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Cortisol levels and leukocyte population values in transported and exercised horses after acupuncture needle stimulation

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ABSTRACT

Road transport and physical exercise represent stressful stimuli that can lead to homeostasis disruption with direct effect on health status, welfare, and physical performance of the athletic horse. Acupuncture is recognized as a practice modulating the physical well-being of athletes. In this study, the effect of acupuncture treatment on some hematochemical parameters was evaluated in 5 thoroughbred horses after road transport and exercise. Horses competed in 2 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 acupuncture (acupuncture group). 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 acupuncture was evaluated on cortisol concentration, white blood cell (WBC) count, and leukocytes population including lymphocytes, neutrophils, monocytes, eosinophils, and basophils. A significant effect of transport ($P < 0.01$) and exercise ($P < 0.001$) was found on cortisol, WBC, lymphocyte, and neutrophil values in both groups. Transported and exercised horses subjected to acupuncture treatment showed statistically significant lower cortisol, WBC, and lymphocyte values ($P < 0.01$). 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 acupuncture practice influences animal's psychological perception of a stressful condition, probably, by modulating the neural, immune, and endocrine control systems.

Introduction

Training, transport, and competition are the most important activities that sports horses undergo during their career and probably represent the major cause of injuries and health problems and of economic loss for horse breeding and industry. For that reason, a higher competence in this field could be useful for equine technicians. Effectively, transport and physical exercise represent stressful stimuli that can lead to homeostasis disruption with direct effect on animal health status and physical performance of the athletic horse (Hinchcliff et al., 2004). Horses are transported for different reasons, including competitions, breeding, pleasure activities, sales, and slaughtering. Travel includes handling, loading, transport in self, unloading, and often adaptation to a new environment; each of these phases affects horse physiology and behavior in a different way (Hinchcliff et al., 2004). Athlete horses are accustomed to travel before competitions but, after transport, some of them show lower performance than usual. Physiological explanations and proper guidelines about this issue could help owners or trainer to better manage this kind of situation, avoiding related poor performance's problems (Padalino, 2014). To limit health problems related to transport and exercise stress, it is

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important to examine the health status of the horses before and after the physical effort to optimize the environmental conditions inside the truck or to provide them with electrolytes and antioxidants. It is recognized that stressors as transport and physical exercise evoke the activation of hypothalamic-pituitary-adrenal axis and the sympathetic nervous system leading to glucocorticoids and catecholamines release, which in turn modulate the inflammatory reaction. Because the immune and endocrine systems interact with each other under a stressful condition to re-establish homeostasis of the organism (Etim et al., 2013), it is important to monitor immunologic and endocrine markers for the assessment of the physical well-being of athletes (Akimoto et al., 2003).

The scientific community is currently interested in studying innovative 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, especially before competition. One of the practices that has been used to modulate the physical well-being of athletes is the acupuncture (Miyamoto, 1997), one of the traditional Chinese medicine techniques. Acupuncture has been used for treatment of injury, reduction of fatigue, and management of physical condition in athletic fields (Karvelas et al., 1996; Miyamoto, 1997). It has been demonstrated that acupuncture treatment leads to alleviation of muscle tension, improvement of local blood flow, increase of pain threshold, and modulation of autonomic nervous system (Knardahl et al., 1998; Barlas et al., 2000). However, only few studies have been actually published on the influence of acupuncture treatment on the physical well-being of transported athlete horses (Angeli et al., 2008; Godoi et al., 2014; Rizzo et al., 2017). To test the hypothesis that stimulation of acupuncture points may improve the recovery abilities of athletic horses by modulating the stress and inflammatory responses, we studied the changes in serum cortisol levels, white blood cell (WBC) count, and leukocyte populations in acupunctured thoroughbred horses after road transport and physical exercise.

Materials and methods

Animals and study design

Five clinically healthy and regularly trained thoroughbred horses (5 gelding, mean age, 4 ± 1 years; mean body weight, 437 ± 15 kg) were enrolled in the study after the informed consent had been provided by 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 (07.00 AM; 05.00 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 2 official 1300-m races at Mediterraneo racetrack (Siracusa, Sicily, Italy). For each race, the 5 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 2 journeys of 145 km. The second journey was made 2 weeks after the first 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 acupuncture treatment (acupuncture group [AG]) and were loaded after 30 minutes onto the truck gently and without the use of force. Horses were subjected to acupuncture only once 30 minutes before being loaded on the truck. A stainless steel needle (diameter, 0.25 mm; length, 30 mm; Dongbang Acupuncture Inc., Chingdao, China) was inserted vertically into a depth of 2-3 cm, and stimulation was produced by bidirectional twisting of needles, as described in previous studies (Lee et al., 2014; Hana et al., 2015). In particular, Figure 1 shows the name and location of the selected acupoints received from AG. For acupuncture stimulation, stainless needles were inserted into the left side or right side of selected acupoints. The locations of acupoints were found according to the anatomical structures.

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 and 21.5°C (maximum/minimum). The relative humidity inside the truck ranged between 68% and 75%.

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 8-mL vacutainer tubes with cloth activator (Terumo Co., Tokyo, Japan) and into 2-mL vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA). Immediately after collection, blood samples were placed in refrigerated bags and transported to the laboratory for the analysis.

Blood samples collected into tubes containing clot activator were centrifuged at 3000 rpm for 10 minutes, and the obtained sera were analyzed within 2 hours to estimate the concentration of cortisol using enzyme-linked immunosorbent assay (ELISA) kit (cortisol [Horse] ELISA kit; Abnova, Walnut, CA) by means of a microtiter plate reader (EZ Read 400 ELISA; Biochrom, Cambridge, UK). All calibrators and samples were run in duplicate, and samples exhibited parallel displacement to the standard curve for the ELISA analysis. The assay sensitivity was 1 ng/mL. The intra-assay and interassay of variation were 6% and 6.8%, respectively.

EDTA whole blood samples were processed in the laboratory within 2 hours for the evaluation of WBC count and leukocyte identification and counting. The evaluation of WBC count was performed by means of an automated hematology analyzer (HeCo Vet C; SEAC, Florence, Italy). To leukocyte identification and counting, a manual analysis was performed on all whole blood samples. Two peripheral blood smears were performed for each sample and, after air drying, the obtained slides were stained through Dif-Stain kit (Titolchimica srl, Rome, Italy). The same laboratory professional has later performed the microscopic analysis of blood films by using an optical microscope (Nikon Eclipse e200; Nikon Instruments Europe BV, Amsterdam, The Netherlands). A manual 200-cell differential count was performed on each blood film. For each animal, the leukocyte differential count was calculated by averaging of the data recorded from each blood film of the same sample.

Statistical analysis

Data, expressed as mean values $\pm$ standard deviation, were tested for normality using the Shapiro-Wilk normality test. All data were normally distributed ($P > 0.05$), and the statistical analysis was performed. A general linear model for repeated measure was applied to assess significant effect of the experimental conditions (road transport, exercise, and acupuncture treatment) on studied parameters. When significant differences were found, Bonferroni post hoc comparison was applied. P values <0.05 were considered statistically significant.