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Effects of conjugated oestrogens and aminocaproic acid upon exercise-induced pulmonary haemorrhage (EIPH)

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Abstract

Aminocaproic acid (ACA) and Premarin® (PRE) are used to treat exercise-induced pulmonary haemorrhage (EIPH) at the racetrack based upon their putative coagulation effects. We hypothesized that neither ACA nor PRE would reduce EIPH because the literature does not substantiate coagulation deficits being manifested in EIPH. Six Thoroughbreds were run from 4 m s^{-1} until fatigue (1 m s^{-1}s × 1 min increments; 6° inclined treadmill) after being treated with placebo, PRE (25 mg) or ACA (5 g) at 2-week intervals in a randomized crossover design. Coagulation and exercise-related variables were measured at rest and maximal effort. EIPH and inflammation were quantified via bronchoalveolar lavage fluid (BALF) 30–60 min post-exercise. EIPH was not altered by either treatment (3.8 ± 1.7 (placebo), 4.6 ± 3.2 (ACA) and 2.4 ± 1.2 (PRE) × 10^6 RBC ml^{-1} BALF; $p = 0.12$), nor was coagulation. However, inflammation was decreased (5.9 ± 0.9 (placebo), 4.4 ± 0.9 (ACA) and 4.2 ± 0.4 (PRE) × 10^5 WBC ml^{-1} BALF; both $p < 0.05$). There was a trend for decreased time-to-fatigue (720 ± 27 (placebo), 709 ± 24 (ACA) and 726 ± 28 (PRE) s; $p = 0.09$ for placebo *vs.* ACA) and a reduction in plasma lactate (19.5 ± 3.0 (placebo), 14.7 ± 1.0 (ACA) and 17.6 ± 2.5 (PRE) mmol l^{-1}; $p < 0.05$ for placebo *vs.* ACA) following ACA administration. ACA and PRE were not effective in reducing EIPH, and ACA may be detrimental to performance. However, both may mitigate exercise-induced pulmonary inflammation.

Keywords: equine; EIPH; bronchoalveolar lavage; aminocaproic acid; conjugated oestrogen

Introduction

Exercise-induced pulmonary haemorrhage (EIPH) is manifested in the majority of racehorses[1,2] and impacts their performance[3–5]. The condition generally worsens with repeated exercise and increased age[1,6] and results in extended breaks from training, additional veterinary care, missed races, track banishment[4,7] and even acute mortality in severe episodes[8]. EIPH has been projected to cost the industry, specifically the Thoroughbred and Standardbred racing industries, in excess of $100,000,000 per year[5].

Accordingly, significant efforts have been made since the 1990s to accurately quantify EIPH[1,2], understand its pathogenesis and create effective therapies. However, available therapeutic interventions reduce but do not prevent EIPH. This is due in part to the complex aetiology of EIPH, as reviewed by Erickson and Poole[9], with multiple factors contributing to its initiation or severity, whereas most therapeutic strategies address only one causative factor. Four treatment strategies have demonstrated scientific evidence for efficacy in the amelioration of EIPH including furosemide[10–12], the Flair™ equine nasal strip[10–14], concentrated equine serum[15–17] and omega-3 fatty acids[17]. The financial expenditure to horse owners and trainers is substantial for the numerous unproven treatments that are marketed today.

Early EIPH research[18–21] suggested the possibility of coagulation deficits in exercising horses potentiating EIPH; however, multiple investigations[22–25] have refuted this theory. Despite this and conflicting reports with regards to clinical efficacy (alone or in combination with furosemide), conjugated oestrogens and aminocaproic acid (ACA) are still used on racetracks in an attempt to control EIPH.

There is a compelling demand for effective prophylaxis and/or treatment that provides better control of EIPH and, in so doing, reduces the susceptibility to sequelae that impact pulmonary health and precipitate premature athletic career termination. An attempt to legalize only scientifically proven EIPH prophylactics by the Racing Medication Testing Consortium is underway with the goal of eliminating the use of expensive, unnecessary and ineffective race-day medications, as well as aiding in the standardization of race-day medications among racetracks across the nation.

