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Effects of doxycycline on heartworm embryogenesis, transmission, circulating microfilaria, and adult worms in microfilaremic dogs

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ABSTRACT

Tetracycline treatment of animals or humans infected with filariae that harbor *Wolbachia* endosymbionts blocks further embryogenesis, and existing microfilariae gradually die. This treatment also kills developing larvae and has a slow-kill effect on adult filariae, all presumably due to elimination of the *Wolbachia*. Also, *Dirofilaria immitis* microfilariae in blood collected from dogs up to 25 days after the last dose of doxycycline developed to infective L_3 that were normal in appearance and motility in mosquitoes but did not continue to develop or migrate normally after subcutaneous (SC) injection into dogs. The present study was designed to determine whether heartworm microfilariae collected at later times after treatment would regain the ability to continue normal development in a dog. The study also was expected to yield valuable data on the effects of treatment on microfilariae and antigen levels and adult worms. The study was conducted in 16 dogs as two separate replicates at different times. A total of five dogs (two in Replicate A and three in Replicate B) infected either by SC injection of L_3 or intravenous transplantation of adult heartworms were given doxycycline orally at 10 mg/kg twice daily for 30 days, with three untreated controls. Microfilarial counts in the five treated dogs gradually declined during the 12–13 months after treatment initiation. Two dogs were amicrofilaremic before necropsy and three had 13 or fewer microfilariae/ml. Only one treated dog was negative for heartworm antigen before necropsy. Overall, treated dogs generally had fewer live adult heartworms than controls, and most of their live worms were moribund. All three control dogs remained positive for microfilariae and antigen and had many live worms. L_3 from mosquitoes fed on blood collected 73–77 or 161–164 days after initiation of doxycycline treatments were injected SC into five dogs. None of the dogs injected with L_3 from mosquitoes fed on blood from doxycycline-treated dogs were ever positive for microfilariae or antigen, and none had worms at necropsy; three control dogs were positive for microfilariae and antigen and had many live worms. These data indicate that doxycycline treatment of microfilaremic dogs gradually reduces numbers of microfilariae and blocks further transmission of heartworms.

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This latter effect should be highly effective in reducing the rate of selection of heartworms with genes that confer resistance to macrocyclic lactone preventives and microfilaricides. The data also suggest that doxycycline has a slow-kill effect on adult heartworms.

1. Introduction

Wolbachia endosymbionts are found in all individuals of the filarial species known to harbor them and are thought to be essential for worm survival (Sironi et al., 1995; Taylor et al., 2001, 2005a, 2005b). Proteins derived from these bacteria have been shown to be directly associated with the inflammatory response, innate and adaptive immune responses, and tolerized immunological phenotype in filarial infections (Bazzocchi et al., 2000, 2003, 2007, 2008; Brattig et al., 2001, 2004; Cross et al., 2001; Genchi et al., 2001; Taylor et al., 2001, 2005a; Kramer et al., 2003, 2005, 2008; Punkosdy et al., 2003; Simon et al., 2003; Hise et al., 2004; Morchon et al., 2004; Grandi et al., 2005; Turner et al., 2006; Morchon et al., 2007a, 2007b; McCall et al., 2008b). The genomes of *Brugia pahangi* (Ghedin et al., 2007) and *Dirofilaria immitis* (Gödel et al., 2012) have been sequenced and annotated, and this information should help in developing novel drugs based on the biochemical reactions necessary for the mutualistic symbiosis between filarial parasites and their *Wolbachia* (Foster et al., 2005; Pfarr and Hoerauf, 2005).

