Status of some oxidative stress biomarkers in sheep naturally infected with theileriosis

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Abstract

The present study was aimed to investigate some oxidative stress indices in the erythrocytes from sheep naturally infected with theileriosis. Thirty adult fat-tailed sheep suffering from theileriosis were selected on the basis of clinical examination and positive peripheral blood smears and twenty clinically healthy animals without parasitaemia (according to the blood smears) served as controls. The oxidative stress indices including enzymatic activities of superoxide dismutase, glutathione peroxidase and catalase, glutathione and malondialdehyde were measured in erythrocyte haemolysate. The activities of antioxidant enzymes including superoxide dismutase and glutathione peroxidase were considerably decreased in infected sheep compared to controls, although the decrease was only significant (P<0.05) for superoxide dismutase activity. By contrast, catalase activity was non-significantly higher in the infected group compared to control group. Malondialdehyde concentration was significantly enhanced in erythrocytes of infected group, whereas glutathione level was significantly reduced in infected group in comparison to controls. Disturbed antioxidant mechanisms of erythrocytes in infected group, accompanied by a significant rise in erythrocytic lipid peroxidation, implied that the oxidative stress may have a pathophysiological role in ovine theileriosis.

Keywords: Theileriosis, sheep, oxidative stress, endogenous antioxidants

Introduction

Theileriosis is a tick-borne infectious, haemoproteozoan disease of wild and domestic ruminants in the tropical and subtropical regions of the world, caused by the genus Theileria. It causes serious economic losses through mortality and loss of productivity (Glass et al., 2003). Theileria spp. are intracellular parasites that complete their life cycle in the mammalian hosts by successively utilizing lymphoid cells and erythrocytes (Soulsby, 1982). T. lestoquardi and T. ovis are suspected to cause ovine theileriosis in Iran (Hashemi-Fesharaki, 1997).

Oxidative stress resulting from increased production of free radicals and reactive oxygen species (ROS), and/or a decrease in antioxidant defence, leads in impairment of DNA, enzymes and membranes and induces changes in the activity of the immune system and in the structure of basic biopolymers which, in turn, may be related to various health disorders (Trevisan et al., 2001; Abd Ellah, 2010). Oxidative stress has been reported in some haemoparasitic diseases of animals such as theileriosis in cattle, buffaloes and sheep (Sahoo et al., 2001; Shiono et al., 2003; Grewal et al., 2005; El-Deeb and Younis, 2009; Nazifi et al., 2011), babesiosis in horses and cattle (Deger et al., 2009; Saleh, 2009) and trypanosomiasis in camels (Saleh et al., 2009). There are some evidences that oxidative damage to cellular components incorporate in pathogenesis of anaemia in theileriosis (Asri Rezaei and Dalir-Naghadeh, 2006; Nazifi et al., 2011). On the other hand, the understanding of the pathophysiology of oxidative stress in theileriosis will allow the design of specific antioxidant therapies.

The present study was conducted to assess the pattern of changes and the relative values of some endogenous antioxidants and the level of malondialdehyde, as a biomarker of lipid peroxidation, in erythrocytes of sheep naturally infected with theileriosis. These studies may be of value for further understanding the pathogenesis of the disease and as an aid in diagnosis and supportive therapy of ovine theileriosis.
**Materials and Methods**

Thirty adult fat-tailed sheep suffering from ovine theileriosis were selected from some farms in Fasa district, Iran, during the peak months of theileriosis occurrence in 2010. Infected sheep were selected on the basis of clinical examination and positive peripheral blood smears. Thin blood smears were prepared from the ear vein, and stained with Giemsa for confirmation of the disease on the basis of microscopical observation of piroplasms of *Theileria* in erythrocytes. On the other hand, 20 clinically healthy animals without parasitaemia located in the same farms served as controls. All sheep included in the study ranged from 2 to 4 years old of both sexes.

Blood samples were collected by venepuncture into EDTA-containing tubes from all infected sheep and controls. The blood tubes were placed on ice until laboratory arrival (<2 h). The samples were centrifuged at 750 g for 20 min, and after plasma separation erythrocyte pellet was washed three times with normal saline solution. The washed centrifuged erythrocytes were haemolysed by the addition of an equal volume of ice-cold distilled water and prepared haemolysate were stored at −70°C until analysis.

Glutathione peroxidase (GPx) activity was measured by the method of Paglia and Valentine (1967) using RANDOX-Ransel enzyme kit. In this method, GPx catalyzes the oxidation of GSH by cumene hydroperoxide. In the presence of glutathione reductase (GR) and NADPH, the oxidized glutathione (GSSG) is immediately converted to the reduced form, with a concomitant oxidation of NADPH to NADP⁺. The decrease in absorbance at 340 nm was measured spectrophotometrically, and the results were expressed as units per gram haemoglobin. Haemoglobin (Hb) concentration was measured by cyanmethaemoglobin method.

