Journal of Veterinary Internal Medicine
Open Access

Standard Article

J Vet Intern Med 2018;32:217–221

Efficacy of Minocycline in Naturally Occurring Nonacute *Ehrlichia canis* Infection in Dogs

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Background: Minocycline has been used in the treatment of *Ehrlichia canis* infection in dogs as an alternative to doxycycline, the recommended treatment. However, efficacy of this alternative therapy is unknown.

Objective: To assess the efficacy of minocycline in the treatment of natural occurring *E. canis* infection in dogs.

Animals: Ten privately owned dogs of mixed breed positive for *E. canis* by blood PCR.

Methods: Prospective, randomized clinical study. Dogs positive for *E. canis* by PCR were housed in a kennel environment and randomly allocated to receive doxycycline 10 mg/kg bodyweight PO once daily ("gold standard" control group) or minocycline (extralabel) 10 mg/kg bodyweight PO twice daily (treatment test group) for 28 days. Blood, analyzed by PCR to determine the presence or absence of *E. canis* DNA, was collected weekly during treatment starting on the first day of treatment and including through day 35, 7 days after the last treatment.

Results: In both groups, one dog tested negative after 7 days of treatment. For the doxycycline group, the latest time to a negative PCR test was after 3 weeks of treatment. For the minocycline group, the latest time was on day 28 of treatment. All dogs tested negative 7 days after the end of treatment.

Conclusion and Clinical Importance: Minocycline can be an effective alternative to doxycycline for clearing *E. canis* from the blood in nonacute infections.

Key words: Antibiotic; *Ehrlichia*; Tetracycline; Ticks.

Canine monocytic ehrlichiosis (CME), caused by *Ehrlichia canis*, is an endemic rickettsial disease present in much of the world.[1] The vector for *E. canis* is *Rhipicephalus sanguineus*, the brown dog tick. In an experimental setting, after exposure to *E. canis*, dogs enter an acute phase of disease (2–4 weeks) followed by a subacute phase of infection. A chronic phase of infection, which manifests as a more complex inflammatory disease, occurs in some dogs. Primary clinical signs of infection include lethargy, weight loss, and complications associated with thrombocytopenia. Death is a potential outcome if effective treatment is not provided.[2,3] Detection of *E. canis* infection can be challenging, with blood smears, serology, and PCR tests all having limitations regarding interpretation of results in relation to status of infection. Prevention is achieved with effective tick control; however, due to the intensity of *R. sanguineus* populations in some geographical locations, transstadial transmission, and potential tick resistance to common acaricides, tick control can be challenging.[4]

Abbreviations:

BW	body weight
CME	canine monocytic ehrlichiosis

The recommended treatment for CME is doxycycline (10 mg/kg bodyweight [BW], PO once daily for 28 days), but other drugs such as amicarbalide, chloramphenicol, imidocarb dipropionate, and tetracycline have been studied.[5] While considered safe and effective, treatment failure at various dosages of doxycycline has been reported.[5–8] Some studies suggest that efficacy is related to the stage of infection, with chronic infections potentially more difficult to treat than acute or subacute.[9] Recent shortages of doxycycline suggest that identification of alternative treatments with similar properties to that of doxycycline would be beneficial.[10–12]

Minocycline, another drug in the tetracycline family, has been used as an alternative to doxycycline in veterinary medical practices, with pharmacokinetic studies performed for some dieases.[13–20] Little information is available regarding efficacy of minocycline for the treatment of rickettsial disease.[11,21] The Centers for Disease Control and Prevention has not encouraged the treatment of human rickettsial infections with minocycline due to the lack of efficacy data.[10] In veterinary medicine, minocycline at 20 mg/kg PO Q12h has been suggested as a potentially effective treatment of CME.[18] The greater lipophilic properties and high tissue concentrations of minocycline could be beneficial in *E. canis* treatment as compared to doxycycline.[19,20] This may be especially true if *E. canis* has invaded the central nervous system.[22,23]

The objective of this study was to obtain preliminary data on the efficacy of minocycline in the treatment of

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This work was conducted on the island of St. Kitts utilizing the laboratory facilities at Ross University School of Veterinary Medicine and the kennels at Ponds Veterinary Clinic, Ponds Estate, Basseterre, St. Kitts.