Based on the current literature, we tested the hypothesis that neither ACA nor Premarin® (PRE) when injected IV 2 or 4 h prior to exercise, respectively, would reduce the severity of EIPH as compared with placebo (0.9% saline injected IV 2 h prior to exercise) when evaluated by the technique of bronchoalveolar lavage (BAL) 30–60 min post-maximal exercise.

Methods

Animals

Six Thoroughbred horses, 5–14 years old and weighing 470–600 kg, with Jockey Club and BAL-documented EIPH of varying severities, were used in this study. The horses were trained on a high-speed treadmill (Sato Inc., Uppsala, Sweden) 3 days $week^{-1}$, using a moderate–heavy intensity exercise regimen (endurance, sprint and interval workouts peaking at speeds of 10 m s^{-1} on the incline and 12 m s^{-1} on the flat) beginning 2 months before and throughout the study to keep the horses conditioned and stable with regards to EIPH. All procedures proposed were approved by the Kansas State University Animal Care and Use Committee.

Treatments

Treatment order was randomized in a crossover design. About 10 ml of 0.9% saline (placebo), 5 gm ACA (Hospira Pharmaceuticals, Lake Forest, IL, USA) or 25 mg conjugated oestrogen (sodium equilin sulphate; Premarin®, Wyeth Pharmaceuticals, Madison, NJ, USA) were administered IV at 2 h prior to exercise for placebo and ACA, and 4 h prior to exercise for PRE. Two full weeks were allowed for washout time between the three experimental trials. Two hours was chosen for ACA instead of the standard 4 h before race because maximum plasma concentration and therapeutic effects are seen in horses within the first 1–2 h after administration of a single bolus, and concentrations fall by 2/3 within 3–4 h[26–28]. Doses were chosen based upon the levels allowed at racetracks that permitted the use of these medications and average doses used by racetrack veterinarians using these pharmacological agents.

Animal preparation

Prior to the exercise protocol, two 7-F introducer catheters were aseptically placed under local anaesthesia (2% lidocaine), in the right jugular vein and one 18-gauge, 2″ catheter (Safelet®, NIPRO Medical Corporation, Miami, FL, USA) was placed in either a previously elevated left carotid artery (five horses) or the transverse facial artery (one horse). A Millar pressure transducer (Millar Model SPC-471A, Millar Instruments, Inc., Houston, TX, USA) calibrated from 0 to 200 mm Hg with a mercury manometer and a thermistor catheter (Model 08 407 Thermal Dilution Catheter, Columbus Instruments, Columbus, OH, USA) calibrated with a Physitemp thermocouple thermometer (BAT-10, Physitemp, Clifton, NJ, USA) were advanced through the introducer catheters into the pulmonary artery to measure pulmonary artery pressure and temperature (for temperature correction of blood gases and pH)[29], respectively. The location of the Millar and the thermistor was verified by cardiac wave form visualization.

Coagulation variables

Arterial blood samples were collected into citrated plasma tubes at rest and during maximal exercise and placed immediately on ice. The plasma was removed within 1 h of completing the run and stored at −70°C until analysed (Cornell University Animal Health Diagnostic Center, Ithaca, NY, USA) for antithrombin III activity (AT III), protein C, plasminogen, fibrin degradation products (FDPs), fibrinogen, prothrombin time (PT), partial thromboplastin time (PTT) and thrombin clot time (TCT). Activated clot times (ACT) and cutaneous template bleeding times (CTBT) were also performed in the laboratory at rest, prior to the exercise test.

Cutaneous template bleeding times were performed using the technique standardized by Kopp *et al.*[30]. Briefly, the template system (Surgicutt® Adult Template Bleeding Kit, International Technidyne Corporation, Edison, NJ, USA) was longitudinally applied to a clipped and shaved area (free of vessels) just distal to the accessory carpal bone with three to four measurements being taken on the caudomedial and caudolateral aspects of both forelimbs and averaged. A sphygmomanometer cuff placed proximal to the carpus was inflated to 50 mm Hg for 60 s to achieve cutaneous venostasis before the standard 5 × 1 mm incision was made by the device. Blood flow from the incision was collected on a No. 40 absorbent filter paper (without touching the incision edges) until no capillary blood flow could be detected on the paper.

