Lipoic Acid as an Antioxidant in Mature Thoroughbred Geldings: A Preliminary Study1,2

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ABSTRACT α-Lipoic acid (LA) has demonstrated antioxidant effects in humans and laboratory animals. The objective of this study was to determine whether the effects of LA are similar in horses. Five Thoroughbred geldings were supplemented with 10 mg/kg/d of α-LA in a molasses and sweet feed carrier and five received only the carrier as a placebo (CON). Blood samples were obtained at baseline (0 d), after 7 and 14 d of supplementation, and 48 h post-supplementation (16 d). Blood fractions of red and white blood cells (RBC and WBC, respectively) and plasma were analyzed for glutathione (GSH), glutathione peroxidase (GPx) and total plasma lipid hydroperoxides (LPO). An experienced veterinarian observed no adverse clinical effects. Plasma LPO baselines differed between groups (P = 0.002). When covariates were used, there was a decrease over time in the LA group (P = 0.015) and concentrations were lower in the LA group than in the CON group at 7 and 14 d (P = 0.022 and P = 0.0002, respectively). At baseline, GSH concentration was 69 ± 7 in WBC and 113 ± 13 mmol/mg protein in the RBC, with no differences resulting from either time or treatment. The GPx activity was 47 ± 4 and 26 ± 5 U/g protein at baseline WBC and RBC, respectively, with a lower concentration in the LA group’s WBC at 7 (P = 0.019) and 14 d (P = 0.013). The results show that 10 mg/kg LA had no evident adverse effects, and moderately reduced the oxidative stress of horses allowed light activity. These findings encourage studying of LA in horses subjected to strenuous exercise.

KEY WORDS: oxidative stress, horse, lipid hydroperoxides, glutathione, dihydrolipoate

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4 Abbreviations used: α-TOC, α-tocopherol; CON, control supplemented group; DHLA, dihydrolipoic acid; GSH, glutathione; GPx, glutathione peroxidase; HBSS, Hank’s balanced salt solution; LA, lipoic acid; LPO, lipid hydroperoxides.
LIPOIC ACID AS AN ANTIOXIDANT

stress, aging and metabolic disease. Khanna et al. (9) compared rested and exercised rats supplemented with or without LA. The LA supplemented rats, both rested and exercised, had a higher GSH concentration, and a lower lipid peroxidation level measured by thiobarbituric acid reactive substances compared to those of nonsupplemented rats. During aging, mitochondria use oxygen inefficiently in ATP synthesis. Old rats supplemented with LA had a higher mitochondrial membrane potential, ambulatory activity, GSH and ascorbate concentration; furthermore, the malondialdehyde concentration was five times higher in the nonsupplemented rats (10). Lipoic acid was also used in humans to test its effectiveness on relieving diabetic symptoms (11). Three different doses of LA were compared to a placebo over a period of 19 d. After 5 d patients dosed with 1200, 600 or 100 mg LA had a greater decrease in the severity of their symptoms than patients given a placebo. Other human tests also show positive results when tested under different diseased states or illness conditions, including Alzheimer-type dementia (12).

Oxidative stress is evident in horses undergoing exhaustive and endurance exercise (13–15); this warrants the testing of antioxidant supplements. The objective of this study was to evaluate the safety and antioxidant potential of LA in horses undergoing light voluntary pasture exercise.

MATERIALS AND METHODS

Ten mature Thoroughbred geldings, 7 to 16 y of age, weighing 626.8 ± 8.3 kg, were used in this study. The protocol was approved by the institutional animal care and use committee and performed at the Virginia Tech Middleburg Agricultural Research and Extension Center.

The geldings were maintained on 30 acres of mixed grass/clover pasture during the month of January with ad libitum access to orchard grass/alfalfa hay. Five of the geldings were randomly assigned to the treatment group (LA) and received 10 mg/kg body weight L-α-lipoic acid (Sigma Chemical, St. Louis, MO) mixed into a carrier (70 g of molasses and sweet feed) offered by hand once/d at 1300 h for 14 d. The other five geldings were assigned to the control group (CON) and received the carrier as a placebo at the same time as the LA supplement.

Blood samples were collected by venous puncture in 10 mL sodium heparin–containing Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ) before supplementation (0 d), after 7 and 14 d of supplementation, and 48 h after termination of the supplement (16 d). Blood samples were taken at 1300 h and immediately processed into red blood cell, white blood cell and plasma aliquots.

For assays using erythrocyte lysate, 500 μL of whole blood was transferred into a microcentrifuge tube and centrifuged at 2500 g for 5 min at 4°C. The plasma was removed and discarded from the sample. Erythrocytes were resuspended in 1 mL ice-cold deionized water, then frozen at −80°C until analysis. For the determinations using white blood cells, theuffy coat, located at the interface of the red cell pellet and the plasma was removed after centrifugation of whole blood at 2500 × g for 5 min at 4°C, and transferred to a tube containing 10 mL of lysis buffer (0.15 M NH₄Cl, 0.01 M NaHCO₃, 0.03 M EDTA free acid). White blood cells were washed once in the lysis buffer to lyse the red blood cells, and then washed twice in Hank’s balanced salt solution (HBSS; Life Technologies, Carlsbad, CA). After the last wash, 1 mL HBSS was added to the pellet and mixed thoroughly, after which 0.5 mL was transferred into microtubes and frozen at −80°C until sample analysis. Plasma aliquots were prepared by centrifuging the Vacutainer tubes at 2500 × g for 5 min at 4°C, then transferring the plasma to microtubes, which were frozen at −80°C until sample analysis.

