Veterinary Parasitology 244 (2017) 35–38

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short communication

Evaluation of cardiopulmonary and inflammatory markers in dogs with heartworm infection treated using the slow kill method

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ARTICLE INFO

Keywords:
Heartworm
Biomarker
Slow kill
Doxycycline
Interleukin-6

ABSTRACT

This study evaluated the changes in the levels of cardiac, hemostatic, and inflammatory biomarkers in 12 dogs with different severities of heartworm infection treated using the slow kill protocol, consisting of 6–10 μg/kg of ivermectin and 10 mg/kg of doxycycline combination. The serum levels of cardiac troponin-I, D-dimer, C-reactive protein, and interleukin-6 were measured on the day of diagnosis (D0), after termination of doxycycline administration (D30), after termination of the slow kill treatment (D180), and 10 months after the initiation of therapy (D300). Heartworm antigenemia was cleared in 4/4 class I dogs, 3/4 class II dogs, and 1/4 class III dogs at the end of the therapy (D180), and in 4/4 class I, 4/4 class II, and 1/4 class III dogs at the end of the study (D300). The serum levels of the markers in class I dogs on the day of diagnosis (D0) were within the reference range, while the levels in class II and III dogs were above the reference range. Further, the serum levels of the markers in all dogs decreased significantly at the end of the study (D300), although some markers in class III dogs remained at pathological levels. This study revealed that the slow kill method should be used only as an alternative therapeutic protocol for dogs with low worm burden (class I and II). As the slow kill method alone may not effectively reduce all pathological changes in dogs with heavy worm burden and severe clinical signs (class III), adjuvant therapies including steroids and anti-thromboembolics should be used to minimize the risk of complications.

1. Introduction

Canine heartworm disease (HWD) is a mosquito-borne parasitic disease caused by *Dirofilaria immitis*. The infection can cause mild to severe cardiopulmonary disease in dogs; the severity depends on the worm burden and host immune reaction (Rawlings and Calvert, 1995). The 2014 American Heartworm Society (AHS) therapeutic protocol consists of three doses of intramuscular melarsomine injection along with steroid and anti-thromboembolic therapies, if necessary; it is the most popular and proven therapeutic protocol for dogs with HWD. Due to complications such as pulmonary thromboembolism and intense pulmonary pro-inflammatory reactions from adulticide therapy with melarsomine (Kramer et al., 2011), several optional therapeutic protocols have been proposed to reduce the risk of complications during treatment (Rawlings et al., 2001; Venco et al., 2004; Grandi et al., 2010; Kramer et al., 2011). However, adverse reactions related to adulticide therapy are inevitable in any type of therapeutic protocol. One study reported that the combination of doxycycline and ivermectin could result in 100% microfilaremia and 72.7% antigenemia clearance at the end of therapy (day 180; 85.7% of positive dogs became negative by day 300; Grandi et al., 2010). Moreover, monthly thoracic radiography and echocardiography have demonstrated progressively improved diagnostic parameters for pulmonary hypertension and pulmonary inflammation in approximately 70% of treated dogs (Mavropoulou et al., 2014). However, the efficacy of this slow kill method may differ according to clinical status and worm burden in dogs with HWD. Therefore, additional controlled studies are necessary to validate the usefulness of the slow kill protocol in dogs.

Biomarkers reflecting cardiopulmonary, inflammatory, and hemostatic changes during HWD infection are useful for assessing the severity of HWD and for monitoring clinical outcomes during the therapeutic period in dogs (Carretón et al., 2011, 2013a, 2013b, 2014a, 2014b; Venco et al., 2014). It has been shown that the clinical outcome, including complication and success rates, is generally correlated to worm burden and the status of the clinical presentation (Bowman and Atkins, 2009; Carretón et al., 2014b). Therefore, we aimed to evaluate the changes in cardiac (i.e., cardiac troponin-I [cTnI]), hemostatic (i.e., D-dimer), and inflammatory biomarkers (i.e., C-reactive protein [CRP] and interleukin 6 [IL-6]) in heartworm (HW)-infected dogs treated with a combination of ivermectin and doxycycline (the slow kill protocol).