Activated clot times were performed by warming the ACT tube (with diatomaceous earth) in a 37° Celsius water bath for 3 min, withdrawing 2.5 ml

of blood from a clean jugular venipuncture (immediately discarding 0.5 ml to eliminate thromboplastin, which is responsible for activating specific clotting factors), and injecting 2 ml of blood into the ACT tube and gently inverting once. Timing started as soon as the blood entered the tube. The tube was held upright in the water bath, and after 1 min the tube was gently tilted every 5 s until initial clot formation was observed and timing was stopped.

Maximal exercise test

After each of the three treatments, Thoroughbreds were warmed up at $3\,m\,s^{-1}$ for 4 min, then run at progressively increasing speeds ($1\,m\,s^{-1} \times 1$ min increments) on a 6° inclined treadmill from $4\,m\,s^{-1}$ to volitional fatigue (maximal oxygen uptake; $\dot{V}O_{2max}$), followed by a 4 min cool-down at $3\,m\,s^{-1}$. Pulmonary gas exchange ($\dot{V}O_{2max}$, maximal carbon dioxide production;$\dot{V}CO_{2max}$) was measured via the open flow system as previously described[25,31]. Additional physiologic variables including respiratory exchange ratio ($\dot{V}CO_2/\dot{V}O_2$; RER), pulmonary arterial pressure (Ppa), respiratory rate (RR), heart rate (HR), pulmonary arterial temperature and time-to-fatigue were obtained and monitored, or calculated continuously during rest, exercise and recovery. A computer-based data acquisition system (WinDaq Pro, DATAQ Instruments, Akron, OH, USA) was used for data collection and analysis. A Fourier analysis of the Ppa waveform was performed and the numerical values of the first (~2 Hz) and second (~3 Hz) peaks were multiplied by 60 cycles s^{-1} to obtain the respiratory and HRs, respectively, since these peaks have been shown to correspond to the fundamental frequencies for these variables[32]. Arterial blood gases (partial pressure of arterial O_2 and CO_2; P_aO_2 and P_aCO_2) and plasma lactate samples were collected anaerobically (rest, near-maximal exercise, maximal exercise and recovery), immediately placed on ice and analysed within 1–2 h of the experiment with a blood gas analyser (Nova Stat Profile, Nova Biomedical, Waltham, MA, USA) and corrected to the horse's pulmonary arterial blood temperature[29].

Bronchoalveolar lavage

About 30–60 min post-exercise, BAL was performed on the horses as a means to quantify the severity of EIPH[12,15,25,33]. Briefly, the horses were tranquilised using detomidine hydrochloride (Dormosedan®, Pfizer Animal Health, Exton, PA, USA; 5–10 $\mu g\,kg^{-1}$ IV) and butorphanol tartrate (Torbugesic®, Fort Dodge Animal Health, Fort Dodge, IA, USA; 5–10 $\mu g\,kg^{-1}$ IV). A BAL tube (Bivona, Smiths Medical, Philadelphia, PA, USA; 3 m long, 10 mm OD) with an inflatable cuff was introduced into the right naris and advanced into a caudodorsal lung lobe[34] until wedged and the cuff inflated to create a seal. A total of 300 ml (in 50 ml aliquots) of 0.9% physiologic saline was infused and aspirated with gentle suction. The BAL fluid was centrifuged (Beckman TJ-6, Beckman Instruments, Inc., Palo Alto, CA, USA), the supernatant decanted and the pellet resuspended in 0.9% saline. Red blood cells (RBCs) and total nucleated cells (TNCs) were counted using a haemocytometer (Fisher Scientific No. 02-671-5, Fisher Scientific, Pittsburgh, PA, USA) and reported as RBCs and TNCs per millilitre of recovered bronchoalveolar lavage fluid (BALF) minus tube dead space.

