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Original Research

Effects of Transportation on Redox Homeostasis and Tracheal Mucus

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ABSTRACT

The aim of this pilot study was to document the effects of transportation on markers of oxidative stress (OS) in blood, exhaled breath condensate (EBC), and saliva and to explore their relationships with transport-related increases in tracheal mucus. Twelve horses, six Standardbred, and six Thoroughbred, aging from 3 to 8 years, underwent an 8-hour journey during which they had no access to food or water. Clinical examinations and sampling of blood, EBC, and saliva were performed preloading, at unloading, 12 and 24 hours, and 5 days after journey. Concentration of oxidants (reactive oxygen metabolites [ROMs], advanced oxidation protein product [AOPP], ceruloplasmin [CP], hydrogen peroxide in the EBC) and antioxidants (plasma total antioxidant status [PTAS] and saliva total antioxidant status [STAS], glutathione) were determined, and the oxidative stress index (OSI = ROMs/PTAS × 100) was calculated. Respiratory endoscopy was performed at preloading and unloading, and tracheal mucus was scored. Oxidative stress variables were analyzed using proc mixed procedure with time as the fixed factor, and the variation in mucus score was analyzed by median test. The relationships between OS markers and mucus score were examined by linear regression analysis. Transportation caused a significant increase in tracheal mucus and in the concentrations of ROMs, AOPP, CP, PTAS, and STAS (all $P > .05$). Tracheal mucus was positively associated with ROMs and OSI ($R^2 = 57.8$, $P = .004$; and $R^2 = 70.3$, $P < .001$, respectively). However, animals did not experience OS, as reflected in the lack of changes in OSI. Overall, although the transported horses experienced oxidative and respiratory challenges, they were able to maintain redox homeostasis and did not develop clinical disease.

1. Introduction

Oxidative stress (OS) occurs as a result of failure of the homeostatic mechanisms regulating the balance of free radicals and antioxidants in the blood [1]. It has been reported that OS may occur as a result of increased free radical production (due to physical and/or psychological stress, inflammation, and infection) or decreased availability of antioxidants (due to decreased dietary intake or

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de novo synthesis of antioxidants, or increased antioxidant turnover) [1]. A free radical, also called oxidant, can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital [2]. The two major groups of free radicals are reactive oxygen species and reactive nitric species [3]. Antioxidants provide defence against oxidants and can be divided into three major groups: enzymatic antioxidants (e.g., glutathione [GSH]), nonenzymatic proteins (e.g., albumin), and nonenzymatic low-molecular-weight antioxidants (e.g., ascorbic acid, α-tocopherol) [1,3]. Direct or indirect measures of oxidants and antioxidants are commonly used to quantify OS. The quantity of oxidants in the blood can be measured by determining the plasma level of reactive oxygen metabolites (ROMs; an indicator of oxygen free radical production) or the concentration of proteins and lipid which have been oxidized (e.g., advanced oxidation protein products (AOPPs), malondialdehyde) [4]. The antioxidant status can be assessed by measuring concentrations of single antioxidants or the plasma total antioxidant status (PTAS) [4,5]. The oxidative stress index (OSI), defined as the ratio between ROMs and PTAS, has been proposed an useful tool to quantify OS [6,7]. Oxidants and antioxidants also can be measured in saliva and exhaled breath condensate (EBC) [8–11]. Oxidative stress may contribute to the development of disease including colic [3], laminitis [12,13], and respiratory disease [6,14–16].

Because of the link between physical or psychological stress, disease, and OS [3,17], variables associated with OS such as ROMs, PTAS, OSI, and AOPP have been proposed as useful biomarkers for the identification of stress and poor welfare and health in animals [1,18,19]. Evidence supporting this view comes from the observation that a relationship between transportation-related mental and physical stress and changes in PTAS and oxidants has been identified in transported horses [20–22]. An increase in PTAS is considered a stress-related physiological response because stressed animals mobilize antioxidants to balance the stress-induced increase in oxidative products [23–25].

Transport increases the susceptibility of horses to disease with the respiratory and gastrointestinal systems most commonly affected [26,27]. The observation that transportation results in increased tracheal mucus may explain the relationship between transport and respiratory disease [28–30]. As transportation causes an increase in oxidants, antioxidants, and tracheal mucus and given that the diseases associated with OS and transportation are similar, it is possible that OS associated with transport could be involved in the development of transport-related disease. The principal hypothesis to be tested in this pilot study was that horses transported for 8 hours without access to food or water would experience a disruption of the balance between oxidants and antioxidants in blood and that a correlation would exist between this disruption and the transport-related change in tracheal mucus.

The first aim of this study was to document the effects of transportation on markers of OS (ROMs, PTAS, OSI, AOPP, ceruloplasmin [CP] in blood, hydrogen peroxide in exhaled breath condensate [EBC H_2O_2], and saliva total antioxidant status [STAS]) and to explore their relationships with the transport-related increase in tracheal mucus. The second aim was to compare the results of EBC and saliva samples with results obtained from plasma samples to explore whether EBC and saliva could be collected to monitor transport-related OS in horses. Oxidative variables might become useful indicators to monitor health and welfare during transportation of horses.

2. Materials and Methods

2.1. Animals

Twelve horses (seven geldings and five mares; six Standardbred and six Thoroughbred), aged from 3 to 8 years (median 4), with mean body condition score (BCS) of 2.2 ± 0.4 [31], were recruited into this study. All were accustomed to handling and had previous experience of transportation. The study was approved by the Charles Stuart University Animal Care and Ethics Committee (Project Number 14/037).

2.2. Experimental Protocol

On day 1, all animals were clinically assessed, weighed, and dewormed by two research team members (B.P. and S.L.R.). Blood samples were collected, and the standard hematological analysis used by the Charles Sturt University veterinary clinic was performed. Blood cell counts and biochemical profiles (data not shown) for all horses were within the normal ranges reported by analyzing in the laboratory, so all horses were considered healthy and suitable for the experiment. The animals then underwent 2 weeks of acclimation. During the first week, the horses were kept on pasture. During the second week, the horses were kept in single boxes (4 × 4 m), with wood shavings as bedding. Except during transportation, they remained stabled for the rest of the experiment, spending at least 1 hour a day in a yard. They were fed lucerne hay and oats twice a day (8 AM and 6 PM) and had water ad libitum. The feed quantities were calculated individually to meet maintenance requirements [32]. During the acclimation period, all horses were handled by one of the researchers (B.P.) and underwent training for saliva and EBC sampling procedures once daily using positive reinforcement.

The horses were transported in consignments of six over two identical trips for 8 hours on different days, 48-hour apart. The horses traveled in the same 6-horse commercial truck (Freighter, Fuso, Mitsubishi, Japan), ventilated using venturi vents and louvres. The horses traveled in individual stalls (0.80 m width × 2.30 m length), in a sideways position, restrained by rubber cords. The cord was attached to the low ring of the head collar, so animals were able to turn their head and lower their head below wither height, enabling them to touch their carpus, but not the floor, with their nose. The truck was driven by the same driver, who had extensive experience of long distance horse transport. To comply with the Occupational Health and Safety Act [33], the driver took a 30-minute rest stop after 4 hours of driving. During the rest stop, the horses were not unloaded and the truck was parked in the shade. Horses were not offered food or water at any time during transport.