SHORT COMMUNICATION

The Effectiveness of Immunotherapy in Treating Exercise-Induced Pulmonary Hemorrhage

Tammi S. Epp, DVM, PhD,[a] Paul McDonough, PhD,[b] Don E. Myers, MS,[c] Danielle J. Carlin, PhD,[a] Brad J. Behnke, PhD,[d] Casey A. Kindig, PhD,[†] David C. Poole, PhD, DSc,[a] and Howard H. Erickson, DVM, PhD[a]

ABSTRACT

Inflammatory airway disease has been linked to exercise-induced pulmonary hemorrhage (EIPH), and consequently, we hypothesized that immunomodulation via concentrated equine serum (CES) treatment would reduce EIPH as evidenced by red blood cell (RBC) concentrations in bronchoalveolar lavage fluid (BALF). Separate trials were conducted on Thoroughbred horses treated with either CES (n = 6) or placebo (0.9% saline; n = 4). All horses completed pre-treatment and post-treatment (2 and 4 weeks after initiating treatment) maximal exercise tests on a 10% inclined treadmill (1 m/s/min increments to fatigue) over a 10-week period (2–3 weeks between tests), with bronchoalveolar lavage (BAL) performed 30 minutes after exercise. Treatment ensued 10 days after the pre-treatment exercise test, with horses receiving a series of five CES or placebo injections 24 hours apart (20 mL intratracheal and 10 mL intravenously), with subsequent weekly injections for 5 weeks thereafter. After CES treatment, both EIPH (RBC in BALF) and inflammation (white blood cell concentration [WBC] in BALF) were significantly diminished by the 4-week post-treatment run, demonstrating 46 ± 12% and 24 ± 11% decreases, respectively ($P < 0.05$). In contrast, EIPH was elevated significantly at the 4-week time point, and inflammation remained constant in the placebo trial. In conclusion, these preliminary data suggest that therapeutic intervention involving immunomodulation may represent a viable approach to reducing the severity of EIPH.

Keywords: Exercise-Induced Pulmonary Hemorrhage; Horse; Bronchoalveolar lavage; Immunomodulation; Inflammatory airway disease

From the Department of Anatomy and Physiology, Kansas State University, Manhattan, KS[a]; Department of Applied Physiology and Kinesiology, University of Florida, Gainesville, FL[b]; Sera, Inc., Shawnee Mission, KS[c]; and the Division of Exercise Physiology, Robert C. Byrd Health Sciences Center, West Virginia University School of Medicine, Morgantown, WV.[d]

[†] *Deceased.*

Reprint requests: Tammi S. Epp, DVM, PhD, 228 Coles Hall, Manhattan, KS 66506-5802.

0737-0806/$ - see front matter

doi:10.1016/j.jevs.2009.04.192

INTRODUCTION

Evidence for exercise-induced pulmonary hemorrhage (EIPH) can be found in virtually all racehorses in training[1-3] and can be a cause for poor performance[4,5] as well as potential premature athletic retirement. In addition, epidemiologic[6] and post-mortem[7-9] studies have identified an association between EIPH and inflammatory airway disease (IAD). Furthermore, it is often necessary for additional veterinary care, extended breaks from training, or permanent racetrack banishment for those horses responding poorly to treatment. Existing therapies for EIPH (ie, furosemide and the equine nasal strip)[10,11] are successful in reducing, but not completely abolishing, EIPH, even when used concurrently, which suggests that EIPH has complex and multifactorial causes. Therefore, it may be beneficial to investigate a therapy that could either provide an alternative strategy (ie, reduce inflammatory component) or additive reductions in EIPH.

Concentrated equine serum (CES; Sera, Inc., Shawnee Mission, KS) is a biologic serum aggregate collected from multiple draft horse donors, containing high levels of immunoglobulins, complement, and other serum proteins. The equine-derived CES (an intravenous and intratracheally administered product) and a goat-derived product called caprine serum fraction (CSF; administered intramuscularly; Colorado Serum Co., Denver, CO), have demonstrated evidence for reducing the chronic inflammation associated with lower airway disease (ie, IAD and EIPH) via immunomodulation in separate studies.[12,13] Additional support comes from endoscopic evidence in racetrack field trials (Sera, Inc., Shawnee Mission, KS).[14] Immunomodulation via intravenous immunoglobulin has been successfully used to treat asthma and systemic inflammatory conditions in humans.[15-19] However, no studies have been conducted to specifically examine the effectiveness of CES as a treatment for EIPH in racehorses. Thus, we hypothesized that CES would reduce EIPH (RBC in BALF) and pulmonary inflammation (decreased WBC in BALF) following maximal exercise.