Statistical analysis

All data are presented as mean ± standard error (S.E.). Differences in measured variables were analysed statistically using a three-period crossover design with horse and run as random effects and treatment as a fixed effect. Three exercising samples (three different horses, one from each treatment) were dropped from the coagulation variable analyses due to inadvertent heparin contamination and assay interference. When significant differences were found, a least-square means post-hoc test was used to determine where differences existed. The statistical analysis program (SAS Program 9.1.2 statistical package, SAS Institute, Inc., Cary, NC, USA) was used to analyse the data. Significance was accepted at the $p \leq 0.05$ level.

Results

Lavage variables

The BALF recovery volume (60.3 ± 1.8%; Table 1) was not different among treatments ($p > 0.05$). The [RBC] in the BAL fluid were not altered ($p = 0.12$) with either ACA or PRE (Fig. 1). However, treatment with both pharmacological agents decreased ($p < 0.05$) [WBC] in the BALF (Fig. 2).

Exercise and metabolic variables

There was a trend ($p = 0.09$) for decreased time-to-fatigue by ACA, and horses consistently performed at least one stage less than runs completed after PRE or placebo administration (Fig. 3). Time-to-fatigue was not affected by treatment with PRE (Fig. 3). Peak plasma lactate was significantly decreased after the administration of ACA, but unaffected by PRE (Fig. 3). None of the other remaining variables measured during maximal exercise (Table 1), including cardiorespiratory (HR, RR, $\dot{V}O_{2max}$, $\dot{V}CO_{2max}$, RER), blood gas, acid–base or haematological variables, were altered as a result of either treatment.

Coagulation variables

Coagulation variables measured at rest in the laboratory before the exercise test, including CTBT and

Table 1 Key variables measured at rest (body weight), during (respiratory, cardiovascular, blood gas, acid–base and haematocrit) and following (BALF) maximal exercise under each condition

Variable	Placebo	Aminocaproic acid	Premarin
Body Weight (kg)	533 ± 19	535 ± 17	535 ± 19
$\dot{V}O_2$ (l min^{-1})	76.1 ± 1.2	77.1 ± 1.3	78.6 ± 1.2
$\dot{V}CO_2$ (l min^{-1})	84.5 ± 2.2	82.0 ± 2.6	85.7 ± 2.7
RER	1.11 ± 0.02	1.09 ± 0.02	1.12 ± 0.01
HR (bpm)	211 ± 3	209 ± 3	210 ± 3
RR (bpm)	120 ± 2	119 ± 2	119 ± 2
mPpa (mmHg)	84.4 ± 4.8	82.4 ± 5.7	82.5 ± 4.2
P_aO_2 (mmHg)	60.4 ± 1.9	61.9 ± 2.3	59.6 ± 2.5
P_aCO_2 (mmHg)	49.8 ± 1.9	48.9 ± 2.5	48.3 ± 1.7
pH	7.27 ± 0.04	7.29 ± 0.02	7.30 ± 0.02
HCT (%)	62 ± 1	60 ± 1	61 ± 1
BALF Recovery (%)	62.5 ± 3.1	58.4 ± 3.1	60.0 ± 3.4

$\dot{V}O_2$, oxygen uptake, STPD; mPpa, mean peak Ppa; HR, heart rate; RR, respiratory rate; $\dot{V}CO_2$, carbon dioxide elimination, STPD; RER, respiratory exchange ratio = $\dot{V}CO_2/\dot{V}O_2$; P_aO_2, temperature-corrected partial pressure of oxygen in the CT terial blood; P_aCO_2, temperature-corrected partial pressure of carbon dioxide in the arterial blood; HCT, haematocrit. The data for six horses are included in the table and values are presented as mean ± S.E. Maximum values are given for $\dot{V}O_2$, $\dot{V}CO_2$, RER, HR, RR, mPpa, P_aCO_2 and HCT. The lowest values achieved are given for P_aO_2 and pH during the exercise bout. Body weight and bronchoalveolar lavage fluid (BALF) recovery % are given to indicate no change across the various treatment conditions. No significant differences were found across the treatments for any of the listed variables.

