African Journal of Biochemistry Research Vol.3 (7), pp.282-287, July, 2009
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 © 2007 Academic Journals

Full Length Research Paper

Influence of road transportation during hot summer conditions on oxidative status biomarkers in Iranian dromedary camels (*Camelus dromedarius*)

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Accepted 7 July, 2009

Transportation causes stress in livestock that may alter numerous physiological variables with a negative impact on production and health. The objective of the current study was to investigate the effects of road transport on oxidative stress biomarkers in camels. Ten Iranian dromedary camels were selected and subjected to a journey of approximately 300 km in a truck by road in August 2008. Blood samples were collected immediately before loading at 8:30 A.M., after 1 h transportation, at 9:30 A.M., and at the end of the journey after unloading at 1:30 PM. Final blood sample was taken 24 h after arrival. Plasma concentrations of malondialdehyde and α-tocopherol, erythrocyte superoxide dismutase and whole blood glutathione peroxidase activities were measured using validated methods. The mean concentration of MDA (1.87 ± 0.26 nmol/mL) and glutathione peroxidase activity (297.86 ± 25.68 U/g Hb) in basal pre-transport conditions show significant increase 24 h after arrival. The mean concentration of α-tocopherol (5.22 ± 0.74 µmol/L) and superoxide dismutase activity (1742.5 ± 74.36 U/g Hb) in basal pre-transport conditions had no significant change during and after transportation. Results suggest that transport stress causes an oxidative challenge in dromedary camels and represent novel biomarkers for stress-associated disease susceptibility and welfare assessment. However, further research efforts should be directed towards understanding the role of particular antioxidants and oxidants on the stressful conditions.

Key words: Dromedary camel, road transportation, oxidative status, malondialdehyde, α-tocopherol, glutathione peroxidase, superoxide dismutase.

INTRODUCTION

The dromedary camel is one of the most important domestic animals in the arid and semi arid regions as it is equipped to produce high quality food at comparatively low costs under extremely harsh environments (Yagil, 1982; Yousif and Babiker, 1989). It can survive well on sandy terrain with poor vegetation and may chiefly consume feeds unutilized by other domestic species (Shalah, 1983). Camel husbandry in Iran is almost localized in southern provinces, while its slaughter and meat consumption is done in most parts of this country. Sale and slaughter are the most usual reasons for transporting camels in Iran.

Farm livestock experience a variety of stressors that can modify normal behavior and growth, leading to losses in performance. Transport is a critical phase in animal production and utilization and often considered as one of the main causes of stress raising considerable interest, both in economic and animal welfare terms (Mormede et al., 1982). During transport, animals are exposed to a variety of potential stressors such as motion of the vehicle, noise, vibrations, centrifugal forces, rapidly changing light conditions, heat, cold, poor air quality, deck height, mixing of unfamiliar groups, poor road conditions and the possible lack of water and feed (Parrott et al., 1998 ; Broom, 2000; Hurtung, 2003). Animal health can be impaired by various pre-transport and transport conditions and may cause injury, reduce performance and can promote the development of diseases in animals (Hurtung, 2003). In addition, the time between removing animals from farms to the slaughter house has a major impact on meat quality (Kadim et al., 2006). Stress is directly related to lipid oxida-

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tion in muscle (McClelland, 2004) and the ratio and the proportions of oxidation in meat are also influenced by pre-slaughter (example, stress) and post-slaughter events (Linares et al., 2007). Signs of transportation stress have been demonstrated in different animal species by, for example, increased adrenal cortical activity (Ruiz-de-la-Torre et al., 2001), decreased humoral immunity (Machenzie et al., 1997), increased morbidity and mortality due to infectious diseases in the few weeks following transportation (Hurtung, 2003; Chirase et al., 2004). Increased susceptibility for infections such as pneumonia following transportation is well documented in animal species such as cattle (Knowles 1999; Hurtung 2003; Chirase et al., 2004), sheep (Hurtung, 2003), goat (Kannan et al., 2000), horse (Smith et al., 1996; Knowles, 1999) and camel (Wernery and Kaaden, 2002). Although there are many factors affecting animal susceptibility during these times, vulnerability may be traced back to biochemical processes within the body.