Red blood cell lysate and white blood cells were analyzed for total glutathione (Oxis Health Products, Portland, OR, Biotech GSH-420, kit no. 51023; interassay CV 7.0%, intra-assay CV 5.6%) and glutathione peroxidase (GPx; Oxis Health Products, Biotech GPx-340, kit no. 51017; interassay CV 4.2%, intra-assay CV 5.0%) using an OxyScan Automated Oxidative Stress Analyzer (Oxis Health Products). Total plasma lipid hydroperoxides (LPO; Oxis Health Products, Biotech LPO-560, kit no. 21025) were also analyzed using a spectrophotometer (interassay CV 3.0%, intra-assay CV 4.6%).

Data were summarized as means ± se. Baseline values for the two groups were compared by Student’s t-test and, when different, were subtracted from values at later times (this assumes a covariance of 1). The effects of time, treatment and time by treatment interaction were evaluated on the raw data or increments by analysis of variance with repeated measures using the general linear models procedures of SAS (16). Significance was inferred at $P < 0.05$.

RESULTS

An experienced veterinarian observed no adverse clinical signs, including gastrointestinal distress, in the group supplemented with LA. Total plasma LPO (Fig. 1) was 9.32 ± 0.78 μM for CON and 13.56 ± 0.45 μM for LA at baseline ($P = 0.0016$). The concentrations decreased over time in the LA group ($P = 0.015$) and were lower in the LA group than in the CON group at 7 and 14 d ($P = 0.022$ and $P = 0.0002$, respectively). There was also an effect of treatment and time by treatment interaction ($P = 0.031$). The area under the LPO curve was found to be greater ($P = 0.004$) for the LA group (96.9 ± 15.7 μM d⁻¹) than for the CON group (28.2 ± 7.1 μM d⁻¹). At baseline, the GSH concentration was 69 ± 7 and 115 ± 13 mmol/mg protein in the white and red blood cells, respectively, and was not significantly different between groups. Red blood cell GSH tended to increase with time ($P = 0.074$) and white blood cell GSH increased with time ($P = 0.017$) and tended to increase with treatment ($P = 0.071$). There were no significant interactions between time and treatment for either red blood cell or white blood cell GSH. The GPx activity in the white blood cells (Fig. 2) was 50.6 ± 1.6 for CON and 43.6 ± 033 mmol/mg protein for LA at baseline ($P = 0.041$). The change in concentration was higher in the LA group at 14 ($P = 0.019$) and 16 d ($P = 0.013$) than in the CON group. The red blood cell GPx showed no differences for time or treatment.

DISCUSSION

The results showed that LA had antioxidant effects on horses, similar to that shown in other species (1). Our study raises the possibility that LA may have potential benefits in horses during periods of oxidative stress, such as endurance...
exercise (13). Oral supplementation of LA at 10 mg/kg daily did not result in any adverse signs over a 14-d period.

The dose of 10 mg/kg body weight was chosen by referring to LA’s effectiveness in other species. It is effective in the rat at a dose of 10 to 100 mg/kg or about 40 to 200 mg/kg (9,10) and in humans at a dose of 600 to 1200 mg (about 8.5 to 17 mg/kg) or about 25 to 50 mg/kg (11). It caused gastrointestinal distress, however, at the high dosage (11). Our hypothesis was that a dose of 10 mg/kg or about 50 mg/kg (9,10) would diminish oxidative stress in horses after being supplemented for 14 d, which is the average duration studied in other species, without causing any clinical signs of toxicity.

In humans LA was used in 260 non-insulin-dependent diabetic patients to determine whether it relieved symptoms of diabetic neuropathy including pain, burning, paralysis and numbness (11). Three different doses of LA were compared to a placebo over a period of 19 d. Patients were tested and symptom score was assigned a total symptom score by a physician three times during this period for improvements in neuropathic symptoms and expectations of time by treatment interaction (P < 0.001) and treatment (P < 0.033) and a significant time by treatment interaction (P = 0.031). Data are shown as means ± SE.

**FIGURE 2** White blood cell (WBC) glutathione peroxidase (GPx) for horses receiving the control supplement (CON; n = 5) or lipoic acid–containing supplement (LA; n = 5). The white blood cell GPx baselines are subtracted from 7-, 14- and 16-d samples assuming a covariance of 1. There were significant effects of time (P < 0.001), and treatment (P = 0.033) and a significant time by treatment interaction (P = 0.031). Data are shown as means ± SE.

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