ACT, were not affected by either treatment (Table 2). In addition, coagulation variables sampled at rest and during maximal exercise, including PT, PTT, TCT, fibrinogen, AT III, plasminogen and protein C, were not affected by either treatment. Due to the method by which the laboratory reported D-dimer, it was not possible to evaluate alterations as a result of treatment as they were reported in ranges of 1000–2000 or < 1000 ng ml^{-1}.

Discussion

The principal original findings of the present investigation were that neither ACA nor PRE effectively reduced EIPH. This was the case despite an acute decrease in pulmonary inflammation (i.e. decreased BALF [WBC]) demonstrated by both ACA and PRE. In addition, anecdotal reports by veterinarians that ACA may impair performance may have some validity as a trend existed for a decreased time-to-fatigue.

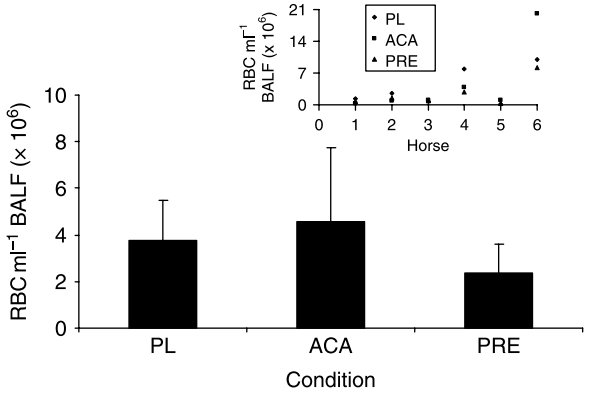

Fig. 1 Severity of exercise-induced pulmonary haemorrhage (EIPH) following maximal exercise ($n = 6$) as determined from the concentration of red blood cells (RBCs) in bronchoalveolar lavage fluid (BALF) after treatment with placebo (PL), aminocaproic acid (ACA) and Premarin (PRE). Data are presented as mean ± S.E. There were no significant differences among conditions ($p > 0.05$). Inset illustrates individual horse results

Rationale for use in treating EIPH

The use of ACA and PRE prior to athletic events has been proposed as prophylactic methods to mitigate EIPH as a result of hypothesized transient coagulation deficiencies in exercising horses[18,21]. Anecdotal reports suggest that ACA may attenuate blood loss or duration of bleeding in a variety of disorders, including guttural pouch mycosis, uterine artery rupture, trauma and EIPH in horses with normal coagulation, though controversy exists among veterinary clinicians regarding the benefits of such use[27]. Antifibrinolytic

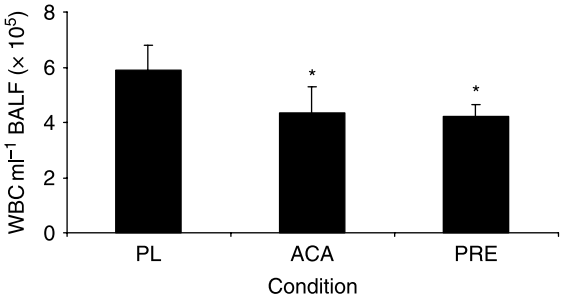

Fig. 2 White blood cells (WBCs) in bronchoalveolar lavage fluid (BALF) following maximal exercise ($n = 6$) after treatment with placebo (PL), aminocaproic acid (ACA) and Premarin (PRE). Data are presented as mean ± S.E. *Denotes significant difference between placebo and treatment conditions ($p < 0.05$)