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http://dx.doi.org/10.1016/j.vetpar.2017.07.015
Received 31 January 2017; Received in revised form 22 June 2017; Accepted 19 July 2017

2. Materials and methods

2.1. Study population

Twelve dogs infected with HW were enrolled in this study. All dogs were brought from a private animal shelter. The owner of the shelter gave consent for the participation of the dogs in this study. The approval of the animal ethics committee at the Kangwon National University was obtained for this study prior to commencement. The HW infection was diagnosed using a commercial enzyme-linked immunosorbent assay (ELISA) kit (SNAP® 4Dx® Plus Test, Idexx Laboratory, Westbrook, ME, USA), according to the manufacturer's instructions. The presence of microfilariae was evaluated using a modified Knott test. Dogs were divided into three groups (n = 4 per group) based on the severity of the disease described in the 2014 AHS guidelines. Class IV dogs (with caval syndrome) were not included in the study, as adulticidal therapy is not recommended for them. The severity of HWD in each dog was confirmed by physical, radiographic, and echocardiographic examinations. Briefly, class I dogs showed no abnormal physical, radiographic, or echocardiographic findings, whereas class II dogs displayed mild clinical signs that included occasional coughing with or without exercise intolerance. These were combined with radiographic abnormalities including mild pulmonary infiltration and pulmonary arterial dilation, and echocardiographic abnormalities including dilation of the main pulmonary artery without interventricular septal flattening. Class III dogs displayed more persistent and severe clinical signs, such as persistent coughing, severe exercise intolerance, weight loss, cachexia, and signs related to right-sided congestive heart failure. These were accompanied by radiographic abnormalities including marked pulmonary infiltration with pulmonary arterial dilation and right ventricular enlargement, as well as echocardiographic evidences of pulmonary hypertension (i.e., dilation of the main pulmonary artery, > 2.8 m/s of peak velocity of tricuspid regurgitation, and/or > 2.2 m/s of peak velocity of pulmonary regurgitation). The degree of worm burden was low in dogs with class I and II, whereas was high in dogs with class III on the right and left parasternal short axis of pulmonary artery plane of echocardiography. All dogs were treated with the slow kill protocol, which consisted of a 4-week oral administration of doxycycline (10 mg/kg, q24hr, Vibravet, Zoetis, Seoul, Korea) and a 6-month oral administration of ivermectin (6–10 μg/kg, q15 day, Heartgard®, Merial, Seoul, Korea). After termination of slow kill therapy (D180), a heartworm preventive drug such as Heartgard® was administered monthly until the end of the study (D300). During slow kill therapy (until D180), dogs were restricted from performing vigorous exercise but allowed to perform mild exercise, such as walking. The HW infection status was re-checked at the end of therapy (D180) and study (D300) using HW antigen test and echocardiography.

2.2. Laboratory evaluations

To measure the concentration of the biomarkers, blood samples were drawn from the cephalic vein of each animal and centrifuged immediately at 4000*g* for 10 min. Subsequently, three aliquots of plasma were stored either at −80 °C until testing (IL-6) or on dry ice until transportation to the reference laboratory (cTnI, D-dimer, and CRP). The cTn-I, D-dimer, and CRP values in this study population were determined by reference laboratories (cTnI by IDEXX Laboratories, Sungnam, Korea; D-dimer and CRP by Neodin Vetlab, Seoul, Korea). Reference ranges of cTnI, D-dimer, CRP, and IL-6 for healthy dogs were established as 0-0.08 ng/mL, 0-0.25 μg/mL, 0–17 mg/mL, and 2–54 pg/mL, respectively, according to the reference laboratory. The canine IL-6 ELISA (Cat. No. MBS2502139, MyBiosource, San Diego, CA, USA) was purchased and used within a week of reception. The inter-assay and intra-assay coefficients of variation were 5.6% and 7.9%, respectively, with a lower and higher limit of detection of 5.0 and 250 pg/mL, respectively. The serum levels of several markers were measured on the day of diagnosis (D0), after termination of doxycycline administration (D30), after termination of slow kill treatment (D180), and 10 months after initiation of therapy (D300).

