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Acute phase protein response in heartworm-infected dogs after adulticide treatment

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ABSTRACT

During the adulticide treatment of *Dirofilaria immitis* the worms die releasing fragments of parasites and causing pulmonary thromboembolisms which could exacerbate the clinical condition. To determine the utility of acute phase proteins (APPs) to monitor the progression of the treatment, different positive [C-reactive protein (CRP), haptoglobin (hp)] and negative [albumin, paraoxonase-1(PON-1)] APPs were measured in 15 heartworm-infected dogs (5 with high and 10 with low parasite burden) following adulticide treatment. The results showed increased concentrations of CRP, decreased concentrations of haptoglobin and PON-1 in infected dogs before starting the treatment. Progressive but not significant increases were observed in PON-1 activity and albumin concentration along the treatment. After the treatment with doxycycline and ivermectine a decrease in CRP and Hp levels was experienced, which could reflect a reduction of the vascular inflammation caused by the elimination of *Wolbachia* and reduction of microfilariae. Fifteen days after the first melarsomine injection, marked increases in CRP and Hp were observed, which could be due to pulmonary inflammation and thromboembolism caused by the post-adulticide death of the worms. This increase was greater in dogs with high parasite burden. As the pathology disappeared, there was an improvement in the concentrations of CRP and Hp, returning into reference values in dogs with low parasite burden at the end of the treatment. The measurement of CRP and Hp could be a resource of support to evaluate the magnitude of the post-adulticide complications during the adulticide treatment of *D. immitis*.

1. Introduction

Adult *Dirofilaria immitis* (heartworm) reside in pulmonary arteries of the canine host, causing severe and potentially fatal disease, first characterized by pulmonary endarteritis which evolves to chronic vascular remodeling, pulmonary hypertension and congestive heart failure (McCall et al., 2008; Simón et al., 2012). This pathogenic reaction is caused by the host's response to the parasite itself, adult or microfilariae, as well as the bacterial endosymbiont *Wolbachia pipientis*, which resides in all biological stages of the parasite, triggering the release of proinflammatory and chemotactic cytokines which induce cellular infiltration and amplification of the inflammatory response (Hise et al., 2004; Kramer et al., 2008). Moreover, during the adulticide treatment the worms die

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releasing fragments of parasites and causing pulmonary thromboembolisms which could exacerbate the clinical condition and symptoms.

It has been reported that there is an acute phase response in dogs with *D. immitis* that is characterized by an increase in C-reactive protein (CRP), a decrease in albumin and paraoxonase-1 (PON-1), as well as by a divergence in the behavior between CRP and haptoglobin (Hp), since increases in CRP were not accompanied by increases in Hp, and Hp even decreased in the dogs with microfilaria (Méndez et al., 2014). Furthermore, it has been suggested that the presence of a moderate increase in CRP and this discrepancy in the results of CRP and Hp could raise the suspicion of heartworm infection in endemic areas.

Besides the utility to support in the assessment of the severity of a disease, the determination of acute phase proteins (APPs) also may be of value to monitor the progression of the disease and the response to the treatment in different inflammatory diseases in the dog (Ceron et al., 2005; Martinez-Subiela et al., 2011; Mylonakis et al., 2011). In the case of canine heartworm disease, it has been recently reported that CRP concentrations are related with the severity of the disease and the development of endothelial damage (Carretón et al., 2014; Venco et al., 2014). However, to the author's knowledge, no information is currently available regarding the utility of APPs to monitor the response to treatment in this disease.

APPs are classified into two groups based on their response to the triggering event; negative APPs are those whose levels are diminished and positive APPs are those whose levels are increased when there is an acute phase response (Ceron et al., 2005; Tvarijonaviciute et al., 2012). The aim of the present study was to evaluate the concentrations of different positive (CRP and Hp) and negative (albumin and PON-1) APPs in heartworm infected dogs following adulticide treatment.

2. Materials and methods

2.1. Animals and samples

Fifteen client-owned dogs brought to the Veterinary Medicine Service of Las Palmas de Gran Canaria University were included in the study. The dogs lived in a hyperendemic area of *D. immitis* (Montoya-Alonso et al., 2011; Carretón et al., 2012).

Nine were males and six were females; age ranged from 2 to 10 years, with a mean age of 5.13 years. Inclusion in the study was based on a positive result for circulating *D. immitis* antigens (Uranotest Dirofilaria®, Urano Vet SL, Barcelona, Spain). Dogs were further evaluated for the presence or absence of microfilariae using a modified Knott test. In addition, echocardiographic findings (Venco et al., 2003) and qualitative antigen testing were evaluated to estimate the worm burden, and according to the results the dogs were divided into two groups (low and high parasite burden).

All the owners provided informed consent for their dogs' enrolment. The study was approved by the Ethics Committee of the Veterinary Medicine Service of Las Palmas de Gran Canaria University and was carried out in accordance to current European legislation on animal protection.

The dogs received adulticide treatment following the American Heartworm Society recommended management protocol (American Heartworm Society, 2014). Briefly, on day 0 the dog is diagnosed and verified as heartworm positive, starting monthly heartworm preventative based on ivermectine (6 μg/kg) and the administration of doxycycline (10 mg/kg BID) for 4 weeks. On day 60 the dog is treated with the first intramuscular injection of melarsomine (2.5 mg/kg), followed on day 90 by a second injection, and a third injection on day 91. On day 120 the dog is clinically examined and discharged. Finally, on day 271, 6 months after completion, an antigen test is carried out to confirm the adulticide efficacy. It was recommended moderate exercise restriction from day 0 until the administration of the first adulticide injection (day 60), and strict exercise restriction afterwards (home rest, always on leash when walking).

Blood samples were drawn from the cephalic vein of each animal on day 0, day 60 (before the first melarsomine injection), day 75 and day 90 (2 and 4 weeks after the first melarsomine injection, respectively) and day 120. Blood was collected into tubes without anticoagulant, serum was obtained and frozen (−20 °C) until analysis.

2.2. Determination of acute phase proteins

The concentration of CRP was measured using a human immunoturbidimetric test (CRP OSR 6147 Olympus Life and Material Science Europe GmbH, Lismeehan, O'Callaghan Mills, Co. Clare, Ireland) previously evaluated for use in dogs (Caldin et al., 2009).

Hp concentrations were measured by a commercially available colorimetric method (kit haptoglobin Tridelta phase range, Tridelta Development Ltd.) that was previously validated in dogs (Martínez-Subiela et al., 2003; Martínez-Subiela and Cerón, 2005).

Albumin was determined using a commercially available Bromocresol green reagent [Albumin OSR 6102 Olympus Life and Material Science Europe GmbH (Irish branch) Lismeehan, O'Callaghan's Mills, Co. Clare, Ireland].

Serum PON-1 activity was determined using *p*-nitrophenyl acetate as substrate, following a previously described method validated for use in dogs (Tvarijonaviciute et al., 2012).

All determinations were performed in serum on an automated biochemistry analyser (Olympus AU600 Automatic Chemistry Analyzer, Olympus Europe GmbH, Hamburg, Germany).

2.3. Statistical analysis

The Graphpad Prism software was used for all data analysis. The results were evaluated for approximate normality of distribution by using the Shapiro–Wilk normality test, giving a nonparametric distribution; therefore, all data were normalized by logarithmic transformation before analysis. Medians and percentiles were calculated using routine descriptive statistical procedures. One-way ANOVA of repeated measures followed by Fisher's LSD test was