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Submitted July 30, 2016; Revised July 29, 2017; Accepted August 28, 2017.

DOI: 10.1111/jvim.14842

dogs with naturally acquired CME as determined by peripheral blood clearance of DNA using PCR. The study was conducted on St. Kitts where there is only one species of *Ehrlichia, E. canis*, and where CME is a commonly diagnosed disease.[24]

Materials and Methods

Study Design, Animals, and Inclusion and Exclusion Criteria

This prospective, randomized, clinical study was conducted under the Ross University School of Veterinary Medicine Institutional Animal Care and Use Committee approved protocol 14-7-034. All animal housing facilities were inspected and approved by the committee. The efficacy of minocycline (extralabel) was tested with doxycycline used as a positive control ("gold standard"). Upon obtaining informed owner consent, male and female dogs of any breed were recruited for use in the study and subsequently returned to the owners after the study. Before enrollment, dogs were screened for *E. canis* exposure by serology by a commercial antibody test.[a] Samples positive for *Ehrlichia* spp. were further tested by PCR. Dogs positive by PCR and deemed healthy to participate, based on a physical examination, were included in the study.

Thirteen dogs were identified for inclusion and enrolled in the study. An acclimation period of 28 days was used to confirm infection status and to ensure dogs were beyond the acute infection period. PCR tests for *E. canis* were performed during the acclimation period to verify continued infection. Also during this time, and throughout the study, dogs were treated for ticks to control infestation.[b] The dogs also received pyrantel pamoate as a general dewormer.[c]

Allocation to treatment group occurred on the day of first treatment (N = 6 per group; one of the 13 dogs died during acclimation due to an unrelated cause). Randomization was performed by generating a coin toss table, utilizing heads for minocycline and tails for doxycycline. Names of the dogs were drawn from a hat and assigned, sequentially, to the head/tail chart. Doxycycline[d] was administered PO as per the recommended dose (10 mg/kg BW once daily); minocycline[e] was administered PO twice daily at 10 mg/kg BW. Treatments were administered at feeding time using capsules of 50 or 100 mg. The first treatment occurred on day 1 with the final treatment on day 28 of the study. Treatments were administered at approximately the same time each day.

Schedule of Events

Thirteen dogs, presenting at the Ponds Veterinary Clinic between October and November 2014, were identified as positive for *E. canis* by PCR. In December 2014, all identified dogs were housed in an approved outdoor kennel facility for an acclimation period (28 days). During acclimation and throughout the study, dogs remained under the primary care of the clinic with secondary veterinary care provided by the Ross University Veterinary Clinic.

A basic physical examination was performed before being placed on acclimation, and a comprehensive examination was performed before treatment. After acclimation, treatment started on day 1 and ended on day 28. Blood collection for PCR analysis occurred on days 1, 7, 14, 21, 28, and 35. Hematology analysis (22-parameter CBC including PLT, PCV, WBC, LYM, NEU, and HGB) was performed on blood before treatment and on days 28 and 35.[f] Clinical chemistry analysis (ALB, ALP, ALT, TBil, BUN, Ca, Phos, Cre, Glu, Na, K, TP, and Glob) was performed on blood collected pretreatment and on day 35.[g] During acclimation and treatment, general health observations (alertness, fecal consistency, feed consumption, eyes [discharge or other abnormality], and respiration) were performed twice daily with any abnormalities noted. The dogs were monitored on days 1–35 for any adverse events, suspected to be related or not related to treatment, and treated as appropriate. Dogs were returned to their owners at the completion of the study.