Superoxide dismutase (SOD) activity was determined by a modified method of iodophenol nitrophenol phenyltetrazolium chloride using the RANDOX-Ransod enzyme kit. This method employs xanthine and xanthine oxidase (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 SOD 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. The results were expressed as units per gram haemoglobin.

Catalase (CAT) activity was measured in the RBC haemolysate by the method described by Claiborne (1986) and expressed as units per gram haemoglobin. The decomposition of H₂O₂ can be directly followed by the decrease of absorbance at 240 nm. The difference in absorbance at 240 nm per time unit allows determining the CAT activity.

Non-enzymatic antioxidant, reduced glutathione (GSH), was assayed by the method previously described by Ellman (1959). In this method, 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) is reduced to 2-nitro-5′-mercaptopbenzoic acid (NMBA) by GSH. The amount of the yellow colour reduced was measured at 412 nm and expressed as micromoles per gram haemoglobin.

Determination of malondialdehyde (MDA) concentration in erythrocyte haemolysate was performed using the method of thiobarbituric acid which measures MDA-reactive products (Placer et al., 1966), as described by Todorova et al. (2005). The concentration of MDA was calculated using a molar extinction coefficient value of 156,000 M⁻¹ cm⁻¹. The results were expressed as nanomoles of MDA per gram haemoglobin.

**Statistical analysis**

All experimental values were presented as mean ± standard error of mean (SEM). The obtained data were analyzed using Student's t-test. The level of significance was set at P<0.05. All calculations were performed using SPSS/PC software.

**Results**

The values (mean ± SEM) of the measured erythrocytic oxidative stress parameters in healthy and affected sheep are presented in Table 1. The activities of antioxidant enzymes including GPx and SOD were considerably decreased in infected sheep compared to controls, although the decrease was only significant (P < 0.05) for SOD activity. By contrast, CAT activity was non-significantly higher in the infected group compared to control group.

As shown in Table 1, MDA concentration was significantly enhanced in erythrocyte haemolysate of infected group whereas GSH level was significantly reduced in infected group whereas GSH level was significantly reduced in infected group in comparison to healthy controls.

**Discussion**

Oxidative stress is an active field of research in ruminant medicine and has been implicated in numerous disease processes. Although the study of oxidative stress is a relatively young field of research in ruminant medicine, the understanding of the role of oxidants and antioxidants in physiological and pathological conditions is rapidly increasing (Cell, 2011). In this study on ovine theileriosis, our results
indicate that infection with *Theileria* led to notable changes in measured oxidative stress indices.

Assaying antioxidant enzymes is among the most widely used methods for determination of oxidative stress. The seleno-enzyme glutathione peroxidase contributes to the oxidative defense of animal tissues by catalyzing the reduction of hydrogen and lipid peroxides (Arthur, 2000). GPx functions in cellular oxidation-reduction reactions to protect the cell membrane from oxidative damage caused by free radicals (Flohe et al., 1973). Various responses of GPx activity in *Theileria* infected ruminants has been reported by various authors. Naziroglu et al. (1999) showed that GPx activities in plasma and erythrocytes did not differ significantly between a group of cattle naturally infected with *T. annulata* and treated with buparvaquone and another group of uninfected untreated controls. Grewal et al. (2005) reported that GPx activity exhibits a significant rise in cattle naturally infected with *T. annulata*. On the other hand, Asri-Rezaei and Dalir-Naghadeh (2006), El-Deeb and Younis (2009) and Nazifi et al. (2011) reported significant decrease in GPx activity in *Theileria* infected cattle, buffaloes and sheep, respectively. Moreover, our results indicated a non-significant decrease in erythrocytic GPx activity in *Theileria* infected sheep in comparison to controls.

Catalase is of equal importance to GPx in the defence of human erythrocytes against H2O2 generating reactions (Harvey, 1997). According to the results of this study, catalase levels were slightly increased in affected sheep. The obtained results were in line with that reported by Asri-Rezaei and Dalir-Naghadeh (2006) who reported significant increase in the activity of CAT in *Theileria* infected cattle with mild or moderate anaemia in comparison to healthy cattle and infected cattle with severe anaemia. Increased activity of catalase had been also reported in *Babesia* infected dogs (Chaudhuri et al., 2008) and *Trypanosoma* infected camels (Saleh et al., 2009). On the other hand, while Grewal et al. (2005) reported no substantial changes in the CAT activity in *Theileria* infected cattle, El-Deeb and Younis (2009) and Nazifi et al. (2011) reported significant decrease in the CAT activity in *Theileria* infected buffaloes and sheep, respectively.

