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Original Research

Acupuncture Needle Stimulation on Some Physiological Parameters After Road Transport and Physical Exercise in Horse

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ABSTRACT

The aim of this study was to evaluate the effect of acupuncture (AG) treatment on some hematochemical parameters in five Thoroughbred horses after road transport and exercise. Horses competed in two official races. For each race, animals were transported from their stables to the racetrack. Horses transported and competed in the first race represent the control group. Two weeks later, the same horses competed in the second race. Before road transport, they were treated with AG. From animals, blood samples were collected at rest (T_{PRE}), after unloaded (T_{POST}), 30 minutes after unloaded (T_{POST30}), at rest in the transit stall (R_{PRE}), at the end of the race (R_{POST}), and 30 minutes after the race (R_{POST30}). The effect of transport, exercise, and AG was evaluated on blood lactate, glucose, red blood cell, hemoglobin, hematocrit, mean corpuscular volume, and erythrocyte osmotic fragility (EOF) values. A significant effect of transport ($P < .05$) and exercise ($P < .01$) was found on all studied parameters in both groups. A significant effect of AG on lactate, glucose, and EOF values was found in transported ($P < .001$) and exercised horses ($P < .05$). The results found in this study showed that transport and exercise are potential stressors for the athlete horse that may affect its welfare and physical performance. The data suggest that AG stimulation promoted the increase of blood glucose values and the reduction of lactate and EOF levels suggesting its role in the improvement of the physiological adaptation to stressful stimuli and of physical performance of Thoroughbred horses.

1. Introduction

Domestic animals are routinely exposed to a variety of anthropogenic stressors. Stress can be defined as physiological response elicited when threat to homeostasis is perceived [1]. The increase in equestrian sports recently has led to a rapid increase in horse transported by road within and between countries [2]. The transport to the race site and race itself can be potential stressors for the athletic horse that may affect health status, welfare, and physical performance [3,4]. Potential stressors for horses during road transport may originate from physical factors such as stocking density, vibration from vehicle, arrangement of the animals, feed deprivation, and road conditions; psychological stressors, which include social regrouping or unfamiliar environment, ambient temperature; or combination of physical and psychological stressors [5,6]. Efforts

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spent by the transported animals for continuous postural adjustment constitute one of the stress factors [7,8]. It has been demonstrated that during road transport, physiological and psychological factors can disrupt homeostasis and metabolism in animals [1,2]. To limit health problems related to transport, it is important to examine the health status of the horses before and after the traveling to provide them with electrolytes and antioxidants and to optimize the environmental conditions inside the truck. Despite the rise of innovative techniques to evaluate musculoskeletal damage [9,10], the most frequently used means to assess threat to homeostasis is blood. In particular, hematology has been widely used to provide information about disease status, performance problems, and fitness in horses [11]. Transport causes oxidative stress and reduction of the content of endogenous antioxidants in the body that could increase the vulnerability of tissues and cellular components to oxygen reactive species [12,13]. Under stress conditions, such as exercise and transport, the alteration in lipid composition of the cell membrane due to blood pH changes and/or oxidative damage affects the cellular osmotic homeostasis and facilitates cellular dehydration. Therefore, the osmotic stress to erythrocytes during transport and/or exercise may increase their susceptibility to irreversible membrane damage and destruction compromising cellular functions [14]. Developing effective methods to help the athletes quickly recover from homeostasis disruption after transport, from muscle fatigue after exercise training or fierce competitions, and to help them achieve the best physiological situations is deemed essential, especially prior to competition. At this regard, Acupuncture, one of the Traditional Chinese medicine techniques, has been used to modulate the physical well-being of athletes [15]. Experiments show that acupuncture (AG) treatment leads to alleviation of muscle tension, improvement of local blood flow, increase of pain threshold, and modulation of autonomic nervous system [16,17]. Acupuncture has been used for treatment of injury, reduction of fatigue, and management of physical condition in athletic fields [15,18]. However, there are a few scientific studies on the physiological effects of AG treatment on the physical well-being of transported athlete horses [19,20].