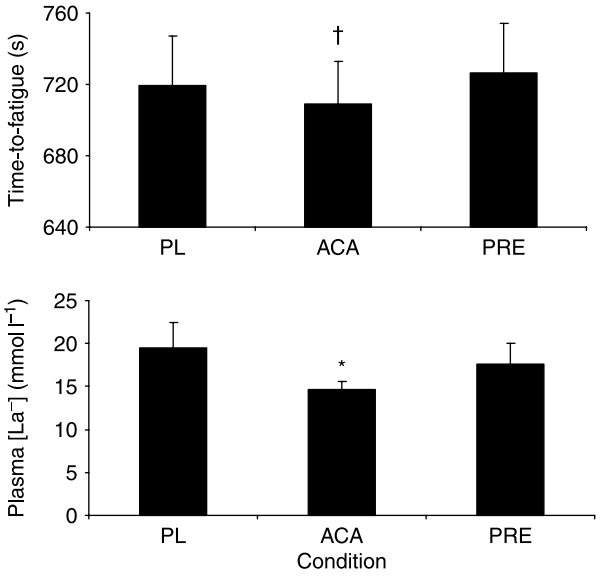

FIG. 3 The top panel represents time-to-fatigue in seconds (s) after horses have been treated with placebo (PL), aminocaproic acid (ACA) or Premarin (PRE). The bottom panel represents maximum plasma lactate concentrations ($[La^-]$) in mmol l^{-1} for horses treated with PL, ACA and PRE. Data are presented as mean ± S.E. for $n = 6$ horses. †Denotes a trend that exists for differences between PL and ACA condition ($p = 0.09$) in the top panel. *Denotes a significant difference that exists between PL and ACA condition ($p < 0.05$) in the bottom panel

treatments inhibit plasminogen activation, fibrinolysis, plasmin and antithrombin, and result in increased stability/lifespan of the clot and control of numerous causes of haemorrhage, benefitting human patients[27,35–39]. Results from Heidmann *et al.*[27] indicate that similar coagulation variables are modified by ACA administration to clinically normal equids, suggesting that it would also benefit horses without coagulopathies (i.e. horses undergoing primary traumatic injury or horses undergoing surgery).

PRE is used as a treatment for EIPH at doses ranging from 0.05 to 0.25 mg kg^{-1} IV[40], but no controlled clinical trials had been conducted in horses. PRE has also been used previously for a wide variety of human conditions requiring haemostasis[36,41–45]. Two primary mechanisms of action for conjugated oestrogens are restoration and/or strengthening of vascular integrity by strengthening collagen and eliminating endothelial discontinuity and degeneration[44,45,46–48]and shortening the bleeding time (>20 min)[36,42,43,45,49]. Other, more controversial, mechanisms include increased platelet aggregation[41,43] and alterations in prostacyclin[43,45], vasoregulation through nitric oxide[36] and coagulation[45,47,48] variables. Onset of action has been reported to occur within 6 h post-administration[41,43,45].

Comparison of coagulation results with the current equine literature

The current study demonstrated no change in any of the coagulation variables (PT, PTT, AT III, protein C, plasminogen, FDPs and fibrinogen) either at rest or during maximal exercise (approximately 2 h (placebo and ACA) and 4 h (PRE) post-administration). Our results are in agreement with the study by Ross *et al.*[28] in non-exercising horses. By contrast, Heidemann *et al.*[27] (non-exercising horses) demonstrated a minor difference with decreases in fibrinogen at 100 mg kg^{-1} ACA at 1 and 5 h post-administration and only at 1 h with the 30 mg kg^{-1} ACA. There was no difference in fibrinogen across treatments in the current study, but the much lower dosage used herein (9.1 mg kg^{-1} ACA) and exercise state may have precluded detection of any effects. Data from the Heidemann *et al.*[27] study as well as the current investigation may suggest that the empirical dose clinically used in horses is too low. However,

