Am. J. Trop. Med. Hyg., 78(5), 2008, pp. 803–805

Short Report: A Doxycycline Hyclate Rodent Bait Formulation for Prophylaxis and Treatment of Tick-transmitted *Borrelia burgdorferi*

Marc C. Dolan,* Nordin S. Zeidner, Elizabeth Gabitzsch, Gabrielle Dietrich, Jeff N. Borchert, Rich M. Poché, and Joseph Piesman

Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Fort Collins, Colorado; Genesis Laboratories Incorporated, Wellington, Colorado

Abstract. The prophylactic and curative potential of doxycycline hyclate formulated in a rodent bait at concentrations of 250 and 500 mg/Kg was evaluated in a murine model of Lyme borreliosis. Both bait formulations prevented tick-transmitted *Borrelia burgdorferi* infection in 100% of C3H/HeJ mice ($N = 16$), as well as cured acute, established infection in mice ($N = 8$) exposed to bait for 14 days. Spirochete infection was cleared in 88.9% to 100% of infected nymphs feeding on mice fed 250 and 500 mg/Kg antibiotic bait formulations, respectively. These data provide evidence for exploring alternative techniques to prevent transmission of Lyme disease spirochetes.

Lyme disease, caused by the spirochete *Borrelia burgdorferi*, is the most commonly reported vector-borne disease in the United States. In the past 5 years an average of greater than 20,000 cases have been reported annually, while the number of reported Lyme disease cases reached an all-time high in 2002 of 23,763 cases.[1] The blacklegged tick, *Ixodes scapularis*, serves as the principal vector in transmission to humans and maintenance of the spirochete in natural reservoirs in the northeastern and upper midwestern United States.[2] The primary reservoirs of *B. burgdorferi* in Lyme-endemic areas include the white-footed mouse, *Peromyscus leucopus*, the Eastern chipmunk, *Tamias striatus*, and shrews, *Sorex cinereus* and *Blarina brevicauda*.[3–5]

Because no human vaccine is currently available, Lyme disease prevention efforts begin with personal protective measures including education, use of repellents, protective clothing, and self tick-checks.[6] Likewise, several tick-control strategies have been shown to significantly reduce vector tick populations in Lyme-endemic areas.[7] These control measures include the use of area-wide acaricides to control questing ticks as well as passively treating rodent reservoirs using host-targeted devices.[7–9] Antibiotic treatment of early, localized symptoms in humans generally includes doxycycline delivered at 100 mg bid for 14 days.[10] Moreover, some success has been obtained using a single dose (200 mg) of doxycycline hyclate to prophylactically treat people exposed to *I. scapularis* tick bites.[11] In this light, an injectable sustained-release formulation of doxycycline hyclate has been shown to be effective in preventing tick-transmitted *B. burgdorferi* and *Anaplasma phagocytophilum* in a murine model.[12,13] The goal of these current studies was to (i) determine the palatability and pharmacokinetics of a doxycycline-laden bait formulation fed to mice and (ii) determine the efficacy of this bait formulation in prevention and cure of tick-transmitted *B. burgdorferi* in a murine model of Lyme borreliosis.

In a pilot study to determine the palatability and consumption rate of doxycycline-laden bait (Genesis Laboratories, Inc., Wellington, CO), groups of 5 specific-pathogen-free, 8-week-old female C3H/HeJ mice (Jackson Laboratories, Bar Harbor, Maine) were exposed to 250 and 500 mg/Kg doxycycline-hyclate baits for 24 hrs.[14] Individual mice from both groups consumed an average of 7 g of bait in a 24-hr period resulting, on average, in 1.8 and 3.5 mg, respectively, of doxycycline ingested per mouse.

In secondary experiments, plasma pharmacokinetic levels were determined for C3H/HeJ mice as previously described[12] by collecting 200 μL of unclotted blood (EDTA microtainers Becton Dickinson) at 2, 4, 8, 24, and 48 hrs after a 24-hr exposure to each bait formulation. Figure 1 demonstrates the comparative pharmacokinetic curves through 48 hrs for both 250 and 500 mg/Kg doxycycline baits ($N = 6$ mice per time point per formulation); the figure also demonstrates that the pharmacokinetic curves follow a similar pattern with plasma concentrations of doxycycline after 250 mg/Kg bait consumption reaching approximately 50% of the blood levels obtained after consumption of the 500 mg/Kg bait. The maximum concentration for 250 mg/Kg bait (0.82 ± 0.19 μg/mL; range, 0.72–0.90 μg/mL) and 500 mg/Kg bait (2.38 ± 1.36 μg/mL; range, 1.88–3.24, Figure 1) occurred at 2 hrs post-bait removal. The minimum concentration occurred at 48 hrs post-bait exposure (250 mg/Kg, 0.06 ± 0.09 μg/mL; range, 0.02–0.10 and 500 mg/Kg, 0.6 ± 0.37 range, 0.46–0.80, Figure 1) respectively. Zeidner and others[12] reported that a sustained-release formulation of doxycycline hyclate provided complete protection from tick-transmitted spirochete infection in mice at plasma levels of doxycycline maintained between 0.1 and 0.5 μg/mL. In the current studies, a 24-hr exposure to 500 mg/Kg bait formulation provided levels at or above 0.1 to 0.5 μg/mL (0.6 μg/mL at 48 hrs) whereas plasma doxycyline levels of mice exposed to the 250 mg/Kg formulation dropped below these putative protective levels after 24 hrs (0.06 μg/mL at 48 hrs, Figure 1).