Based on the hypothesis that stimulation of AG points may improve the recovery abilities of athletic horse after road transport and physical exercise, we aimed to study changes in some erythrocyte parameters, focusing on erythrocyte osmotic fragility test (EOF), in acupunctured Thoroughbred horses after road transport and physical exercise.

2. Materials and Methods

2.1. Animals and Study Design

All treatments, housing and animal care reported below were carried out in accordance with the standards recommended by the EU Directive 2010/63/EU for animal experiments. Five clinically healthy and regularly trained Thoroughbred horses (five gelding, mean age 4 ± 1 year; mean body weight 437 ± 15 kg) were used with the informed consent of the owners. Animals were stabled in individual boxes (3.5 × 3.5 m) at the same training center located in Sicily, Italy (38°00′49″N, 15°25′18″E, 80 m above sea level), under natural photoperiod (sunrise at 06:11 AM, sunset at 05:13 PM; mean temperature 23°C, relative humidity 70%). Horses were fed, twice a day (7 AM and 5 PM), a total food amount of about 2.5% of horse body weight (forage:concentrate ratio 70:30) and water was available ad libitum. The horses competed in two official 1,300-meter races at “Mediterraneo” racetrack (Siracusa—Sicily, Italy). For each race, the five horses were transported from their stables to the racetrack in a 5-horse truck. Each animal traveled, tethered with a 50-cm rope on each side of the halter, in an individual tie stall (length 2.3 m; width 0.85 m), giving a total space of about 2 m^2 and made two journeys of 145 km. Each journey was made 2 weeks after the previous one. The driver and the route were always the same. Horses transported and competed in first official race represent the control group (CG). Two weeks later, the same horses competed in the second official race. Before road transport, they were subjected to AG treatment and were loaded after 30 minutes onto the truck calmly and without the use of force. Acupuncture was performed once a day for 30 minutes across all animals. A stainless-steel needle (diameter 0.25 mm, length 30 mm, Dongbang Acupuncture Inc, Chingdao, China) was inserted vertically into a depth of 2 to 3 cm, and stimulation was produced by bidirectional twisting of needles, as described in previous studies (Hana et al., 2015; Lee et al., 2014). For AG stimulation, stainless needles were inserted into the left or right side of selected acupoints. The locations of acupoints were found according to the anatomical structures (Table 1). Thermal and hygrometric records throughout the experimental period were carried out inside the truck for the whole study by means of a data logger (Gemini, UK). Temperatures inside the truck ranged between 24°C and 21.5°C (maximum/minimum). The relative humidity inside the truck ranged between 68% and 75%.

2.2. Blood Sampling and Laboratory Analysis

From each animal, blood samples were collected by the same operator at rest in their stall at 08:30 AM (T_{PRE}), after being unloaded from the vehicle and housed in the transit stalls (T_{POST}), 30 minutes after unloaded (T_{POST30}), at rest in the transit stall (R_{PRE}), at the end of the race (R_{POST}), and 30 minutes after the race (R_{POST30}). Blood samples were collected by jugular venipuncture into vacutainer tube containing EDTA (Terumo Co, Tokyo, Japan) and tested for blood lactate, blood glucose, red blood cell (RBC), hemoglobin concentration (Hb), hematocrit (Hct), mean corpuscular volume (MCV), and EOF. The blood lactate and glucose levels were evaluated by means of a small handheld meter (Accutrend Plus, Roche Diagnostics, Switzerland). The RBC, Hb, Hct, and MCV values were measured by means of an automated blood cell counter (HeCo Vet C, SEAC, Florence, Italy). The determination of the EOF was performed using a natrium chloride (NaCl) solution prepared as described by Faulkner and King [21]. Particularly, 10 different concentrations of NaCl solution ranging from 0.0% to 0.9% were prepared. A set of 10 test tubes containing 10 mL of different