Oxidative stress resulting from increased production of free radicals and reactive oxygen species (ROS), and/or a decrease in antioxidant defense, leads to damage of biological macromolecules and disruption of normal metabolism and physiology (Trevisan et al., 2001). When reactive forms of oxygen are produced faster than they can be safely neutralized by antioxidant mechanisms, oxidative stress results. These conditions can contribute and/or lead to the onset of health disorders in animals (Miller et al., 1993; Chirase et al., 2004). Oxidative stress has been implicated in the pathophysiology of transport-related maladies (McBride et al., 2001; Chirase et al., 2004; Pregel et al., 2005; Urban-Chmiel, 2006; Wernicki et al., 2006; Burke et al., 2009). Diminished antioxidant defenses or excess oxidative species resulting from transport stress may be deleterious to tissues, and may be linked to the manifestation of disease. Stress of any origin is capable of depleting the body's antioxidant resources (Sconberg et al., 1993). Serum concentrations of the antioxidant vitamin E are reduced in transported steers, and exposure to simulated dust storm after being transported further decreases these concentrations (Chirase et al., 2001). Vitamin E addition to receiving diets seemed beneficial for increasing average daily gain and decreesing shipping fever mortality (Galyean et al., 1999), highlighting the importance of antioxidants to health. Physical and psychological stressors have an influence on the increase of catabolic reactions, which may cause an increase of ROS production (Wernicki et al., 2006). Concentration of ROS is increased in white blood cells isolated from calves after shipping, likely as a result of enhanced respiratory burst (Wernicki et al., 2006). Chirase et al. (2004) observed decreased serum total antioxidant capacity in transported calves. Similarly, Pregel et al. (2005) surmised that total antioxidant status of serum was a useful tool for measuring stress in transported calves. Moreover, heat stress has been recognized as one of the most common problems encountered during road transportation of livestock (Mitchell and Kettlewell, 1998) and contributes to transportation-induced stress during summer months (Stull and Rodiek, 2000).

It is well established that different animal species and even animals of the same species but different genetic background respond differently to the same stressor (Hall et al., 1998). No reference is available about oxidative changes under stressful conditions in dromedary camels. As camels are normally raised and thrive under harsh environmental and dietary conditions, we thought it worthwhile to assess if these desert animals would respond differently to the stressful stimulus of transportation. The objective of the study reported here was to determine the effect of transportation under hot summer conditions on oxidative stress biomarkers in Iranian dromedary camels. Plasma malondialdehyde (MDA) concentration was measured because MDA is one of the end-products of lipid peroxidation, and the extent of lipid peroxidation is most frequently measured by estimating MDA levels (Lata et al., 2004). Estimating the activities of enzymatic antioxidants, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), is another means of evaluating oxidative stress (Kleczhowski et al., 2003). α-tocopherol measurement also was done because of its antioxidant properties.

MATERIALS AND METHODS

Animals and transportation

The 10 clinically healthy Iranian dromedary camels (*Camelus dromedarius*), 5 males and 5 females, ranging in age from 3 - 4 years and weighing about 300 kg were selected for the study. The camels had been reared at the Camel Research Institute in Yazd province of Iran, which is supervised by an experienced veterinarian. Preliminary procedures (handling, physical restraint, loading, and unloadding) were undertaken by the same staff and blood sampling was always carried out by the same operator. The journey took place on one day in August 2008. Transportation of the camels was conducted between Camel Research Institute in Baphgh to Yazd and back to Institute, on smooth roads in an open truck which took 5 h (about 300 Km). Stocking density was about 1 m^2 per animal. Environmental temperature and relative humidity during the journey was 32 - 36°C and 17 - 25%, respectively. The camels had similar feeding and watering conditions *ad libitum* before and after the journey. During the journey there was no feed, water or unloading for rest.

Blood sampling and processing

Blood samples were collected immediately before loading, at 08:30 am (T1), after 1 h transport, at 09:30 am (T2) and immediately after transport termination and unloading, on arrival at Institute (T3) at 01:30 pm. Final blood sample was taken 24 h after arrival (T4). Blood samples were collected by jugular venepuncture into vacuum containers containing EDTA (Becton Dickinson, NJ, USA). The blood tubes were placed on ice until laboratory arrival (<2 h). The samples were centrifuged at 750 g for 20 min, and then the plasma was pipetted into different aliquots and stored at -70°C until analysis for plasma content of MDA and α-tocopherol. Haemoglobin concentration was measured by Cyanmethaemoglobin method. SOD activity was measured by a modified method of iodophenyl nitrophenol phenyltetrazolium chloride (RANSOD kit, Randox Com United Kingdom). This method employs xanthine and xanthine oxidase

Table 1. Mean (± SE) of circulating oxidative status indices in female and male dromedary camels(*n*=5 in each gender) before transport (T1), 1 h after transport initiation (T2), on the end of transportation (T3) and 24 h after arrival (T4).

