Acta Veterinaria (Beograd), Vol. 57, No. 4, 351-356, 2007.

DOI: 10.2298/AVB0704351N UDK 619:612.015.11

INVESTIGATION OF THE ANTIOXIDATIVE METABOLISM IN SHEEP WITH *PESTE DES PETITS* RUMINANTS

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(Received 12. November 2006)

The aim of this study was to examine the changes in the indicators of free oxygen radicals and antioxidant activity, i.e. malondialdehyde (MDA), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) levels, in sheep with peste des petits ruminants (PPR). Twenty sheep with serologically confirmed PPR and ten clinically healthy sheep comprised the study cohort. Serum levels of MDA and activities of GSH-Px and SOD (mean ± standard deviation) in animals with PPR were 1.93±0.11 (nmol/mL), 0.24±0.05 (NADPH+H^+/min/mg-protein) and 4.69±0.53 (U/g-protein) whereas in the control group they were 1.49±0.06 (nmol/mL), 0.81±0.12 (NADPH+H^+/min/mg-protein) and 7.54±0.06 (U/g-protein). Mean MDA level of the patient group was significantly higher than for the control group ($p<0.05$). Mean GSH-Px and SOD activities were significantly lower than those of the control group ($p<0.01$, $p<0.001$, respectively).

These results show that in many tissues the oxidant-antioxidant balance is disrupted which in turn can cause further oxidative damage in PPR.

Key words: antioxidant, peste des petitis ruminants, antioxidative metabolism

INTRODUCTION

Peste des petits ruminants (PPR) is a highly contagious viral disease of sheep and goats caused by the PPR virus (PPRV) (Gibbs *et al*., 1979). The PPR virus causes a marked immunosuppression as evidenced by leukopenia and lymphopenia (Heaney *et al*., 2002). Clinically, PPR is characterized by pyrexia, necrotic stomatitis, catarrhal inflammation of the ocular and nasal mucosa, enteritis and pneumonia followed by death or recovery from the disease. The disease is associated with high morbidity and mortality and is considered to be one of the main constraints in improving small ruminant productivity (Rajak *et al*., 2005). Reactive oxygen species (ROS) are produced in the organism as a natural byproduct of normal metabolic processes, as well as a result of foreign agents and diseases (Heaton, 2002; Agarwal, 2005). Free radicals are scavenged by antioxidants. However, when free radicals are produced in abundance and

exceed the antioxidant capacity, these free radicals disrupt the cytoarchitectonic structures of cells such as proteins, lipids, enzymes, carbohydrates, nucleic acids and their compounds, and cause various metabolic and functional injuries (Halliwell, 1994; You *et al*., 2003).

Oxygen radicals created in biological systems by various mechanisms and malondialdehyde (MDA), a product of lipid peroxides created by reactions of the polyunsaturated fatty acids of the cell membrane phospholipids, increase the permeability of the cell membrane to ions, bind to amino groups of proteins, phospholipids or nucleic acids and cause damage to the membrane receptors and DNA chain (Holley *et al*., 1993). Superoxide dismutase (SOD) is an important antioxidant enzyme that protects the cell integrity structurally and metabolically from pathological conditions by catalyzing the conversion of superoxide anions (O_2^-) to hydrogen peroxide (H_2O_2) to protect the cells from the harmful effects of O_2^- (Cottin *et al*., 1996; You *et al*., 2003; Agarwal *et al*., 2005). On the other hand, the formed H_2O_2 is eliminated by a cytosolic enzyme, GSH-Px, which causes reduction of hydroperoxides and protects fagocytic cells and tissues against oxidative stress (Hogan *et al*., 1990; Cottin *et al*., 1996; Chrobot *et al*., 2000; You *et al*., 2003).

In the present study, we aimed to assess reactive oxygen products and antioxidant enzyme activities by exploring the changes in serum MDA, SOD and GSH-Px in sheep infected by PPR.

MATERIALS AND METHODS

Animals and blood sampling

The study cohort comprised 30 sheep. Experimental and control groups were matched in terms of age and sex. Twenty sheep with clinical signs of PPR and serologically confirmed to be positive comprised the experimental group and 10 clinically healthy sheep comprised the control group. Physical examination and history of the control group animals did not show pyrexia, loss of appetite, fatigue or abortion. None of the experimental groups were previously immunized. Serum samples were obtained from the jugular vein and centrifuged at 3000 rpm for 10 minutes. The resultant sera were stored at -30°C until subsequent analysis.