Ehrlichia canis Detection

Ehrlichia canis exposure was determined by an antibody test for *E. canis* and *Ehrlichia ewingii,* and infection was confirmed by PCR using a previously published protocol for *Ehrlichia* species.[25,26] *Ehrlichia ewingii* nor the vector, *Amblyomma americanum,* is not known to exist on St. Kitts and, therefore, positive results with both tests were used to confirm *E. canis* infection.[27] For the PCR analysis, DNA was extracted from 100 μL of blood or buffy coat with a standard kit.[h] Elution was performed with 200-μL manufacturer's buffer or 50 μL, followed by 100 μL buffer. Presence of DNA in the samples was confirmed by NanoQuant absorbance.[i] Positive and negative controls were included and consisted of DNA from a confirmed positive dog and the reagents with DNase-free distilled water, respectively. PCR was performed by "Kiss" Fluorescence Resonance Energy Transfer (FRET) and a Roche LightCycler 2.0 for DNA amplification and software analysis.[j] PCR analysis was performed, targeting the 16s rRNA (*rrs*) gene with the following primers: forward primer (5′-GAGGATTTTATCTTTGTATTGTAGC TAAC-3′), reverse primer (5′-TGTAAGGTCCAGCCGAACTG ACT-3′), fluorescein probe (5′-ACGCGAAAAACCTTACCACTT TTTGAC-6-FAM-3′), and the LCRed 640 probe (5′-LCRed640-X_n-phosphate-3′)g with a sensitivity of 5 copies per PCR.[k] A positive amplification curve and the melting temperature were used to confirm the presence of *E. canis* DNA.[25]

Outcome Evaluation and Statistical Analysis

The primary outcome of this study was to assess the treatment success of minocycline with success defined as negative PCR results from peripheral blood. Statistical comparisons between groups (Mann–Whitney test)[l] were conducted to determine whether there was a difference in the time to clearance between antibiotics.

Results

In this study, 2 dogs tested negative for *E. canis* by PCR on day 1 and were therefore excluded from statistical analysis, resulting in five dogs (4 males and 1 female) per treatment group. In the minocycline group, dogs ranged from 1 to 6 years of age and 3 to 21 kg BW. In the doxycycline group, dogs ranged from 3 to 6 years of age and 6 to 32 kg BW. One dog in each group was *Anaplasma* spp. antibody positive based on the commercial kit. Treatment administration was successful for all dogs on all days with the average dose of doxycycline being 12 mg/kg BW and for minocycline being 11 mg/kg BW. There were no adverse events related to treatments administered during the study. In addition to the tick control and endo-parasite treatments, 1 dog in the doxycycline group received cephalexin (10 mg/kg BW BID × 14 d). None of the concomitant treatments administered have known efficacy against or interaction with *E. canis*.

Treatment success, narrowly defined as negative for *E. canis* in peripheral blood by PCR by end of 28 days

Table 1. Time to *Ehrlichia canis* clearance from blood based on PCR after commencement of treatment on day 0.

Cumulative Number of Dogs Negative at Each Time Point		
Days After Treatment Commenced	Doxycycline Treated (N = 5)	Minocycline Treated (N = 5)
7	1	1
14	4	4
21	5	4
28	5	5
35	5	5

Results were not statistically significantly different.

of treatment, was 100% in both groups (Table 1). In the doxycycline group, the earliest time to a negative test was after 7 days of treatment and the longest after 21 days of treatment (mean 2; median 2 weeks). In the minocycline group, the earliest time to a negative test also was after 7 days of treatment and the longest after 28 days of treatment (mean 2.2; median 2 weeks). No significant difference was found in the time to a negative test (Mann–Whitney test; $P > 0.1$). All dogs remained negative for *E. canis* in peripheral blood 7 days after the last treatment.

Hematology and clinical chemistry revealed across both treatment groups the presence of thrombocytopenia, lymphocytosis, anemia, and hyperglobulinemia, which are all common parameters affected by *E. canis* infection. Table 2 provides an overview of how the parameters changed by end of the 28-day treatment period and their status 7 days after treatment was completed.