Parameter*		T1	T2	T3	T4
Plasma malondialdehyde	Female	1.67 ± 0.24	1.62 ± 0.36	1.95 ± 0.11	2.12 ± 0.27
(nmol/mL)	Male	2.07 ± 0.49	1.72 ± 0.51	2.16 ± 0..39	2.35 ± 0.28
	Total	1.87 ± 0.26 [a]	1.67 ± 0.29 [a]	2.06 ± 0.19 [a,b]	2.23 ± 0.21 [b]
α-tocopherol	Female	5.51 ± 1.28	4.43 ± 0.95	4.32 ± 0.74	5.18 ± 0.98
(µmol/L)	Male	4.94 ± 0.92	5.2 ± 0.83	4.11 ± 0.97	3.56 ± 1.09
	Total	5.22 ± 0.74	4.8 ± 0.6	4.22 ± 0.57	4.37 ± 0.74
Glutathione peroxidase	Female	317.52 ± 38.71	242.6 ± 33.68	279.86 ± 27.42	343.84 ± 28.96
(U/g Hb)	Male	278.2 ± 35.73	297.24 ± 29.11	301.44 ± 21.16	322.14 ± 25.44
	Total	297.86 ± 25.68 [a]	269.92 ± 22.88 [a]	290.65 ± 16.72 [a]	332.99 ± 18.53[b]
Superoxide dismutase	Female	1837 ± 82	1436 ± 90	1950 ± 40	1823 ± 116
(U/g Hb)	Male	1648 ± 117	1740 ± 146	1810 ± 173	1814 ± 97
	Total	1742 ± 74	1588 ± 96	1880 ± 87	1819 ± 71

*No significant difference was observed in any parameter between sexes at all sampling times. Time □ gender interaction was not significant for all parameters ($P < 0.05$). [a,b]Mean (± SE) in each row with no common superscript differ significantly ($P < 0.05$).

(XOD) to generate superoxide radicals which react with 2-(4- iodophenyl)-3-(4-nitrophend)-5-phenyltetrazolium chloride (INT) to form a red formazan dye. The superoxide dismutase activity was then measured by the degree of inhibition of this reaction. One unit of SOD was considered a 50% inhibition of reduction of INT under the condition of the assay. Glutathione peroxidase activity (GSH-Px) was measured by the method of Paglia and Valentine (1967) (RANSEL kit, Randox Com, United Kingdom). GSH-Px catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidised glutathione (GSSG) is immediately converted to the reduced form with a concomitant oxidation of NADPH to $NADP^+$. The decrease in absorbance was measured at 340 nm by UV-visible spectrophotometer (Pharmacia LKB Biochrom, England). Total MDA concentration of the plasma was measured by reverse phase high-pressure liquid chromatography with UV detection at 310 nm after derivatisation with 2, 4-dinitrophenylhydrazine as described by Pilz et al. (2000). α-tocopherol content of plasma was measured in deproteinized, hexane-extracted plasma using reverse phase high-pressure liquid chromatography with UV detection at 295 nm (Solichova et al., 2003).

Statistical analysis

The data are presented as mean ± standard error (SE). A two way (time, gender) repeated measures analysis of variance was applied for statistical analysis. The level of significance was set at $P < 0.05$. Significances between means were assessed using the least-significant-difference procedure. All calculations were performed using SPSS/PC software.

RESULTS

The mean ± SE of the measured parameters of oxidative status in Iranian dromedary camels subjected to road transportation are presented in Table 1. Mean concentration of MDA increased significantly 24 h after arrival. No significant change was observed in plasma concentrations of α-tocopherol during and after transportation. Erythrocyte glutathione peroxidase activity showed a significant increase 24 h after arrival. The increase in superoxide dismutase activity on arrival and 24 h later was not significant. No significant difference was observed in any parameter between sexes at all sampling times. Time × gender interaction was not significant for all studied parameters.

