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Short communication

Oxidative stress associated with road transportation in ewes

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ABSTRACT

The influence of road transportation on reactive oxygen species (dROMs), antioxidant barrier (Oxy-adsorbent) and thiol antioxidant barrier (SHp) was evaluated in 20 healthy Comisana ewes. The animals were divided into two groups: A ($n = 10$), the experimental group, was transported by road for 6 h over a distance about 490 km with an average speed of 80 km/h, and B ($n = 10$), the control group, was not subject to road transportation. Blood samples were collected via jugular venepuncture before and after the road transportation as well as after 8, 12, 24 and 48 h post-transport. Two-way repeated measure analysis of variance (ANOVA) was performed to determine the effects of sampling time, the differences between treatments, and the interaction between Time and Treatment. There was a significant Time × Treatment interaction for all oxidative stress parameters ($P < 0.05$), indicating that time course of the studied parameters differed across the treatment Groups. In Group A, there was an increase due to the effect of sampling time on dROMs ($P < 0.05$), Oxy-adsorbent ($P < 0.05$) and SHp ($P < 0.05$). Road transport caused an increase in catabolic reactions, which may cause an increase in reactive oxygen species and anti-oxidant substances. In conclusion, the transport has negatively affected the oxidant/antioxidant status in ewes showing that is very stressful and constitutes a crucial welfare and economic problem to animals and farmers.

1. Introduction

Oxidative stress in veterinary medicine and particularly in ruminant health is a relatively recent field of research (Celi, 2010, 2011). Oxidative stress occurs when the antioxidant defence system is overwhelmed by an increased oxidant burden or a reduced antioxidant supply (Kirschvink et al., 2008). It is know that a stressful condition leads to the imbalance between oxidants and antioxidants in favour of oxidants at the cellular or individual level (Khadija et al., 2009). The alteration of oxidative balance, if not adequately restored by the antioxidant barrier, induces an oxidative stress with cellular damage (Trevisan et al., 2001), which makes the organism sensitive to serious degenerative diseases (McCord, 2000). Measure of oxidative stress allows estimation of the real status of psychological defence and prevention of the appearance of correlated pathologies (Piccione et al., 2007). Various studies were carried out to monitor the oxidative stress parameters in ewes when the homeostasis is altered in this species (Piccione et al., 2006, 2008). For example, during road transportation the animals are exposed to a variety of potential stressors which can be highly stressful and compromise welfare causing severe modifications (Hurtung, 2003; Zhong et al., 2011). Several researchers have confirmed that transportation for short or long periods can impose a variety of physical and psychological stimuli that disrupt homeostasis and metabolism of different animal species by, for example, increases in the activity of enzymes and hormones (Adenkola and Ayo, 2010), changes in heart

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rate (Waran et al., 1996), mobilisation of energy and protein metabolism (Todd et al., 2000), increased adrenal cortical activity (Ruiz-de-la-Torre et al., 2001), a challenged immune system (Early and O'Riordan, 2006) and a positive response of acute phase proteins (Giannetto et al., 2011). Psychological stress due to road transportation or inappropriate housing elevates oxidative stress in ewes as measured by serum total antioxidant capacity (Pregel et al., 2005). The plasma/serum ability to oppose the massive oxidative action of a hypochlorous acid solution is evaluated by means of the oxy-adsorbent test (Oxy-adsorbent), and a significant component of the plasma/serum barrier to oxidation is thiol. It is to oppose the propagation step of peroxidative processes by inactivating either alcohoxyl or hydroxyl radicals (SHp test). The effect of road transport on oxidative status biomarkers has been evaluated only in horses (Onmaz et al., 2011) in dromedary camels (Nazifi et al., 2007) and in goats (Idrus et al., 2010) showing that transportation may cause injury, reduce performance, cause increased morbidity and mortality rate and consequently substantial economic losses due to loss of live weight and poor meat quality, too (Minka and Ayo, 2009). The aim of the present study was to evaluate the influence of the road transportation on reactive oxygen species (dROMs), Oxy-adsorbent and SHp, in order to improve animal health and welfare and meat quality.

2. Materials and methods

2.1. Animals

The study was carried out on 20 non-pregnant, clinically healthy Comisana ewes during the mild-dry season of March–May in Sicily (Italy). The animals were divided into two groups: Group A ($n = 10$, aged 3–4 years old, average LW 55.40 ± 3.6 kg) was transported by road for 6 h over a distance of approximately 490 km at an average speed of 80 km/h, and Group B ($n = 10$, aged 3–4 years old, average LW 55.80 ± 4.2 kg), the control group, was not subject to road transportation. The animals of Group B were located at the destination site of transported sheep in the same environmental conditions.

