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Effects of intravenous and subcutaneous heartworm homogenate from doxycycline-treated and untreated donor dogs on bronchial reactivity and lung in cats

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ABSTRACT

A controlled, blind research study was conducted to define the innate response of lungs in specific pathogen free (SPF) cats to intravenous ($n = 10$) or subcutaneous ($n = 4$) administration of homogenate of adult *Dirofilaria immitis* from donor dogs compared with lung response in control cats ($n = 6$). There was no difference in cats that received heartworm homogenate IV for 18 days from donor dogs treated with doxycycline for 1 month compared with cats given heartworm homogenate from untreated donor dogs. Cats did not develop clinical signs, and no radiographic changes were noted. Cats given SC heartworm homogenate at lower concentration than IV groups did not develop histologic changes. Cats that received IV heartworm homogenate for 18 days developed mild interstitial and peribronchial myofibrocyte proliferation and smooth muscle proliferation of the pulmonary arteries.

Bronchial ring contractility *in vitro* was blunted in the IV homogenate cats to the agonists acetylcholine and 5-hydroxytryptamine. Cats in the SC group had increased sensitivity to histamine at high concentrations but normal contractility and relaxation responses to other agonists. No increase in mast cells was noted in lung tissues of cats given homogenate. In the absence of bronchial wall remodeling, cats given IV homogenate had blunted responses to bronchial constriction, but normal relaxation to nitroprusside and substance P and increased sensitivity to histamine. In the absence adult heartworms, the homogenate of adult heartworms in the circulation of SPF cats induced a direct effect on lung parenchyma and altered bronchial ring reactivity.

1. Introduction

Heartworm disease in cats results in an intense inflammatory response (Dillon, 2008; Lee and Atkins, 2010; Holmes et al., 1992). The interstitial lung disease and pulmonary arterial involvement often develop in lobes and vessels where the parasite does not physically reside (Dillon et al., 2007; Dillon et al., 2014). The concept that

Abbreviations: 5-HT, 5-hydroxytryptamine; ACh, acetylcholine; HIS, histamine; ISO, isoproterenol; PSR, picrosirius red; SMA, α-smooth muscle actin; SNP, sodium nitroprusside; SubP, substance P; TB, toluidine blue.

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the physical presence of the worm induces only local direct injury does not correlate with the pattern of the lung disease observed in heartworm-infected cats. The death of the heartworms is associated with an intense focal and downstream effect, but the clinical disease suggests a more complicated pathophysiology than simple embolization. Blunting of bronchial ring reactivity has been demonstrated in cats infected with adult heartworms, but peribronchial smooth muscle proliferation and fibrosis were also noted (Wooldridge et al., 2012).

The purpose of this study was to demonstrate an innate pulmonary response of heartworm-naïve specific pathogen free (SPF) cats exposed to daily doses of adult heartworm homogenate. The hypothesis was that physical presence of the adult heartworm was not required for heartworm byproducts to induce pulmonary pathology, and homogenate in cats without prior sensitization would alter normal bronchial ring reactivity. The role of *Wolbachia* in direct lung pathology was evaluated by treating one donor dog with doxycycline for 31 days prior to homogenate preparation for injection in one group of cats.

2. Material and methods

2.1. Preparation of homogenate

The homogenate was prepared from separate donor dogs for each treatment group (Table 1). On the day of collection, *Dirofilaria immitis* adult worms were collected separately for each dog, washed immediately in modified Hanks Balanced Salt Solution (HBSS, Mediatech, Manassas, VA), and viable male and female worms were separated. From each dog, equal numbers of male and female worms were collected, washed with sterile saline, and homogenized with a rotor tissue homogenizer at 10,000 rpm for 20 min, and the supernate was filtered through 18-μm filters. The ultra-filtrate was diluted with saline to a quantity for daily injections of that group. The homogenate for the two intravenous (IV) groups had the same protein concentration and volume and same total adult heartworm exposure over the 18 days of administration. The protein concentration was determined by validated techniques for cerebral spinal fluid total protein determination (Clinical Pathology Laboratory, College of Veterinary Medicine, Auburn University, AL). Dose aliquots were drawn into individual syringes and stored at 45 F. Each day, homogenized aliquots in the syringes were administered as a single dose to cats in each group.

For the IV homogenate 18-day study, two heartworm donor dogs were utilized (9 months post infection with 200 L_3, MP3 strain). In one dog, doxycycline (Mutual Pharm Co, Philadelphia, PS) was administered (20 mg/kg divided BID orally) for 31 days prior to heartworm collection. The other dog received no treatment before collection of heartworms. Donor dogs with heartworms for subcutaneous (SC) homogenate injections received no treatments before heartworm collection.

2.2. Animal model

Age-matched 6-month-old SPF cats ($n = 20$) were divided into groups: untreated controls ($n = 6$), IV homogenate via indwelling 18-ga catheters for 18 days ($n = 4$), IV homogenate from doxycycline treated dogs via indwelling 18-ga catheters for 18 days ($n = 4$), IV homogenate for 9 days ($n = 2$), and SC homogenate for 18 days ($n = 4$) (Table 1). One day prior to initiation of injections, catheters were introduced into the jugular vein with the cats under sedation with an intramuscular dose of medetomidine (Dormitor®, Pfizer Animal Health, NY), butorphanol (Torbugesic®, Fort Dodge, IA), and ketamine (ketamine hydrochloride, Abbott Lab, Chicago, IL), and secured with bandaging for the duration of the study. On each day of treatment, the catheters were flushed with 1 ml of sterile saline before and after homogenate administration. Cats were monitored daily, and physical examinations performed weekly.

Cats were housed as isolated groups in an environmentally isolated AAALAC accredited facility of the Auburn University Laboratory Animal Health Veterinary Research Building to prevent exposure to mosquitoes. The protocol was approved by the Auburn University Institutional Animal Care and Use Committee.

2.3. Digital radiology

Thoracic radiographs at the completion of the 18-day IV homogenate studies were acquired on randomly selected cats and graded for pulmonary artery, bronchial pattern, interstitial pattern, and mixed bronchial-interstitial-alveolar pattern using a 0–3 scoring system (0 = normal;

Table 1
Treatment groups for heartworm homogenate evaluations in cats.

Group	Route	No. of cats	No. of homogenized heartworms/cat[a]	Dose	Days of treatment (mg/dl)	Homogenate total protein (mg/dl)	Homogenate sodium (mmol/l)
Control	None	6	0	0	18	–	–
Homogenate/ Doxycycline[b]	IV	4	6	1.6	18	26.4	150
Homogenate	IV	4	6	1.6	18	22.4	147
Homogenate	IV	2	3	2.2	9	42.2	150
Homogenate	SC	4	2	0.3	18	11.0	148

IV = intravenous; SC = subcutaneous

[a] All adult worms collected were 9 months post L_3 infection

[b] Heartworms were collected from a donor dog after the dog received 31 days of treatment with doxycycline