Table 2 Coagulation variables

Variable	Placebo	Aminocaproic acid	Premarin
Cutaneous bleeding time (s)	417 ± 87	561 ± 236	497 ± 139
Activated clot time (s)	158 ± 4	162 ± 4	160 ± 3
Partial thromboplastin time (s)	86 ± 10 (rest)	70 ± 8 (rest)	103 ± 18 (rest)
	61 ± 2 (max. ex.)	79 ± 9 (max. ex.)	69 ± 9 (max. ex.)
Prothrombin time (s)	16 ± 1 (rest)	16 ± 1 (rest)	16 ± 1 (rest)
	17 ± 2 (max. ex.)	16 ± 1 (max. ex.)	23 ± 8 (max. ex.)
Thrombin clot time (s)	18 ± 4 (rest)	14 ± 1 (rest)	22 ± 6 (rest)
	14 ± 1 (max. ex.)	20 ± 4 (max. ex.)	15 ± 2 (max. ex.)
Fibrinogen (mg dl^{-1})	346 ± 27 (rest)	373 ± 29 (rest)	342 ± 19 (rest)
	315 ± 39 (max. ex.)	365 ± 41 (max. ex.)	359 ± 40 (max. ex.)
Antithrombin III activity (%)	81 ± 8 (rest)	86 ± 9 (rest)	88 ± 5 (rest)
	99 ± 8 (max. ex.)	83 ± 12 (max. ex.)	91 ± 9 (max. ex.)
Plasminogen (%)	97 ± 11 (rest)	102 ± 11 (rest)	105 ± 12 (rest)
	100 ± 15 (max. ex.)	82 ± 11 (max. ex.)	107 ± 10 (max. ex.)
Protein C (%)	103 ± 7 (rest)	107 ± 10 (rest)	106 ± 6 (rest)
	104 ± 9 (max. ex.)	97 ± 9 (max. ex.)	105 ± 11 (max. ex.)
D-dimer (ng ml^{-1})	1000–2000 (rest)	<1000 (rest)	<1000 (rest)
	1000–2000 (max. ex.)	<1000 (max. ex.)	<1000 (max. ex.)

Values are means ± S.E. No significant differences found among conditions in any variable measured at rest or during exercise.

very high doses are contraindicated clinically due to the potential for induction of transient hypocoagulability as a result of dramatically decreased fibrinogen levels[27]. Additionally, small decreases in PT and PTT were demonstrated, which were neither seen in the current study nor in any of the human studies[27]. Heidmann *et al.*[27] questioned the decreases observed in PT and PTT in their study as no changes in intrinsic or extrinsic coagulation would be expected since antifibrinolytics act only after clot formation.

Potential explanations for ineffectiveness of treatments

Prolonged blood coagulation during exercise was once theorized to contribute to the severity of EIPH[18–21] by allowing a larger volume of blood to be extravasated from the pulmonary capillaries before effective sealing occurred.

However, previous studies suggesting compromised platelet function and/or coagulation in horses with EIPH were most likely flawed by classification of bleeders[1,2], effects of sample timing[23] and processing of samples[23], as well as by choice of anticoagulant in evaluating platelet function[24,50,51]. In fact, these studies in addition to the study of Kociba *et al.*[22] have refuted the dogma of diminished haemostatic function as an aetiological factor in EIPH, and, if anything, propose that horses are transiently hypercoagulable during exercise. In the current study, there was no difference in any of the measured coagulation variables between either of the treatment groups and the placebo horses.

When considering that enhanced coagulation is the major mechanism through which ACA and PRE are hypothesized to work, it is not surprising that negligible improvements in EIPH severity are seen with their use. Further evidence refuting the effectiveness of coagulation-enhancing agents for treating EIPH comes from Epp *et al.*[25] examining the effects of herbal formulations designed to reduce EIPH via enhanced platelet function/coagulation, in which no effect on [RBC] in the BAL fluid 30–60 min post-exercise was observed, despite a potentially shortened bleeding time.

Furthermore, ultrastructural observations suggest that capillary breaches are dynamic, meaning that once threshold pressure is achieved rupture occurs, the elevated pressure sustains haemorrhage and once the horse ceases to maintain the elevated pressure (stops exercising), the tear reseals and leakage of blood stops. This resealing occurs within 2–3 min of end-exercise, which is long before clotting is completed in the horse, and platelets are seen covering the endothelial blood gas barrier breaks[52,53]. However, conjugated oestrogens may be beneficial from this standpoint in that they have been theorized, after long-term administration, to decrease bleeding by strengthening the Type IV collagen in the basement membrane.