Competitive Enzyme Linked Immunosorbent Assay (c-ELISA) for PPRV antibodies

Competitive enzyme linked immunosorbet assay (cELISA) was used to detect antibodies against PPRV as described in the manual of Pestes des Petits Ruminants enzyme linked immunosorbent assay kit and the Office International des Manual of Standards (OIE, 2004). At the end of the test the wells were read on a plate reader (DAF, Italy) and the absorbance (OD) of wells was determined at 492 nm. The absorbance is converted to percentage inhibition (PI) using the formula.

$$PI = 100 - (OD \text{ of test wells} / OD \text{ of the MAb control wells}) \times 100$$

Serum MDA, SOD and GSH-Px measurements

Serum MDA levels were measured as the thiobarbituric acid reactive species by the method of Yoshioka et al. (1979). To determine SOD activity, we used a method based on conversion of superoxide radicals generated through xanthine and xanthine oxidase to H_2O_2 by SOD, which in turn reduces nitroblue tetrazolium (NBT) (Sun *et al*.,1988). GSH-Px activity was measured using the method reported by Paglia and Valentine (1967). This method is based on the conversion of GSSG to GSH to achieve the catalysis of H_2O_2 to water. This conversion, in the presence of reduced NADP, is mediated by glutathione reductase. Therefore the activity of GSH-Px is determined by measuring the decrease in NADPH.

Statistical analyses

Statistical comparisons were made using the Mann-Whitney *U*-test. (Rao, 1973). Results are shown as the mean ± standard deviation. Statistical significance was assumed at $p<0.05$.

RESULTS

Serological findings

Eighty sheep sera were tested to antibodies against PPRV with c-ELISA and 22 of 80 sera (27.5%) were found positive.

Serum MDA, GSH-Px and SOD levels

The changes in serum MDA, GSH-Px and SOD in the infected and the control group are presented in Figure 1. Mean serum MDA, GSH-Px and SOD

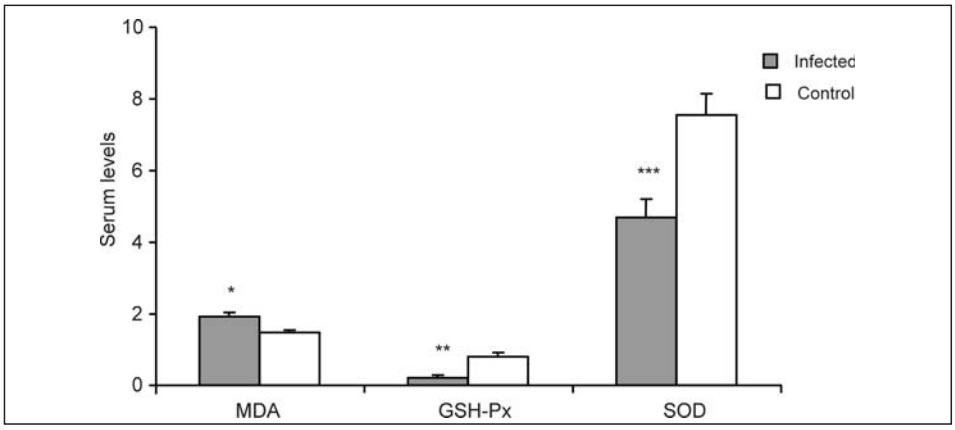

Figure 1. Serum MDA (nmol/mL), GSH-Px (NADPH+H^+/min/mg-protein) and SOD (U/g-protein) levels in infected and control sheep. Asterisks indicates statistical significance (***) $p<0.001$ and (**) $p<0.01$ and (*) $p<0.05$ (Mann-Whitney *U*-test)

levels in animals with PPR were 1.93±0.11 (nmol/mL), 0.24±0.05 ($NADPH+H^+$/min/mg-protein), and 4.69±0.53 (U/g-protein), respectively. In the control group, we found the mean MDA, GSH-Px and SOD levels to be 1.49±0.06 (nmol/mL), 0.81±0.12 ($NADPH+H^+$/min/mg-protein), and 7.54±0.06 (U/g-protein), respectively. In the infected group, mean MDA, GSH-Px and SOD were significantly different than those of the control group ($p<0.05$, $p<0.01$, $p<0.001$, respectively).

