Na^+/K^+ATPase Activity in Sheep with Natural Babesiosis

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Abstract

The aim of this study was to determine the Na^+/K^+ATPase activity in the erythrocytes of sheep naturally infected with *Babesia ovis* before and after treatment. Seven diseased sheep and seven control animals were used for the study. Babesia infection was confirmed with Giemsa's staining of blood smears. Na^+/K^+ATPase activity in erythrocyte was determined colorimetrically by the release of inorganic phosphate from ATP in the presence and absence of ouabain. A marked decrease of Na^+/K^+ATPase activity ($p < 0.05$) was obtained in infected sheep (0.81 ± 0.28 μmol inorganic phosphate/h.mg protein) compared to control animals (3.63 ± 0.72 μmol Pi/h.mg protein). Beside this decline, Na^+/K^+ATPase activity of treated animals (1.29 ± 0.81 μmol Pi/h.mg protein) were significantly ($p < 0.05$) decreased compared to control animals. It can be concluded that decreased erythrocyte Na^+/K^+ATPase activity in *babesia ovis* may be due to the usage of new permeation pathways (NPPs) for the flux of ions instead of Na^+/K^+ pump.

Babesia ovis, Sheep, Na^+/K^+ATPase

Babesiosis is an infection caused by species of tick-borne, intraerythrocytic and generally host-specific protozoan parasites of the genus Babesia (Wormser 2006). It occurs in a wide variety of vertebrate hosts and is very widely distributed around the world (Mehlhorn 2008). Ovine babesiosis is a haemoparasitic tick-borne disease of small ruminants caused by *Babesia ovis, Babesia motasi* and *Babesia crassa* (Uilenberg 2001). *Babesia ovis* is highly pathogenic especially in sheep, and causes severe infections characterized by fever, anaemia, icterus and haemoglobinuria (Almeria et al. 2001). Ovine babesiosis is the most important seasonal sheep disease and has been observed in all geographical regions in Turkey (Sayın 1997).

Na^+/K^+ATPase (EC 3.6.3.9) is present in the membrane of most eukaryotic cells and couples the energy released in intracellular hydrolysis of ATP to the export of three intracellular Na^+ ions and the import of two extracellular K^+ ions. The continuous operation of this macromolecular machine ensures the generation and maintenance of concentration gradients of Na^+ and K^+ across the cell membrane. This electrochemical gradient provides energy for the membrane transport of metabolites and nutrients, e.g., glucose and amino acids, and such ions as protons, calcium, chloride and phosphate. The electrochemical gradient is essential also for regulation of cell volume and for the action potential of muscles and nerves (Rose and Valdes 1994).

Alteration of this transport enzyme is thought to be linked to several parasitic and viral diseases (Baghian and Kousoulas 1993; Iizumi et al. 2006; Ulug et al. 1996; Kuralay et al. 1998; Ravikumar and Kurup 2001). However, the effect of *Babesia ovis* infection on Na^+/K^+ATPase activity in sheep has not been reported previously.

The present study was aimed to examine the relationship between babesiosis and the Na^+/K^+ATPase activity.

Materials and Methods

Fourteen Akkaraman female sheep weighing 25-30 kg, aged 4-5 years and localized in different regions of Van were used as subjects for this study. All the sheep included in this study were submitted to clinical and parasitological examinations. The whole flock was examined for babesia and 7 infected sheep were randomly selected as patient

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grooup. Seven clinically and parasitologically healthy sheep were selected as control. Seven sheep were infected naturally with *Babesia ovis* and 7 control animals were clinically healthy. The sheep were kept under their natural conditions. The daily feed ration and feeding regimen was uniform for all animals. Treatment of seven infected animals was conducted with diminazene aceturate (Berenil®) 7% solution) once at a dose of 3.5 mg/kg, intramuscularly. Blood samples of all animals were taken into plain and EDTA containing vacutainer tubes from the jugular vein. Samples were first taken at the onset of the disease during the disease season of babesiosis (June - August), then one week following the treatment of the diseased animal. At this time, the treated animals did not display any clinical signs of babesiosis. All treatments of the animals were performed according to National Ethical Rules. Na^+/K^+ATPase activity in the erythrocytes was measured as the release of inorganic phosphate from hydrolysis of ATP in the presence and absence of oubain. Erythrocytes were incubated at 37 °C for 60 min in 1 ml of a solution containing 3 mM ATP (pH = 7.0), 50 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 100 mM tris-HCl (pH = 7.4). To inhibit the Na^+/K^+ATPase activity, 1 mM oubain was added. The reaction was stopped by the addition of trichloracetic acid. ATPase activity was expressed as nanomoles of phosphorus released per mg protein/h (Serpersu and Ciliv 1978; Dasmahapatra et al. 1985).

The results were expressed as means ± standard deviation. Duncan's test was used for statistical analysis, setting $p < 0.05$ to establish significant differences.

Results

The biochemical findings obtained in the study are summarised in Table 1.

Blood smears prepared from the seven diseased animals showed the presence of *Babesia ovis* in the red blood cells with different degrees of parasitaemia. On the other hand, no piroplasm was detected in control animals. After recovery (i.e. following the treatment), piroplasms were still detected in all infected animals by direct microscopic examination with very low degree of parasitaemia. Mean Na^+/K^+ATPase activities of the diseased animals were significantly lower than in the controls ($p < 0.05$) (Fig. 1). Also Na^+/K^+ATPase activities of treated animals (one week after treatment) were significantly decreased in sera compared to the control animals ($p < 0.05$) (Fig. 1).