DISCUSSION

The transportation stress can be viewed as a combination of a number of concurrent stressors that are likely to operate during and around transportation, and which exert both ‘‘physical’’, ‘‘physiological’’ and ‘‘psychological’’ stressful stimuli (Knowles, 1998). Oxidative stress may exacerbate psychological and physiological demands brought about by stressful conditions. Oxidative stress, involving an imbalance between the production of free radicals and the capability of an organism to absorb their excess, has been proposed to play a role in the pathogenesis of several infectious diseases of domestic animals (Miller et al., 1993; Lykkesfeldt and Svendsen, 2007). Oxidative stress is extremely dangerous because it does not exhibit any symptoms and is recognizable with great difficulty by means of common methods of analysis. Oxidative stress promotes the insurgence of serious pathologies as a result of the degenerative damage of cellular structures (Freidovich, 1999; Matsuo and Kaneko, 2000; McCord, 2000). Measure of oxidative stress allows es-timation of the real status of physiological defense and prevention of the appearance of correlated pathologies (Piccione et al., 2007). The inevitability of livestock transport makes stress associated with transportation an appropriate field of focus. Physiological stress due to transportation or inappropriate housing elevates oxidative stress in calves as measured by plasma ascorbate levels or serum total antioxidant capacity (Cummins and Brunner, 1991; Tyler and Cummins 2003; Chirase et al. 2004; Pregel et al. 2005; Wernicki et al., 2006).

Lipid peroxidaion is a general mechanism whereby free

radicals induce tissue damages, and implicated under several diverse pathological conditions (Halliwell and Chirico 1993). Malondialdehyde (MDA) has been widely applied as the most common biomarker for the assessment of lipoperoxidation in biological and medical sciences (Bird and Draper, 1984; Suttnar et al., 2001). MDA concentration in the present study at basal pre-transport state was lower than values (30.44 ± 2.89 µmol/L) obtained by Mohamed (2008) in healthy dromedary camels. The mentioned difference may be due to different methodologies utilized in two trials. In the present experiment significant increase in plasma MDA concentrations was observed 24 h after transport termination. Our finding is in conformity with the reported results in cattle (Chirase et al. 2004; Wernicki et al. 2006). Chirase et al. (2004) found that serum MDA concentrations in calves tripled after transportation. Wernicki et al. (2006) reported large increases in plasma thiobarbituric acid reactive substances on the first 3 days after transportation, with a gradual decline on the sixth day, and a return to baseline levels on the ninth day post-transport. Oxidative stress associated with cattle transport has also been evidenced by excessive accumulation of leukocyte lipid oxidation products (Urban-Chmiel, 2006). In horses, after the transportation and training exercises a significant increase of lipid peroxidetion was observed (Ishida et al. 1999). Burke et al. (2009) fail to detect any increase in plasma MDA 7 days after transport in beef calves and reported a decline in MDA concentration 7 days after transport. The week of recuperation time given to steers in that experiment may have been sufficient for recovery from oxidative insult and return to normal redox balance.

Higher concentrations of MDA in dromedaries after transportation under hot temperatures may be explained by higher levels of glucocorticoids and adrenaline-induced pathways of aerobic energy production associated with stress, which generate reactive oxygen metabolites and thus lipid peroxidation (Freeman and Crapo, 1982; Nockels et al., 1996). Glucocorticoids, as the final effectors of the hypothalamic–pituitary–adrenal (HPA) axis, participate in the control of whole body homeostasis and the organism's response to stress. In mammals, lipid peroxidation was induced while the nonenzymatic antioxidant capacity was decreased, and enzymatic antioxidant systems were suppressed in the liver (Ohtsuka et al., 1998), erythrocytes (Orzechowski et al., 2000), skeletal muscle and lymphoid organs (Pereira et al., 1999) after administration of glucocorticoid hormones. Furthermore, presence of heat stress conditions in the present study, in addition to other stressful stimuli, can induce the metabolic changes that are involved in the induction of oxidative stress. Heat stress can enhance the formation of ROS and induce oxidative stress in cells (Flanagan et al., 1998; Lord-Fontaine and Averill-Bates, 2002) and intact animals (Hall et al., 1994; Harmon et al., 1997; Bernabucci et al., 2002; Lin et al., 2006).