Discussion

This pilot study demonstrates that minocycline, at 10 mg/kg twice daily for 28 days, successfully cleared or suppressed *E. canis* below the detectable limits of the PCR method used in dogs with nonacute natural infections. Whereas the follow-up samples 7 days after treatment remained below detectable limits, conclusions regarding complete clearance of the infections were not feasible. Even with the sensitivity of PCR, bacteria numbers can be suppressed below the detection limit with the use of antibiotics. This can lead to less reliable conclusions regarding complete bacterial clearance. Four dogs in the doxycycline treated group and two in the minocycline group were still thrombocytopenic at the end of treatment, suggesting that suppression occurred with both treatments versus clearance. However, the positive percent change in platelets, as well as one of the doxycycline treated thrombocytopenic dogs having normal platelet counts 7 days after completion of treatment, might also suggest a slow recovery.

Splenic and bone marrow PCR testing may have enabled detection of noncirculating *E. canis* to determine whether suppression or clearance occurred. An alternative would have been the use of immunosuppressive steroids to force recrudescence of infection for improved PCR detection. Due to the use of private dogs and limitations imposed by the IACUC and the dog owners, biopsies and use of steroids were not permitted. Further retesting of dogs once returned to their owners was not an option due to the exceptionally high tick pressure found on St Kitts. If follow-up samples were collected after return of the dogs to their owners

Table 2. Median value (minimum; maximum) of selected blood parameters of dogs with *Ehrlichia canis* and number of dogs below (↓) or above (↑) the normal range.

Parameter (Normal Range)	PCV (37.5–55.0%)	Lymphocytes (1–4.8 × 10^9)	Platelets (200–500 × 10^9)	Globulin (2.3–5.2 g/dL)
Doxycycline treated (N = 5)				
Pretreatment	40 (31; 44) 1 ↓	2.8 (0.9; 7.0) 1 ↓; 1 ↑	86 (55; 225) 4 ↓	7.1 (3.8; 7.8) 4 ↑
Day 28 of treatment	40[b] (38; 45) –	2.6 (1.2; 6.4) 1 ↑	175 (71; 285) 4 ↓	Not determined
7 days post-treatment	44 (38; 45) –	2.8 (0.7; 4.4) 1 ↓	225[c] (90; 268) 2 ↓	4.7 (2.5; 6) 1 ↑
% change[a]	0 (−5; 42)	−0.7 (−38; 84)	27 (4.7; 362)	−34.2 (−15.5; −39.7)
Minocycline treated (N = 5)				
Pretreatment	34 (28; 52) 4 ↓	2.1 (1.1; 4.9) 1 ↑	141 (104; 261) 4 ↓	5.8 (2.6; 6.1) 3 ↑
Day 28 of treatment	40 (34; 53) 2 ↓	1.8 (0.2; 4.8) 1 ↓	298 (144; 337) 2 ↓	Not determined
7 days post-treatment	40 (30; 50) 1 ↓	2.0 (1.2; 3.1) –	279 (152; 323) 2 ↓	3.7[b] (2.4; 5.3) 1 ↑
% change[a]	14.3 (−4; 24)	12.3 (−52; 36)	61.5 (6.9; 211)	−21.2[b] (−7.7; −43.1)

[a]Percent change pre- to 7 days post-treatment calculated for each dog and then averaged for the treatment group. A negative number indicates a decrease in the value.

[b]Data for one dog missing.

[c]Platelet reading was 1 for one dog; a follow-up reading was 244. Due to the discrepancy, this dog is omitted from the analysis on this day.

and found to be positive, reinfection could not be excluded.