Table 1. Na^+/K^+ ATPase activity in the erythrocytes of healthy sheep (control group) and naturally infected sheep with *Babesia ovis* (infected group) at the onset of the disease and one week after treatment by diminazene aceturate parasites (mean ± SD)

Na^+/K^+ ATPase activity (µmol Pi/h.mg protein	Control group (n = 7)	Infected group (n = 7)	
		During disease	After treatment
	3.63 ± 0.72	0.81 ± 0.28*	1.29 ± 0.81*

* ($P < 0.05$)

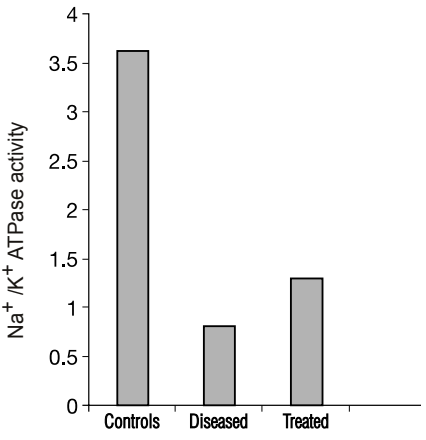

Fig. 1. Na^+/K^+ ATPase activity of controls, diseased and treated sheep with *Babesia ovis* (µmol Pi/h.mg protein).

Discussion

There are important changes in the biochemistry of hosts suffering from parasitic invasions depending on the species of the parasites and the sites of the hosts they invade (Özer et al. 1995; Russel and McDowell 1989). *Babesia* agents are intraerythrocytic parasites that have destructive effects on the erythrocytes of the hosts (Ginsburg and Atamina 1994).

Na^+/K^+ATPase is present in the plasma membrane of most eukaryotic cells and exchanges intracellular Na^+ for extracellular K^+ using the energy of ATP hydrolysis. Na^+/K^+ATPase controls directly or indirectly many essential cellular functions and regulation of this enzyme is believed to play a key role in the etiology of various pathological processes (Kyunglim 2001).

The present study was conducted to examine

the effect of babesia infection on erythrocyte Na^+/K^+ATPase enzyme activity. Although the evaluation of Na^+/K^+ATPase activity has not been reported previously in babesia species, we found several studies carried out on malaria species which are intra-erythrocytic protozoan parasites like babesia.

In an early study, Dunn (1969) postulated that increased erythrocyte Na^+/K^+ ratio of monkeys infected with *Plasmodium knowlesi* is due to the impairment of the Na^+/K^+ATPase pump activity. Besides, Ginsburg et al. (1986) put forward that erythrocytes infected with *Plasmodium falciparum* gain Na^+ and lose K^+ ions because of inhibition of erythrocyte Na^+/K^+ATPase activity.

The mechanism(s) underlying the inhibition of the Na^+/K^+ pump is unclear. Kirk (2001) informs that after malaria infection, an erythrocyte undergoes many modifications of its physical/chemical properties, any of which might be expected to alter the activity of endogenous transport systems. The lipid composition of the erythrocyte membrane is altered, as are the cytoplasmic ion and, perhaps, protein concentrations. All of these are known to influence the activity of endogenous transporters and channels including Na^+/K^+ATPase activity.

In addition to this, it is claimed that new permeation pathways (NPPs) induced by the parasites may affect the activity of endogenous transporters (Cabantchik 1990; Staines et al. 2000; Kutner et al. 1985). Merckx et al. (2009) also report that in order to survive within erythrocytes, parasites alter the permeability of the host plasma membrane, either by upregulating existing transporters, or by creating NPPs.

Staines et al. (2001) found that Na^+/K^+ATPase activity is increased in human erythrocytes infected with the malaria parasite *Plasmodium falciparum* in the period 24–36 h post invasion to hinder the leakage of ions via the NPPs. However, in the latter 12 h of the parasite's occupancy of the erythrocyte (36–48 h post invasion), the flux of ions via the NPP increases whereas the Na^+/K^+ATPase activity undergoes a progressive decrease.

In this study, babesia infection induced a significant decrease of erythrocyte Na^+/K^+ATPase enzyme activity. Besides, the activity of this enzyme after a two-week treatment was still significantly low compared to controls. It is well known that after recovery, animals become carriers of the parasite (Kaufmann 1996) and the decreased erythrocyte Na^+/K^+ATPase enzyme activity was probably due to the carrier state of treated animals.

On the other hand, Tanabe et al. (1983) found no change in Na^+/K^+ATPase activity of the *Plasmodium chabaudi* infected mice using membrane vesicles prepared from parasitized erythrocytes. Accordingly, Mohan et al. (1994) notified that erythrocyte Na^+/K^+ATPase activity did not reveal any significant change in human infected with *Plasmodium falciparum*.

The results of this study suggest that it is likely that the decreased erythrocyte Na^+/K^+ATPase activity in *Babesia ovis* may be due to the usage of NPPs for the flux of ions instead of Na^+/K^+ pump.

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