2.3. Statistical analyses

All statistical analyses were performed using the statistical software MedCalc 12.1.3.0 for Windows (MedCalc Software, Ostend, Belgium). Descriptive statistics were generated, and normality testing with the Kolmogorov–Smirnov test for all continuous variables was performed. Data are reported as median and range unless otherwise stated. Differences in continuous data among different HWD classes were determined by the Kruskal–Wallis test. The median of each biomarker at baseline (D0) was compared to each time point after initiation of therapy using a Mann–Whitney *U* test. A P-value < 0.05 was considered significant.

3. Results

We were not able to determine the ages of the dogs enrolled in this study, although all were mature. The average body weights in the class I, II, and III groups were 6.7 ± 1.4 kg (2 males and 2 females), 11.3 ± 7.4 kg (2 males and 2 females), and 7.9 ± 3.6 kg (2 males and 2 females), respectively. All dogs survived and tested negative for microfilariae at the end of therapy (D180) and the study (D300). However, 1/4 dogs in class II, and 3/4 dogs in class III tested positive for the heartworm antigens at the end of therapy (D180). In addition, 3/4 dogs in class III still tested positive at the end of the study (D300). There were significant differences in all biomarker levels among the study groups on the day of diagnosis (D0; P < 0.05; Fig. 1).

The cTnI levels on the day of diagnosis (D0) were significantly higher in dogs with more severe clinical signs (P < 0.05; Fig. 1). Pathological levels of cTnI were found in 3/4 dogs in class II, and 4/4 dogs in class III on the day of diagnosis (D0), but not at the end of therapy (D180) or the study (D300). The cTnI levels of all class groups significantly decreased after initiation of therapy (P < 0.05; Fig. 1). The median cTnI levels returned to non-pathological levels at the end of the therapy (D180) in all classes (Fig. 1).

D-dimer levels on the day of diagnosis (D0) were significantly higher in dogs with more severe clinical signs (P < 0.05; Fig. 1). Pathological levels of D-dimer were found in 2/4 dogs in class II and 4/4 dogs in class III on the day of diagnosis (D0), and in 2/4 dogs in the class III group at the end of the study (D300). Although D-dimer levels in all classes significantly decreased after the initiation of therapy, they were consistently higher than the reference range even after the end of therapy (D180) in class III dogs.

The CRP levels on the day of diagnosis (D0) were significantly higher in dogs with more severe clinical signs (P < 0.05; Fig. 1). Pathological levels of CRP were found in 2/4 class II dogs and 4/4 class III dogs on the day of diagnosis (D0). The CRP level in class III dogs was persistently higher than the reference range during the study period (Fig. 1), although it tended to decrease after therapy. At the end of therapy (D180), 2/4 dogs in class II and 4/4 dogs in class III displayed pathological levels of CRP. Furthermore, 1/4 dogs in class II and 4/4 dogs in class III showed pathological levels of CRP at the end of the study (D300; Fig. 1).

The IL-6 levels on the day of diagnosis (D0) were significantly higher in dogs with more severe clinical signs (P < 0.05; Fig. 1). Pathological levels of IL-6 were found in 2/4 dogs in class II and 4/4 dogs in class III on the day of diagnosis (D0). The IL-6 levels in class III dogs were persistently higher than the reference range during treatment (Fig. 1), although the levels tended to decrease after therapy. At the end of therapy (D180), 1/4 dogs in class II and 4/4 dogs in class III had pathological levels of IL-6. Furthermore, 1/4 dogs in class II and 2/4 dogs in class III had pathological levels of IL-6 at the end of the study