Superoxide dismutase is important in the antioxidative defence mechanism and protects against lipid peroxidation (Halliwell and Chirico, 1993; Miller et al., 1993). According to our data, erythrocytic SOD activity in *Theileria* infected sheep was significantly lower than the parasitologically free controls. Similar findings had been reported in bovine (Asri-Rezaei and Dalir-Naghadeh, 2006; El-Deeb and Younis, 2009) and ovine theileriosis (Nazifi et al., 2011). Decreased erythrocytic SOD activity has been reported in *Trypanosoma* infection in humans (Wen et al., 2004) and camels (Saleh et al., 2009) and *Plasmodium* infection in humans (Erel et al., 1997). However, the obtained results were different from that reported by Grewal et al. (2005) who reported no substantial changes in the activity of SOD in cattle naturally infected with *Theileria*. In addition significant increases in erythrocytic superoxide dismutase activity have been reported in babesiosis in dogs (Chaudhuri et al., 2008) and anaplasmosis in cattle (Nazifi et al., 2008).

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. Considering the fact that mature red cells lack protein synthesis machinery and cannot replace damaged proteins, it can be assumed that SOD and GPx are consumed to scavenge ROS and their decline in infected animals may be due to degradation by ROS during the detoxifying process. Since both CAT and GPX are responsible for enzymatic removal of H2O2, the higher activity of CAT in infected group may provide some extra protection against H2O2 to increase the lifespan of erythrocytes. Non-significant increase in CAT activity might be due to increased specific activity of pre-existing enzyme as an indirect compensatory response to increased oxidant challenge.

GSH is required for the disposal of H2O2 by the reaction catalysed by GPx. Depletion of tissue GSH is one of the primary factors that permit lipid peroxidation (Konukoglu et al., 1998). The significant reduction in GSH amount was in line with the findings of El-Deeb and Younis (2009). They reported a significant reduction in the levels of glutathione in *T. annulata*.
infected buffaloes compared with healthy buffaloes. It was reported also that babesiosis in sheep (Bicek et al., 2005) and horse (Deger et al., 2009) and trypanosomiasis in camel (Saleh et al., 2009) can lead to significant reduction in GSH levels, thus supporting the findings of this study.

Among the known biological molecules, lipids are one of the most susceptible substrates to free radicals damage and biomarkers of lipid peroxidation are considered the best indicators of oxidative stress (Georgieva, 2005). Lipid peroxidation is a non-enzymatic chain reaction based on oxidation of mainly unsaturated fatty acids and is associated with the presence of reactive oxygen species. Malondialdehyde 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). Several lines of evidence have suggested an important role for lipid peroxidation in the pathogenesis of several parasitic diseases (Bagchi et al., 1993; Deger et al., 2009). Based on the present results, a significant increase of MDA concentration was found in the erythrocytes of Theileria infected sheep in comparison to the control group that indicate a high production of free radicals in diseased sheep. Our finding of increase in erythrocytic MDA is consistent with the results of Naziroglu et al. (1999), Sahoo et al. (2001), Grewal et al. (2005), Asri-Rezaei and Dalir-Naghadeh (2006) and El-Deeb and Younis (2009), who observed an increase of MDA in red blood cells in bovine theileriosis. Similar finding had been reported by Nazifi et al. (2011) who reported the increased levels of MDA in ovine theileriosis. Also there are several reports that infection with some other haemoparasites is associated with a marked elevation in lipid peroxidation (Murase et al., 1996; Griffiths et al., 2001; Eze et al., 2008; Chaudhuri et al., 2008; Deger et al., 2009; Saleh, 2009; Saleh et al., 2009). The increased MDA content observed in this study may not only be due to increased free radical generation but also be exacerbated by the inefficient endogenous antioxidant capacity.

Oxidative injury to the erythrocyte membrane is evidenced by increased formation of MDA during progress of infection. The erythrocyte membrane is rich in polyunsaturated fatty acids, a primary target for reactions involving free radicals (May et al., 1998; Devasena et al., 2001). Moreover, repeated exposure to high concentration of oxygen or presence of iron renders erythrocytes more susceptible to peroxidative damage (Clemens and Waller, 1987). The higher production of ROS and consequent elevated lipid peroxide concentrations renders the erythrocytes more fragile and prone to lysis which might have an important role in the pathogenesis of anaemia in case of theileriosis (Grewal et al., 2005; Asri-Rezaei and Dalir-Naghadeh, 2006).

In conclusion, the glutathione level and the activities of measured antioxidant enzymes excluding CAT have been shown to be reduced which indicates that erythrocytes have depleted antioxidant mechanisms in a response to Theileria infection in sheep. Disturbed antioxidant mechanisms of erythrocytes, accompanied by a significant rise in lipid peroxidation of erythrocytes, implied that the oxidative stress may have a pathophysiological role in ovine theileriosis.

Acknowledgement

This research was financially supported by grant (NO. 3444) from Ferdowsi University of Mashhad, Mashhad, Iran.

References