Within the limits of the study, the effect of minocycline and doxycycline was not different, providing evidence that minocycline could be an alternative treatment for *E. canis* infections when doxycycline cannot be used or is not available. As stage of infection might influence the efficacy of treatment, additional studies with experimental infections with known day of infection and a quantified infection dose may be necessary to validate true clearance.[3]

In a clinical setting, twice daily administration of minocycline might result in owner compliance issues when compared to doxycycline.[28] However, studies have shown that there is variability with doxycycline therapy in both peripheral blood and tissue clearance, resulting in the potential for re-emergence from tissues once treatment has ended.[9] If organisms are surviving treatment in tissues, minocycline might be more effective due to the higher concentrations in critical tissues.[19,20] Although the "gold standard" dosage given once daily was followed for this study, twice daily dosing (as was required by the pharmacokinetics of minocycline) might have altered the efficacy of doxycycline, possibly changing our current results.

Whereas this study provides evidence that minocycline clears or suppresses PCR detection of *E. canis*, the group size was small and there was no untreated control group. These limitations occurred due to the limited housing capacity of the animal facility. We had intended to include six dogs per group, which based on estimates of power calculations would have allowed further comparisons between the two treatments. Unfortunately, one dog from each group had to be excluded due to negative PCR tests after acclimation was started. The spontaneous clearance of *E. canis* in the peripheral blood of the two excluded dogs is a phenomenon that has been previously reported.[6,29] The infection could have entered a chronic stage with *E. canis* organisms residing in tissues. However, we are not able to specifically explain this observation further from our data and results.

In conclusion, this study provides evidence that minocycline can clear or suppress circulating *E. canis* and might be an alternative treatment to the "gold standard" doxycycline. Minocycline, being a close relative to doxycycline, is a logical choice for not only *E. canis* but also likely for other members of the Anaplasmataceae and family Rickettsiales. Doxycycline shortages have occurred in the human medical and veterinary field, driving elevated prices.[10,12] Minocycline as an alternative is widely available and may be remarkably much less expensive. With other drug characteristics such as high lipophilicity, low protein binding, and improved penetrance into tissues such as the brain,[19] minocycline could be more suitable to doxycycline in treating these elusive bacteria. More clinical studies and experimental studies, however, are required to evaluate minocycline in treating severe disease caused by *E. canis* infections and in assessing the impact on clearance.

Footnotes

[a] IDEXX SNAP® 4Dx® Plus Test (Westbrook, ME)
[b] Frontline® Tritak® (Merial, Duluth, GA), NexGard chewable (Merial, Duluth, GA), Seresto® collars (Bayer AG, Leverkusen, Germany) and biweekly Frontline Plus® topical (Merial, Duluth, GA)
[c] Strongid® (Zoeitis, Florham Park, NJ)
[d] Doxycycline Monohydrate capsules (PAR Pharmaceutical Companies Inc., Spring Valley, New York, NY)
[e] Minocycline HCl capsules (Ranbaxy Pharmaceuticals, Princeton, NJ)
[f] Abaxis Vet Scan HM5 (Abaxis, Union City, CA)
[g] Abaxis Vet Scan VS2 (Abaxis, Union City, CA)
[h] Qiagen® DNeasy® Blood and Tissue Spin-Column kits (Rohm and Haas Company, Philadelphia)
[i] TECAN infinite M200 PRO NanoQuant (Tecan Group Ltd. Männedorf, Switzerland)
[j] Light Cycler 2.0 (Roche Molecular Systems, Inc., Pleasanton, CA)
[k] IDT (Integrated DNA Technologies, Inc. Coralville, Iowa)
[l] VassarStats: Website for Statistical Computation. http://vassarstats.net/utest.html

Acknowledgments

The authors acknowledge Trellor Fraites and the Diagnostic Services team at Ross University School of Veterinary Medicine. We also acknowledge Dr. Burnell Nisbett and Kurtis Greenaway at Ponds Veterinary Clinic. We thank the Devry Education Group and Ross University School of Veterinary Medicine for funding this research. The work was supported by Ross University School of Veterinary Medicine intramural grants.

Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Minocycline was used extralabel.

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