2.2. Procedures

Before the road transportation, all ewes underwent individual clinical examinations in order to exclude ewes with any clinical signs of diseases. All haematological and haematochemical parameters measured were within the physiological range for Comisana ewes (Jackson and Cockcroft, 2002; Kaneko et al., 1997). Group A were transported by road for 6 h over a distance of 490 km involving a combination of road surfaces ranging from small country lanes (5 km), secondary roads (40 km) to motorways (445 km). They had no previous experience of road transport. All animals were transported in accordance with the Directives 64/432EEC and 93/119EC (Council Regulation). Temperature and relative humidity inside the vehicle during the transportation ranged from 23 to 25 °C and 80 to 84%, respectively. For all of the experimental period temperature and relative humidity were continuously recorded with a data logger positioned in the pen (Gemini, Chichester, West Sussex, UK). The animals were transported at an ambient temperature of 21 °C and relative humidity of 68% at loading time and during the first stage of the transport, and at 20 °C and 73% in the final stage of the transport and unloading. After the transport the animals were confined to paddock where they were fed hay (2 kg), wheat straw (1 kg) and wheat concentrate (0.5 kg). Water was available *ad libitum*. All animals were fed with the same power before, during and after the experimental period.

2.3. Blood sampling and analysis

For all ewes, blood samples were collected via jugular venipuncture into vacutainer tubes with no additive immediately before and after the road transportation, and 8, 12, 24 and 48 h post-transport. All samples were centrifuged at 3000 × g for 20 min and the serum obtained were immediately analyzed by means of a UV spectrophotometer (model Slim SEAC, Firenze, Italy) for the assessment of dROMs, Oxy-adsorbent and SHp. These techniques are based on the "spin traps" system, molecules which react with free radicals, creating complexes revealed by spectrophotometry. The dROMs test (Alberti et al., 2000) is a colorimetric test that is based on the capacity of transition metal ions to generate in vitro alkoxyl and peroxyl radicals in the presence of hydroperoxides (R-OOH). A chromogenic reagent (N,N-diethylparaphenylen-diamine) is then added to this solution. This chromogen possesses the feature of being oxidized by hydroperoxyl and alkoxyl radicals and transformed into a pink to red coloured cation. The concentration of the coloured complex is directly related to the R-OOH levels of the sample. The concentration of ROMs, that directly parallels with colour intensity, is expressed as Carratelli Units (1 CARR $U = 0.08$ mg% hydrogen peroxide). Increased values directly correlate to increased levels of oxidative stress. The oxy-adsorbent test (Gerardi et al., 2002) evaluates the ability of plasma to oppose the massive oxidant action of an excess of hypochlorous acid (HClO) in water solution by assessing photometrically the residual unreacted radicals of the acid. The sample is subjected to massive oxidation by HClO; the antioxidant substances contained in the sample react with the acid and can be quantified by measuring the excess of HClO. Decreased values directly correlates to the injury severity of "plasma barrier to oxidation". When the "excess" of radicals of HClO after massive oxidation is high, the plasma barrier is reduced and vice versa. The quantification of the unreacted acid is carried out by the spectrophotometric method (reading at $\lambda = 490$ nm), after addition of suitable buffered chromogenous agent, an aromatic alkyl diamine (N,N-diethylparaphenylendiamine). The concentration of the coloured cation is directly proportional to the concentration of HClO and is indirectly related to the antioxidant capacity. The SHp test (Carratelli et al., 2001) is a colorimetric determination of plasma/serum thiol antioxidant barrier, which opposes peroxidative processes inhibiting both alkoxyl and hydroxyl radicals. This test is based on the ability of thiol groups, which are in a biological sample, to develop in an adequately buffered solution, a photometrically detectable coloured complex (maximum peak of absorbance, 405 nm), when react with 5,5-dithiobys-2-nitrobenzoyc acid (DTNB), which is solubilized as chromogenic mixture. The intensity of photometrically detected colour is directly proportional to the concentration of thiols, according to the Lambert–Beer's law. Protocols of animal husbandry and experimentation were reviewed and approved in accordance with the standards recommended by the Guide for the Care and Use of Laboratory Animals and by Directive 86/609 CEE (European Communities, 1986).

2.4. Statistical analysis

All the results were expressed as mean values ± standard deviation (SD). Data were normally distributed ($P < 0.05$, Kolmogorov–Smirnov test). Two-way repeated measure analysis of variance (ANOVA) was performed to determine the statistically significant effect of sampling time, the significant differences between the Groups A and B, and the interaction between Time × Treatment. The level of significance was set at <0.05. Bonferroni's multiple post hoc comparison test was applied. The data were analyzed using the software STATISTICA 8 (Stat Soft Inc.).

3. Results

Fig. 1 shows the mean pattern (±SD), together statistical significances, of studied stress oxidative parameters in Groups A and B.

There was a significant Time × Treatment interaction effect for all oxidative stress parameters ($P < 0.05$), indicating that time course of the parameters studied differed across the treatment Groups. In Group A, there was an increase due to the effect of sampling time on dROMs ($F_{(4,36)} = 6.89$, $P < 0.05$), Oxy-adsorbent ($F_{(4,36)} = 6.89$, $P < 0.05$) and SHp ($F_{(4,36)} = 6.89$, $P < 0.05$) and differences were found between treatments for all parameters 12, 24 and 48 h post transport ($P < 0.05$).