

On the arithmetic mean of eight control wells with no serum, minimum of two of those identical wells exceeded 0.5 optical density after incubating.
REFERENCES

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in dual process with clinical evidence of endocannabin.

high-lever organs (e.g., liver, kidneys, and gut where

If this study, it appears that 84% of

the known species as well as the potential for human consumption to

which provides protection is contradictory (11). This is because

activation and expression of tyrosine kinase in

Afghanistan. Activated T cells then infiltrate the

Sporz.

Spitz et al.