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Increase nitric oxide and oxidative stress in dogs experimentally infected by *Ehrlichia canis*: Effect on the pathogenesis of the disease

Aleksandro S. Da Silva [a,*], Thiago D. Munhoz [b], Joice L.M. Faria [c], Giovanni Vargas-Hérnandez [b], Rosangela Z. Machado [d], Taís C. Almeida [e], Rafael N. Moresco [e], Lenita M. Stefani [a], Mirela Tinucci-Costa [b,**]

[a] Department of Animal Science, Universidade do Estado de Santa Catarina (UDESC), Chapecó, SC, Brazil
[b] Department of Veterinary Clinical and Surgery, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brazil
[c] Universidade de Uberaba (UNIUBE), Uberaba, MG, Brazil
[d] Department of Veterinary Pathology, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista (UNESP), Jaboticabal, SP, Brazil
[e] Department of Clinical and Toxicological Analysis, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil

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ABSTRACT

The aim of this study was to evaluate nitric oxide levels, lipid peroxidation, protein oxidation and glutathione reductase activity in serum of dogs experimentally infected by *Ehrlichia canis*. Banked serum samples of dogs divided into two groups were used: negative control ($n = 5$) and infected by *E. canis* ($n = 5$). The concentration of nitrite/nitrate (NO_x), lipid peroxidation (TBARS), advanced oxidation protein products (AOPP), and glutathione reductase (GR) activity in sera were evaluated. Samples were collected on days 0, 3, 6, 18 and 30 post-infection (PI). NO_x and TBARS levels were significantly ($P < 0.05$) higher in the infected group at 18 and 30 days PI, as well as AOPP levels at 30 days PI when compared to samples from control group. The GR activity was significant ($P < 0.05$) increased in serum of dogs infected by *E. canis* on days 18 and 30 PI. Based on the increased levels of NO_x, TBARS, AOPP and GR activity we concluded that dogs experimentally infected by *E. canis* develop a state of redox imbalance and that these changes might be involved in the pathophysiology of the disease.

1. Introduction

Ehrlichia canis is an obligate, intracytoplasmic parasitic disease that affects the canidae and is the causative agent of canine monocytic ehrlichiosis (Woody and Hoskins, 1991; Bulla et al., 2004). The disease has a worldwide distribution, and is transmitted through the saliva of the brown dog tick, mainly *Rhipicephalus sanguineus* (Smith et al., 1976; Bulla et al., 2004). This disease has no age or sex preference and compromises its host in several different ways, with varying degrees of severity (Castro et al., 2004; Nakaghi et al., 2008).

E. canis infection results in parasite replication inside dog mononuclear phagocytic cells with the formation of morulae or colony of bacteria surrounded by a vacuolar membrane (Cohn, 2003), resulting in an asymptomatic, acute or chronic infection. Dogs infected by *E. canis* may have hyperthermia, anorexia, weight loss, edema,

* Corresponding author. Tel.: +55 55 3220 8958.
** Corresponding author.
E-mail addresses: dasilva.aleksandro@gmail.com, aleksandro_ss@yahoo.com.br (A.S. Da Silva), mirelatc@fcav.unesp.br (M. Tinucci-Costa).

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hemorrhage, uveitis and blindness, mild anemia, thrombocytopenia and leukopenia (Neer, 1998; Sangione, 2011), clinical signs that may vary according to the clinical phase of infection. Oxidative damage may contribute to the worsening of various infectious diseases, including canine babesiosis and canine rangeliosis (Chaudhuri et al., 2008; Franca et al., 2012). One study concluded that oxidative stress not resulted from only ehrlichiosis, but when ehrlichiosis is associated with babesiosis, the oxidative damage may appear (Kumar et al., 2006).

According to the literature, free radicals and other reactive oxygen species play an important role in tissue damage in a variety of pathological processes (Nohl et al., 1996), resulting in enhanced lipid peroxidation, protein oxidation and DNA breakage (Halliwell, 1994; Witko-Sarsat et al., 1998). To counteract oxidative damage during infections, there is a multilayered defense system including DNA repair systems, scavenging substrates and antioxidant enzyme systems such as superoxide dismutase, catalase and glutathione (Callahan et al., 1988; Gaté et al., 1999). Nitric oxide (NO) reacts with oxygen species and biological molecules, such as dioxygen, superoxide anion and oxyhemoglobin to form a variety of products, including nitrites and nitrates that at high concentrations can be toxic to cells and thus be an indicator of oxidative stress (Beckman and Koppenol, 1996).