Limitations of the current study that could have impacted our ability to detect a statistically significant treatment difference included a small number of horses (half of which were relatively light bleeders), a single dose of PRE and ACA that may have been too low to elicit coagulation and EIPH alterations and the use of BAL. Therefore, a larger dose–response PRE study in heavy bleeders may be warranted. The data suggest that if the six horses evaluated herein are indeed representative of the entire horse population, the small effect of PRE on EIPH would require $n = 24$ to obtain statistical significance at the $p < 0.05$ level, and whether this effect would be more readily apparent in a larger study with heavier bleeders and larger doses remains to be determined. The limitations and advantages of the BAL technique have been discussed previously[11,33,54].

Anti-inflammatory effect with no impact on EIPH severity

In the human literature, there is evidence that ACA and conjugated oestrogens both have anti-inflammatory properties. In this respect, ACA acts via the prevention of complement-mediated release of cytokines and leukocyte activation[55,56], and conjugated oestrogens act by inhibition of expression and/or production of NF-κB, reactive oxygen species, cyclooxygenase-2 metabolism, pro-inflammatory cytokines and cell adhesion molecules, ultimately resulting in less neutrophil and monocyte recruitment and migration to the lungs[57–59]. Therefore, it was not surprising that a reduction in WBC was observed in the BALF of horses treated with ACA as well as with PRE.

The paradox that exists in this study is that inflammatory airway disease has previously been demonstrated to be an important contributor to the severity of EIPH[1,15,60–65]. Therefore, it is necessary to address why a reduction in EIPH did not follow a decrease in [WBC] in the BALF in the present investigation. One plausible explanation for this occurrence may be that both treatments to date, which have evidenced reductions in EIPH mediated via reductions in inflammation (i.e. CES and omega-3 fatty acids), have required a time span of week to months to become effective[15–17]. ACA and PRE are acute treatments that may not be in circulation long enough to reduce EIPH via the potential mechanism of enabling alveolar macrophages more rapidly to clear haemorrhage, which ultimately decrease the prolonged inflammatory response and scar formation. Oestrogen probably has time-dependent effects on inflammatory diseases. In some diseases, oestrogen delays the onset of disease but has no significant effect after onset[59]. Reductions in EIPH may be seen with prolonged

administration of these medications, but this would be expensive and would probably result in undesirable side effects.

Contraindications for using ACA for treating EIPH

With regards to EIPH, transient hypercoagulability during intense exercise[22–24,66–68,50,51] may be troublesome when combined with coagulation-enhancing drugs. Thrombi may form and may act to increase Ppa by lodging in the microvasculature and consequently increasing EIPH[69]. In fact, the tendency for EIPH to be increased with herbal treatments supports the theory that haemostatic enhancement may be detrimental to attenuation of EIPH[25]. Similar concerns have been raised in human medicine regarding risk of thrombotic complications (specifically pulmonary microthrombi) due to ACA's effect of inhibiting fibrinolysis without suppressing thrombin formation.

When considering performance ability, there was a trend ($p = 0.09$) for a decreased time-to-fatigue and an associated decrease in plasma lactate in horses treated with ACA. Lower plasma lactate makes sense as these horses consistently ran one stage less on ACA than either placebo or PRE trials, and the reduced plasma lactate may be a reflection of this decreased time-to-fatigue or an altered balance between lactate production and removal. These data corroborate anecdotal reports from track veterinarians indicating detrimental effects of ACA on performance.

Conclusions

Conjugated oestrogens and ACA are currently used on racetracks in an attempt to control EIPH. However, the scientific evidence herein does not justify their use. Specifically, neither of these treatment strategies significantly decreased EIPH, and there is a potential for negative sequelae such as microvascular thrombi, increased bleeding and even decreased performance (ACA). These results are extremely important with regards to official standardization and use decisions of scientifically proven race-day medications across race tracks nationwide by racing jurisdictions and the Racing Medication Testing Consortium.

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