Antioxidants are implied in the inactivation or transformation of oxidants, which can either be transformed by antioxidant enzymes into less reactive forms or which can react with antioxidant molecules that are chemically stable.α-tocopherol is considered to be the most biologically active of several forms of vitamin E. It is an effective lipophilic antioxidant which protects lipid membranes against peroxidation (Solichova et al., 2003). Cattle that were stressed or were given a stress treatment of ACTH and epinephrine injections were found to have reduced α-tocopherol concentrations in their plasma, neutrophils, and red blood cells (Sconberg et al., 1993). After stress cattle may have reductions in α-tocopherol concentrations in certain tissues. Supplemental vitamin E may be required after stress to restore α-tocopherol in tissues (Nockels et al., 1996). The importance of antioxidant status during shipping and receiving can be further substantiated by work demonstrating decreased average daily gain and increased bovine respiratory disease in conjunction with decreased post-transport concentrations of serum vitamins A and E (Chirase et al., 2001). Based on our results no significant difference was observed in plasma α-tocopherol concentration during and after transportation in dromedary camel. However, caution should be observed in interpreting vitamin E status based solely on plasma α-tocopherol values, because of plasma vitamin E values may not accurately reflect body status of vitamin E (Sconberg et al., 1993; Nockels et al., 1996).

Antioxidant enzyme levels are sensitive markers of oxidative stress. Both increased and decreased antioxidant enzyme levels have been reported in different conditions as a consequence of enhanced ROS production either by up-regulation of enzyme activity or utilization of the antioxidant enzymes to counter the ROS. Superoxide dismutase (SOD) is a metalloenzyme which catalyses the dismutation of $O_2^{\bullet-}$ into O_2 and H_2O_2. It is important in the antioxidative defense mechanism and protects against lipid peroxidation (Halliwell and Chirico, 1993; Miller et al. 1993). Erythrocytes, due to their role and their propensity to generate radical species, may be considered as sensitive and intermediate cells in oxidative reactions (Sato et al., 1998). Various kinds of stressors increase lipid peroxidation levels and therefore SOD activity (Gaal et al., 1993; Lata et al., 2004). The higher erythrocyte SOD activity attributed to elevated temperatures during summer months has been reported in cattle (Bernabucci et al., 2002). At the present study no significant increase was observed in erythrocyte SOD activity due to road transporttation stress. This could be due to the fact that we mea-sure erythrocyte SOD activity, and perhaps whole blood SOD activity might be a better measure.

The seleno-enzyme glutathione peroxidase, as one of the primary antioxidant enzymes, contributes to the oxidative defense of animal tissues by catalyzing the reduction of hydrogen and lipid peroxides (Arthur 2000). GSH-Px functions in cellular oxidation-reduction reactions to protect the cell membrane from oxidative damage caused by free radicals (Flohe et al., 1973). Whole blood G-SH-Px activity at basal conditions reported here is in line with the data reported for the dromedary camel by Corbera et al. (2001). GSH-Px ac-

tivity show significant increase 24 h after arrival in comparison with previous activity concentrations and this is different from results of Burke et al. (2009) that reported GSH-Px activity does not fluctuate appreciably in calf leukocytes due to two-stage weaning and subsequent transport. This difference might be due to different sampling schedule because in the mentioned trial sampling was done 7 days post-transport and this time might be sufficient for recovery from oxidative damage as Wernicki et al. (2006) showed a post-transport decline in lipid oxidation on the sixth day. We speculate that the significant increase in GSH-Px activity might be an indirect compensatory response of cells to increased oxidant challenge due to stressful stimuli of transportation under hot temperatures. Increased GSH-Px activity attributed to elevated temperatures during summer has been reported in cattle (Bernabucci et al., 2002). In humans, GSH-Px activity increases after long-term exercise training regimens, a response which may be an adaptive mechanism against physical stressors (Tessier et al., 1995; Tauler et al., 2006). Stabel et al. (1989) reported that signs of morbidity coincided with elevated plasma GSH-Px in weaned and transported calves challenged with *Manhemia hemolytica*.

In line with the data reported for other species mentioned above, our findings show significant increased concentrations of plasma MDA due to transportation stress in dromedaries which, in parallel with increased whole blood GSH-Px activity supported the hypothesis that transport stress represents an oxidative challenge for livestock. These findings suggest novel biomarkers for stress-associated disease susceptibility and welfare assessment. Better understanding of the mechanisms of ROS production and further investigation of their effects on the stress-related maladies are essential to obtain new insight into this issue and eventually develop new diagnostic, prophylactic and therapeutic strategies. However, further research efforts should be directed towards understanding the role of particular antioxidants and oxidants on the stressful conditions because of this fact that susceptibility to disease is enhanced when physiological stress increases oxidative stress. In the other hand, because of the potential effects of lipid peroxidation on tissue types, it would be interesting and economically relevant to find out whether plasma MDA concentrations are related to carcass characteristics of meat animals.

ACKNOWLEDGMENT

The authors gratefully acknowledge the staff of the Camel Research Institute for their kind helps in this study.

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