Studies of the effect of oxidative stress on the pathophysiology of canine ehrlichiosis are rare. Therefore, the aim of this study was to evaluate the nitric oxide levels, lipid peroxidation, protein oxidation and glutathione reductase activity in serum of dogs experimentally infected by *E. canis* in order to investigate the role of oxidative stress in the pathophysiology of canine ehrlichiosis as well as some of the mechanisms involved in this condition.

2. Materials and methods

2.1. Serum samples

Banked canine serum samples stored at −80 °C collected on days 0, 3, 6, 18 and 30 after *E. canis* inoculation from a previously reported studies (Faria et al., 2011; Munhoz et al., 2012) were used in the current study. The stored serum from five *E. canis* infected dogs and five matched normal controls were used to evaluate NO_x, TBARS and AOPP levels and GR activity. Experimental *E. canis* infection was confirmed by PCR (Munhoz et al., 2012), blood smear, and serology.

2.2. Nitric oxide levels

Nitric oxide levels in serum of dogs infected with *E. canis* were evaluated indirectly, by nitrite/nitrate (NO_x) quantification according to the technique described by Tatsch et al. (2011), and NO_x levels were expressed in micromoles per liter.

2.3. Lipid peroxidation and protein oxidation

Lipid peroxidation (TBARS levels) was measured in sera as described by Jentzsch et al. (1996). Results were obtained by spectrophotometry at 535 nm and expressed as nanomoles of malondialdehyde per milliliter of serum. In addition, protein oxidation was assessed through measurement of AOPP concentrations by a method previously described by Witko-Sarsat et al. (1998). Results were expressed as micromoles per liter.

2.4. Glutathione reductase activity

Serum glutathione reductase activity was assessed by the automated technique using Cobas Mira® analyzer (Hermes et al., 2013) and results were expressed as U/L.

2.5. Statistical analysis

Data were presented as mean values ± standard deviation (SD). NO_x, TBARS, AOPP and GR results were subjected to Student's *t*-test. Values with probability (p) less than 5% were considered statistically different.

3. Results

3.1. Nitrite/nitrate levels

The NO_x levels did not differ ($P > 0.05$) between groups on days 0, 3 and 6 PI. Dogs infected by *E. canis* showed a significant ($P < 0.05$) increase in NO_x levels on days 18 and 30 PI when compared to healthy animals (Fig. 1).

3.2. TBARS and AOPP levels

The TBARS levels did not differ ($P > 0.05$) between groups on days 0, 3 and 6 PI. TBARS level, indicated by MDA concentration, was higher in the infected group on days 18 ($P < 0.05$) and 30 PI ($P < 0.01$; Fig. 2A). On the other hand, AOPP levels in infected dogs increased significantly ($P < 0.05$) only on day 30 PI (Fig. 2B), when compared to not-infected animals.

3.3. Glutatione reductase activity

The glutatione reductase activity did not differ ($P > 0.05$) between groups on days 0, 3 and 6 PI. The GR activity was increased in sera of dogs infected by *E. canis* on

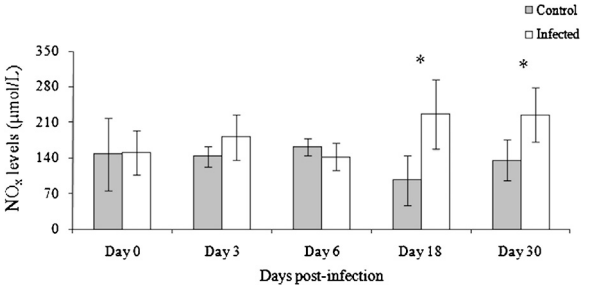

Fig. 1. Dogs experimentally infected by *Ehrlichia canis*. Nitric oxide level in serum samples indicated by nitrite/nitrate (NO_x) concentration of infected dogs compared to not-infected (control) on days 0, 3, 6, 18 and 30 PI. Asterisks indicate statistical difference (*$P < 0.05$) between infected and not-infected groups.