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Oxidative stress in Shaal sheep of different age groups

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Abstract: Oxidative stress plays an important role in the etiology and/or progression of a number of diseases and aging. In this cross-sectional study, some oxidative stress biomarkers (malondialdehyde [MDA] as a lipid peroxidation biomarker, ferric reducing/antioxidant power [FRAP], and total nonprotein SH groups) in plasma samples of Shaal ewes of different age groups were assessed. For all 3 measured parameters, the youngest age group (10-30 months old) had the lowest amounts. Although FRAP levels increased slightly and age dependently, nonprotein SH group content showed a decreasing trend after a peak in the 31-50 months group. The highest MDA concentration was observed in the oldest group. Our results suggest a relation between age and oxidative stress parameters in the early life of Shaal ewes. It also showed an age dependent increase in FRAP levels.

Key words: Oxidative stress, free radicals, aging, sheep, lipid peroxidation, ferric reducing/antioxidant power

Introduction

Formation of reactive oxygen/nitrogen species (RONS) that exceed the body's antioxidant capacity has been termed oxidative stress (1). RONS are produced in the body as the result of normal cellular metabolism as well as through exposure to a variety of environmental (e.g. some toxicants, ozone, and certain nutrients) and physiological (e.g. physical and mental stress) challenges. The precise cellular damage and disease generation that may accompany oxidative stress are related specifically to the macromolecules (nucleic acids, proteins, lipids) that are targeted by RONS, the frequency and duration of attack, and the tissue-specific antioxidant defenses present. The body's total antioxidant capacity serves to protect cells from excess production of RONS (2,3).

However, in several conditions, such as human aging or ewe milking, the rate of damage by oxygen and oxygen-derived radicals increases and causes oxidative stress (4,5).

It is now commonly recognized that RONS are involved in a variety of physiological and pathological processes, including gene transcription, regulation of soluble guanylate cyclase activity in cells (6), cellular signal transduction, cell proliferation and differentiation (7), nucleic acid lesions, gene damage, and gene repair activity, leading to subsequent cell death by necrosis or apoptosis (8) and also playing an important role in the progression of a number of diseases (9,10), including cancer, cardiovascular diseases, cataracts (11), mutagenesis (12), and

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ageing (10-14). One explanation of the causes of aging is the damage of the biological systems caused by free radical processes (15). It has been postulated that if free radical reactions were the major cause of aging, a reduction in their levels by antioxidants (e.g. vitamins E and C and glutathione) and antioxidant enzymes (e.g. catalase [CAT], superoxide dismutase [SOD], and glutathione peroxidases [GPx]) should, in principle, retard aging (16). However, levels of antioxidants and activities of antioxidant enzymes in different mammalian species appear to be constant. This relationship has been explained by considering the concentrations of antioxidants and antioxidant enzymes relative to the specific metabolic rate rather than absolute concentrations or activities (17).

Because of free radicals' involvement in the pathogenesis of some diseases or in the toxicity of xenobiotics and ageing, there is a great need for biomarkers of radical damage, which can be used to monitor the involvement of oxidative stress in such damages.

This study aims to discover preliminary and comparative pieces of information on biomarkers of oxidative stress in ewes of the Shaal breed. We compared 3 blood oxidative stress biomarker levels, namely MDA concentration, total nonprotein SH group contents, and the ferric reducing/antioxidant power of plasma (FRAP), in clinically healthy Shaal ewes from different ages groups. This study especially focused on the age-related variations of oxidative stress biomarkers in plasma.

Materials and methods

This study was carried out during October-December 2008 at the Animal Research Institute, Faculty of Veterinary Medicine, Tehran University, Iran.

Forty clinically healthy dry female Shaal sheep of different age groups (10-90 months old) were fed and bred in similar standard conditions. The sheep feeding ratio consisted of corn, alfalfa hay, soy beans, sugar beet molasses, DCP, trace mineral supplements, and ammonium sulfate. Water was given ad libitum. All animals were divided into 4 groups in relation to age: Group 1 (10-30 months old), Group 2 (31-50 months old), Group 3 (51-70 months old), and Group 4 (71-90 months old). After overnight fasting, blood samples were collected from the jugular vein into heparinized evacuated tubes and centrifuged at 1000 × g for 10 min. The isolated plasma samples were stored at –70 °C until tested.

Blood plasma samples were assessed in duplicates to determine the FRAP, MDA concentration, and total nonprotein SH groups content of plasma.

Thiobarbituric acid reactive substances (TBARS) measurement

Blood plasma samples were mixed with trichloroacetic acid (20% w/v) and the precipitate was dispersed in H_2SO_4 (0.05 M). TBA (0.2% w/v in sodium sulfate, 2M) was added and heated for 30 min in a boiling water bath. TBARS adducts were extracted by n-Butanol and measured at 532 nm (18).

FRAP assay

The antioxidant capacity of blood was determined by measuring the ability of plasma to reduce Fe^{3+} to Fe^{2+}. The complex between Fe^{2+} and TPTZ gave a blue color with absorbance at 593 nm (19).