DISCUSSION

PPR is a febrile disease that is characterized by respiratory distress, stomatitis and gastroenteritis.There is a balance in the organism between production of free radicals and enzymatic and non-enzymatic anti-oxidant defense mechanisms. Oxidative stress due to an increase in reactive oxygen species or a deficiency in antioxidant defense mechanisms, causes structural and functional modifications of lipid, protein and DNA-containing macromolecules of the cell (Cottin *et al*.,1996; Heaton *et al*., 2002; Agarwal *et al*., 2005). It has been reported that hydroperoxides and MDA levels were higher than in the controls in human immunodeficiency virus (HIV) infection (Peterhans, 1997). In another study, MDA activity was found higher in patients with adult dengue disease compared to the controls. The authors have argued that increased oxidative stress may be underlying the etiopathogenesis of the disease (Gil *et al*., 2004). Ko *et al*. (2005) reported that MDA levels were increased in patients with viral hepatitis C. In the present study, we found that MDA levels in sera samples of the animals with PPR were higher than the controls (1.93±0.11 nmol/mL vs. 1.49±0.06 nmol/mL). In many studies (Biesalski *et al*., 1993; Peterhans, 1997; Lawrence, 2002; Ko *et al*., 2005), the researchers argued that serum MDA level was high secondary to lipid peroxidation due to oxidative damage. As a result of lipid peroxidation, unsaturated fatty acids present in the cell membrane are converted into water-soluble products and cause reactions that would disrupt the integrity of the membrane (Holley *et al*., 1993). Therefore, determination of MDA level could be used as an indicator of free-radical mediated tissue injury. This supports the view that PPR infection causes oxidative stress and consequently lipid peroxidation and the by-products may be used as indicators of damage in various tissues. Chrobot *et al*. (2000) found SOD activity in patients with chronic viral hepatitis B and C decreased compared to the controls. On the other hand, SOD activity was found higher than the controls in another study on viral hepatitis C patients (Ko *et al*., 2005). In our study, SOD activity in the patient group was significantly lower than the control group (4.69±0.53 U/g-protein, 7.54±0.06 U/g-protein, respectively). These results may be indicating that the balance between the antioxidant enzymes and the free radicals was altered, the SOD enzyme was inhibited as a result of the increase in toxic radicals and the antioxidant efficacy was decreased (Peterhans, 1997; Chrobot *et al*., 2000).

Gil *et al*. (2004) reported that serum GSH-Px levels in the sera of adult dengue patients were lower than the control group. This finding was replicated in Hepatitis C patients as well by Ko *et al*. (2005) who observed that GSH-Px levels

were lower than the controls. Beck *et al*. (2001) conducted a study on rats infected with Coxsackie virus in which they observed that GSH-Px level decreased concomitantly with selenium levels. In the present study, serum GSH-Px activity of PPR sheep was lower than in healthy animals (0.24 ± 0.05 NADPH$+H^+$/min/mg-protein versus 0.81 ± 0.12 NADPH$+H^+$/min/mg-protein). This supports the view that the antioxidant activity was not functional enough due to the increase in free radical production or the antioxidant response was not sufficient once the damage was initiated (Atroshi *et al*., 1996; Chrobot *et al*., 2000). The decrease in GSH-Px, which protects the fagocytic cells and neutrophils from oxidative damage caused by free radicals, shows that the host's defense system is affected by oxidative stress and that there is damage of cells of the immune system (Hogan *et al*., 1990; Atroshi *et al*., 1996; Heaton *et al*., 2002; Agarwal *et al*., 2005).

In this study we determined whether an imbalance in oxidant-antioxidant activity may be involved in the pathogenesis of PPR. It is believed that the increase in MDA, when the membrane lipids undergo lipid peroxidation, is the most important step in the development of tissue damage secondary to free radicals. Low GSH-Px and SOD activities also support this view. The results show that in PPR disease, the oxidant-antioxidant balance which, in turn, can cause oxidative damage in many tissues is disrupted. It can be concluded that free radicals play a role in the pathogenesis of PPR, as well as in many other diseases.

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ISPITIVANJE ANTIOKSIDATIVNOG STATUSA OVACA OBOLELIH OD KUGE MALIH PREŽIVARA

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SADRŽAJ

Cilj ove studije je bio da se ispitaju koncentracija malondialdehida i aktivnost enzima glutation peroksidaze (GSH-Px) i superoksi-dismutaze (SOD) u serumu ovaca obolelih od kuge malih preživara. U ispitivanja je bilo uključeno dvadest obolelih i deset zdravih životinja. Koncentracija malondialdehida u serumu je bila povećana u grupi obolelih životinja, dok je aktivnost ispitivanih enzima bila značajno smanjena. Ovi rezultati ukazuju da je kod obolelih ovaca poremećen oksidativno-antioksidativni balans što može imati uticaja na razvoj patogenog procesa.