MATERIALS AND METHODS

Animals and Preparation

Ten Thoroughbred geldings (4–12 years old; 470–620 kg) with a history of EIPH were divided into

placebo (n = 4) and treatment (n = 6 CES) groups in this preliminary investigation. The horses were housed on dry lots with loafing sheds on the Kansas State University campus, and had ad libitum access to water and salt. They were fed alfalfa and grass hay, as well as concentrate (Strategy, Purina Mills Inc., St. Louis, MO) twice daily. All procedures were approved by the Kansas State University Animal Care and Use Committee. Horses were instrumented with a Millar pressure transducer (for measurement of pulmonary artery pressure [Ppa; Millar Model SPC-471A, Millar Instruments, Incorporated, Houston, TX]) and a thermistor (for measurement of core body temperature and temperature correction of blood gases and pH; Model 08407 Thermal Dilution Catheter, Columbus Instruments, Columbus, OH) that were calibrated and placed in the pulmonary artery through jugular vein introducer catheters and verified by cardiac wave form visualization. Arterial blood gases and plasma lactate samples were collected via an arterial catheter placed in a previously elevated carotid artery.

Administration of CES or Placebo

Dosage was based on field studies completed by Sera, Inc., Shawnee Mission, KS. Twenty milliliters CES or placebo (0.9% saline) was administered by intratracheal injection with an 18-G 1.5-inch needle in the proximal one third of the trachea (between tracheal rings) after aseptic preparation. This was followed by slow intravenous administration of 10 mL of CES or placebo. The horse's head was elevated for 10 minutes after injection to allow gravity-dependent flow into the lungs and to minimize mucociliary clearance of CES or placebo. The procedure was repeated every 24 hours for 5 days with weekly injections (both the intravenous and intratracheal) given for 5 weeks thereafter. On the week of an exercise test, the CES or placebo was administered 24 hours before exercise. No adverse effects were noted in any of the experimental animals.

Experimental Protocol

A timeline of the experimental protocol is provided in Figure 1. The horses were conditioned on a high-speed treadmill (Sato, Inc, Uppsala, Sweden) using a moderate-to-heavy intensity exercise regimen ($\leq$10 m/s on flat; $\leq$7 m/s on inclined treadmill) 3 days/week before and throughout the study. On experimental days, horses performed a maximal exercise test on a 10% inclined treadmill, consisting of a warmup at 3 m/s for 4 minutes, followed by progressive increases in speed (1 m/s × 1-minute increments) until volitional fatigue, and were recovered at 3 m/s for 4 minutes. Pulmonary gas exchange was measured with an open-flow system, and cardiorespiratory variables (ie, oxygen uptake [$\dot{V}O_2$], carbon dioxide elimination, heart rate, respiratory rate, and Ppa), blood gases, plasma lactate, and time-to-fatigue were measured at rest, throughout the exercise test, and during recovery as described previously by Epp et al.[20]

Bronchoalveolar Lavage

Baseline (resting; with no exercise 7–10 days prior), 30 to 60 minutes post-exercise, and 1 week post-exercise BALs were performed (seven in total, as numbered in Fig. 1) as previously described[10,20] as a means to establish a nonexercising baseline RBC concentration in BALF, quantify the effects of CES on the severity of EIPH immediately post-exercise, and to confirm a return of RBC concentration in BALF to baseline before the next exercise test. The horses were tranquilized using detomidine hydrochloride (Dormosedan, Pfizer Animal Health, Exton, PA; 5–10 μg/kg intravenously) and butorphanol tartrate (Torbugesic, Fort Dodge Animal Health, Fort Dodge, IA; 5–10 μg/kg intravenously). A BAL tube (Bivona, Smiths Medical, Philadelphia, PA; 3 m long, 10-mm OD) was advanced blindly through the right naris, wedged in the caudodorsal lung lobe, and sealed with an inflatable cuff located at the end of the tube. A total of 300 mL (50-mL aliquots) of 0.9% saline was infused and aspirated with gentle suction. The BALF was centrifuged (Beckman TJ-6), the supernatant decanted, and the pellet resuspended in 0.9% saline. RBC and WBC concentrations were determined via a hemocytometer (No. 02-671-5, Fisher Scientific, Pittsburgh, PA) and presented as RBC/mL of recovered BALF, accounting for tube dead space.

Statistical Methods

All data are presented as mean ± standard error (SE). Data were analyzed via repeated measures analysis of variance using SAS PROC MIXED with treatment and time as fixed effects and horse as a random effect (SAS Program 9.1.2 statistical package, SAS Institute, Incorporated, Cary, NC). The relationship between variables was determined via a Pearson product-moment correlation analysis (Sigma Stat 3.0, SPSS, Inc., Chicago, IL). Significance was accepted at $P < 0.05$, and a one-tailed test was used for a priori directional hypotheses involving EIPH and inflammation.

RESULTS AND DISCUSSION

The novel finding of the current study is that CES significantly reduced EIPH and associated inflammation after maximal exercise in Thoroughbred horses after 4 weeks of therapy. This was evidenced by a 46 ± 12% decrease in RBC concentration in the BALF between the pre-treatment and the 4-week post-CES treatment runs (Fig. 2). Inflammation after maximal exercise also declined 24 ± 11%, as evidenced by the WBC concentration in the BALF (Fig. 3). In comparison, EIPH significantly increased by 245 ± 113%, whereas no change was demonstrated in WBC concentration in the BALF (Fig. 3) for horses under placebo conditions.