Nonprotein SH groups assay

Total SH groups of plasma were measured spectrophotometrically at 412 nm using DTNB as the reagent (20). After adding Tris buffer (pH 8.2, 0.25 M) to plasma, the first absorbance was read at 412 nm (A1). Then DTNB was added and a second absorbance was read at 412 nm (A2). The concentration of total SH groups was measured (mM) based on the following formula:

$$(A2 - A1 - B^{*}) \times \frac{1.07/0.05}{13{,}600^{**}} = (A2 - A1 - B^{*}) \times 1.57 \text{ mM}$$

* B = Blank of DTNB

** 13,600 $cm^{-1} M^{-1}$ is the molar absorption concentration of total thiols.

Statistical analysis

One-way analysis of variance (ANOVA), followed by Tukey's test, was used to compare the results. P values greater than 0.05 were considered insignificant. The results were reported as mean ± SD.

Results

As shown in the Table, the lowest and the highest MDA concentrations were observed in the youngest and the oldest age groups, respectively. FRAP levels increased age dependently, and the lowest concentration of FRAP (μmol L^{-1}) was observed in group 1 (10-30 months old).

The lowest content of nonprotein SH groups was observed in the youngest ewes. However, after a peak in the 31-50 months group, it decreased age dependently in older age groups.

As demonstrated in the Table, among all oxidative stress parameters, the lowest values belong to the youngest group. Although these values for nonprotein SH group content and FRAP show the least antioxidant defense power in the youngest age group, the lowest oxidative stress biomarker, MDA concentration, was also seen in this group.

Discussion

In this study on female Shaal sheep, our results suggest a relationship between age and oxidative stress parameters, at least in the early life stage. In the youngest age group, nonprotein SH group content and FRAP showed the least antioxidant defense power. However, the lowest concentration of MDA, which is widely used as a biomarker of lipid peroxidation, was also seen in the youngest age group. It seems that in the early growth phase, these measured parameters are significantly lower than in other growth phases.

Similar to our research, Nussey et al. (21) had some experience with wild Soay sheep of different ages. They found that among females, lambs had significantly elevated phospholipid oxidative damage in plasma in comparison with other age groups. Their study showed that there was no evidence of increasing damage with age among older sheep, but life history had a correlation with oxidative damage in blood. Among lambs, levels of oxidative damage increased significantly with increasing growth rates over the first 4 months of life. Their findings included a correlation between oxidative damage levels and growth rates during first 4 months of the lives of the lambs (21). Our findings show that although increasing damage with age among ewes is not clearly significant, the youngest ewes had the lowest MDA levels in plasma in comparison with other age groups, and the oldest age group had the highest levels of MDA. A contrary relationship has been reported between TBA and FRAP (22,23), but we did not find any significant relationship.

Much research has been carried out on the effects of age on oxidative stress biomarkers in different tissues, but there is not a consensus among the researchers. Junqueira et al. (24) reported a mild and gradual oxidative stress status in aged human subjects. Pansarasa et al. (23) investigated the age-related variations in free radicals, antioxidant enzymes, and oxidative stress biomarkers in human skeletal muscles in different age groups and concluded that oxidative stress plays an important role in muscle aging in human subjects.

Some studies have shown that the effects of oxidative stress increase with age, but not all tissues, including plasma, can fully reflect it (24). However, Mézes and Sályi (25) observed that the liver, red blood cells, and plasma are the best tissues to show the ROS-derived changes. In addition, there has been an

Table. MDA, FRAP, and non-protein SH group assessment in Shaal sheep of different age groups.

Test	Group 1	Group 2	Group 3	Group 4
MDA (μmol L^{-1})	0.627 ± 0.071	0.920 ± 0.089	0.827 ± 0.078	0.939 ± 0.091
FRAP (μmol L^{-1})	186 ± 8	191 ± 6	192 ± 4	193 ± 6
SH groups (mmol L^{-1})	0.12 ± 0.01	0.20 ± 0.01	0.14 ± 0.03	0.13 ± 0.02

emphasis on the impact of lipid peroxidation on aging (25). Valls et al. (26) indicated an increase in lipid peroxidation products and glutathione peroxidase, a decrease in antioxidant enzymes CAT and SOD, and an unchanged level of glutathione content in rat liver with age. In contrast, Pansarasa et al. (23) observed no change in GSH-PX, CAT, GSHtot, and GSH levels, but reported increased SOD and GSSG levels. Inversely, some studies have shown weak evidence about age and oxidative stress biomarker increases. Valls et al. (26) studied the age-related variations in antioxidant status and lipid damage and concluded that the mitochondrial lipid peroxidation index was not different in young and old mice. Muradian et al. (27) showed decreased activity of antioxidant enzymes in young rats compared with old ones, although the variations were statistically significant only for SOD. Argüelles et al. (28) suggest that oxidative stress biomarkers in plasma or serum cannot fully reflect the status of biomarkers in all sensitive organs, including the liver and kidneys, and there is not always a direct relation among them.

Biomarkers assessed in this study have been named in many different studies, and despite the belief that their concentration in plasma does not reflect their antioxidant status and/or body defense against ROS (18,29), they are still being extensively used as biomarkers. Any discussion of the relationship between oxidative stress and aging is complicated by the considerations, first, that there is no general agreement as to what aging is, and, second, that oxidative stress occurs by multiple mechanisms (30). We suggest further studies to discover the relationship between aging and oxidative stress biomarkers in blood and plasma along with other organs, especially the liver and kidneys. It should be investigated whether lifestyle can affect these parameters. Our results can be useful for identifying the biomarkers of radical damage indicators that, in Shaal sheep breeding, are useful to prevent diseases caused by oxidative stress, which may arise with milk production and in the management of older animals.

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