Status of Lipid Peroxidation, Some Antioxidant Enzymes and Erythrocytic Fragility of Crossbred Cattle Naturally Infected with Theileria annulata

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ABSTRACT

Erythrocytic lipid peroxidation, activities of some antioxidant enzymes and osmotic fragility of red blood cells was studied in adult (> 1 year) crossbred cattle naturally infected with Theileria annulata. Twenty clinically healthy animals (group I) and 15 clinical cases (group II) of tropical theileriosis were selected. Cattle suffering from theileriosis had significantly higher (p<0.01) erythrocytic lipid peroxidation and osmotic fragility. Activities of antioxidant enzymes, viz. glucose-6-phosphate dehydrogenase (G6PD) and glutamate peroxidase (GPx) were also significantly increased (p<0.01) in group II. However, superoxide dismutase and catalase did not show significant changes. The results indicated that infection with theleria led to increased oxidative stress to the animals, and even a significant rise in the activities of antioxidant enzymes. G6PD and GPx could not lower this oxidative stress. However, the increase in the activities of antioxidant enzymes pointed towards the body’s defence mechanism against lipid peroxidation during oxidative stress in theileriosis.

Keywords: erythrocytic lipid peroxidation, antioxidant enzymes, osmotic fragility, crossbred cattle, Theileria annulata

Abbreviations: LPO, lipid peroxidation; G6P, glucose-6-phosphate; G6PD, glucose-6-phosphate dehydrogenase; GPx, glutathione peroxidase; SOD, superoxide dismutase; Hb, haemoglobin

INTRODUCTION

Bovine tropical theileriosis is recognized as a disease of major economic importance. It causes heavy losses due to mortality, decreased production and reduced working efficiency of affected animals in tropical and subtropical regions of the world, including India, where ticks are more prevalent. Since the introduction of a bovine crossbreeding programme in India, this haemoproteozoan disease has gained great importance because exotic and crossbred cattle are more susceptible to Theileria annulata infection than are indigenous stock.

Most of the previous studies on biochemical changes in T. annulata infection have been carried out on experimentally infected calves (Yadav and Sharma, 1986; Sandhu et al., 1998; Singh et al., 2001). A recent study reported biochemical profiles in Friesian cattle during natural infection with T. annulata (Omer et al., 2003). However, very little
information is available regarding oxidative stress and antioxidant enzymes during natural cases of theileriosis. Since theileria organisms spend part of their life cycle inside erythrocytes, we thought it worthwhile to investigate several aspects of oxidative activities. Thus we monitored key antioxidant enzymes like G6PD, GPx, SOD and catalase. We also assayed lipid peroxidation and the osmotic fragility of erythrocytes during natural infection with *T. annulata*.

**MATERIALS AND METHODS**

*Source of animals and samples*

Fifteen adult cattle ( >1 year) suffering from theileriosis (group II) were selected from some farms in Punjab during disease outbreak. Twenty healthy animals (group I) selected from the same farms served as controls.

Blood samples were collected from the jugular vein, in heparinized glass-stoppered vials. Blood (5 ml) taken in a graduated tube, was centrifuged at 700g for 15 min in a refrigerated centrifuge (REMI, C-30) at 4°C. Plasma was separated and the erythrocyte pellet was washed three times with normal saline solution. Then distilled water was slowly added to the erythrocyte pellet and with constant stirring up to the 5 ml level. The resulting haemolysate was used for estimation of lipid peroxidation and activities of antioxidant enzymes.

*Clinical examination*

A thorough clinical examination was performed on all the animals. Clinical signs of *Theileria annulata* infection, namely high temperature, swelling of lymph nodes, oedema, dyspnœa, nasal discharge and lacrimation were recorded. Haemolytic anaemia and icterus developed in the later stages. Thick and thin blood smears were prepared for confirmation of disease. Giemsa staining of slides revealed piroplasms of *T. annulata* in erythrocytes of all group II animals.

*Biochemical assays and analysis*

Lipid peroxidation (LPO) was measured in haemolysate according to Placer and colleagues (1966). Activities of the antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PD) and catalase were estimated in the haemolysate by the methods of Nishikimi and colleagues (1972), Hafeman and colleagues (1974), Deutsch (1978) and Hugo (1984), respectively. Osmotic fragility of erythrocytes and blood haemoglobin concentration were determined according to Dacie and Lewis (1973) and Jain (1986), respectively. Means and standard errors were calculated using a digital scientific calculator. The data was subjected to Student’s *t*-test analysis (Snedecor and Cochran, 1994).