Tetracyclines have been shown to inhibit development of larval stages in vertebrate hosts, block embryogenesis, and have a slow-kill effect on adult worms in human filariae, *Onchocerca ochengi* in cattle, and filariae in animal models (Bosshardt et al., 1993; Hoerauf et al., 1999, 2000, 2001, 2003a, 2003b; McCall et al., 1999; Langworthy et al., 2000; Townson et al., 2000; Volkmann et al., 2003; Foster et al., 2005; Taylor et al., 2005b; Debrah et al., 2007). When given daily to heartworm-microfilaremic dogs for 4 weeks, tetracycline drugs, particularly doxycycline, have been shown to be highly effective against third-stage (L_3) and fourth-stage larvae (L_4), at least moderately effective against juvenile heartworms (McCall et al., 2011), highly effective in blocking embryogenesis (Bandi et al., 1999; McCall et al., 2011), and gradual elimination of circulating microfilariae (McCall et al., 2008a), presumably by killing their *Wolbachia* endosymbionts. These drugs also have been shown to kill adult worms of other filarial species, but months to years are often required for this stage to die in these species (Langworthy et al., 2000; Chirgwin et al., 2003; Gilbert et al., 2005; Debrah et al., 2007).

Tetracycline drugs, particularly doxycycline, are becoming widely used by veterinarians prior to adulticidal therapy to reduce the resulting inflammatory response associated with dead heartworms in dogs (American Heartworm Society, 2014). The adulticidal and microfilaricidal activities of these tetracycline drugs have been greatly enhanced by adding ivermectin to the treatment protocol (Bazzocchi et al., 2008; McCall et al., 2008a, 2008b; Grandi et al., 2010). Administration of weekly prophylactic doses of ivermectin along with intermittent doses of doxycycline over a period of 8.5–9.0 months was 78.3% effective in killing adult heartworms (McCall et al., 2008a).

Many heartworm-positive dogs undergoing melarsamine dihydrochloride therapy harbor larval and/or juvenile stages of the parasite, as well as adult worms and microfilariae. A protocol supported by the American Heartworm Society includes pretreatment with doxycycline for 1 month and prophylactic doses of macrocyclic lactone preventives for 2–3 months before administering melarsomine dihydrochloride, which should kill susceptible heartworm larvae that are 1–3 months old (Atkins and Miller, 2003). Pretreatment of heartworm-positive dogs with ivermectin and doxycycline prior to receiving three arsenical (melarsomine dihydrochloride) injections to kill adult heartworms in dogs resulted in less pulmonary pathology associated with worm death in 60% of the dogs, compared with the worm-associated pathology in the dogs treated with melarsomine alone (Dzimianski et al., 2006; McCall et al., 2008a). Additionally, general lung histopathology is significantly reduced (Kramer et al., 2008). Nelson and Sellers (unpublished data) have found that using this pretreatment protocol with doxycycline and ivermectin resulted in fewer moderate-to-severe episodes of thromboembolism and no deaths of dogs for the past 6–7 years.

However, recent concern about the possibility of the existence of populations of heartworms that are resistant to macrocyclic lactones preventives encourages the search for alternative drugs that could be used to kill any heartworm larvae carrying resistant genes at the time of adulticide treatment (American Heartworm Society, 2014).

It has also been shown that *D. immitis* microfilariae (in blood taken from dogs relatively soon after they had been treated with a low dose of doxycycline) fed to mosquitoes developed to third-stage larvae that appeared to be normal in motility and appearance but did not complete their development in the vertebrate host (McCall et al., 2008a). A similar observation also has been made on *Litomosoides sigmodontis*, which is transmitted by mites (Arumugam et al., 2008) and *B. pahangi*, which is transmitted by mosquitoes (McCall, unpublished data).

The main objectives of this study were to determine whether microfilariae in blood collected at later time points during their decline after doxycycline treatment at a relatively high dosage would regain their ability to develop to L_3 that were capable of continuing their development in a dog and to assess the effects of this treatment on post-treatment microfilaria counts and antigen scores and adult worm counts at necropsy. The dosage and treatment schedule for doxycycline used in this study is widely used by veterinarians in adulticide therapy for heartworm-positive dogs (*i.e.*, 10 mg/kg body weight administered twice daily for 1 month). This present report describes partial results from a complex study, which is still ongoing.