For challenge studies, laboratory-reared *I. scapularis* nymphal ticks infected with *B. burgdorferi* strain B-31 were raised and maintained as previously described.[15] The infection rate in these nymphs was previously determined to be 72% by culture and dark-field microscopy. The prophylactic challenge experiments were conducted by placing 5 infected nymphs between the scapulae of specific-pathogen-free, 8-week-old female C3H/HeJ mice using fine forceps and allowing these ticks to feed to repletion. Mice were then separated into 4 groups of 16 mice each (including 2 control groups) and exposed to either 250 or 500 mg/Kg doxycycline bait for the duration of the tick challenge ($\leq$ 96 hrs). Control

* Address correspondence to Marc C. Dolan, Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, 3150 Rampart Rd, Fort Collins, CO 80521. E-mail: mcd4@cdc.gov

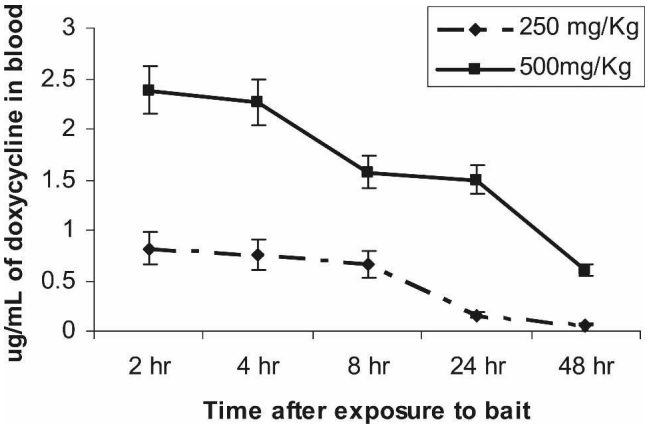

FIGURE 1. Plasma doxycycline levels in C3H/HeJ mice over a 48-hr period after exposure to 250 or 500 mg/Kg doxycycline-laden bait. Each data point is the mean per time point of 6 mice. Standard error bars represent the standard errors of the mean for each group.

mice received a normal maintenance diet of rat chow (Laboratory Diet 5001, PMI Nutrition International, LLC, Brentwood, MO). Ticks that fed to repletion from individual mice were collected and held at 23°C for 14 days until placement in culture. No difference was noted in tick feeding, as an average of 3.28 ticks fed to repletion on mice exposed to doxycycline bait compared with an average of 3.38 for mice exposed to control feed (data not shown). At 4 weeks post-bait exposure, an ear biopsy was obtained and cultured in Barbour-Stoenner-Kelly medium[16] to determine infection with *B. burgdorferi*.[17] Replete ticks collected from individual mice were surface sterilized and cultured in BSK media to determine *B. burgdorferi* infection status as described previously.[18] At 6 weeks post-bait exposure, control mice that were determined to be *B. burgdorferi*-infected by skin culture were then exposed to either 250 or 500 mg/Kg doxycycline bait for 14 days. After removal from treated bait, mice were again biopsied and skin was cultured in BSK media to determine infection status.

In challenge experiments, 100% of mice exposed to either 250 or 500 mg/Kg doxycycline bait formulations were prophylactically protected from tick-transmitted *B. burgdorferi* infection (Table 1). In addition, none of the replete ticks (0/55 [0%]) recovered from mice exposed to 500 mg/Kg doxycycline bait were positive by culture as compared with 44 of 58 (76%) of the ticks recovered from untreated control animals. Replete ticks collected from mice exposed to the 250 mg/Kg formulation demonstrated an infection rate of 8 (4/50), compared with an infection rate of 77.5% (36/50) in ticks recovered from control mice that were fed a control formulation. In the curative trial, 100% of culture positive mice derived from the control groups that were then exposed to either 250 or 500 mg/Kg doxycycline bait for 14 days were cleared of detectable infection as determined by skin biopsy culture. In total, the 500 mg/Kg doxycycline bait formulation afforded 100% prophylactic protection and cure of acute *B. burgdorferi* infection, while effectively sterilizing infected nymphs that fed to repletion. In contrast, exposure of mice to the 250 mg/Kg doxycycline bait resulted in 100% prophylaxis and cure in mice, while affording an 88% clearance (Modified Abbott's Formulation[19]) of *B. burgdorferi* in previously infected ticks. The inability of the 250 mg/Kg doxycycline bait formulation to completely clear ticks of spirochete infection could be due to plasma doxycycline levels dropping below previously determined therapeutic levels, or possibly a failure of mice to consume enough bait daily to sustain therapeutic plasma levels.

TABLE 1

Efficacy of 250 and 500 mg/Kg doxycycline-laden bait for prophylaxis and cure of *Borrelia burgdorferi* infection in mice

	Prophylactic trial*		Curative trial†
	Pos mice/total (%)	Pos ticks/total (%)	Pos mice/total
250 mg/Kg	0/16 (0%)	4/50 (8%)	0/16
500 mg/Kg	0/16 (0%)	0/55 (0%)	0/16
0.0 mg/Kg	28/32 (88%)	80/108 (74%)	NA†

* Mice were exposed to either 250 or 500 mg/Kg doxycycline-laden bait for the duration of the nymphal tick feeding, ~96 hrs (4 d). The control group received a normal maintenance diet of rat chow.

† Five weeks after infestation, control mice that were ear biopsy positive on culture from the Prophylactic Trial were exposed to either 250 or 500 mg/Kg doxycycline-laden bait for 2 wks. Mice were ear biopsied again and evaluated for spirochete clearing (cure) on culture. NA = not applicable.

In summary, a novel doxycycline hyclate impregnated bait formulation was shown to be efficacious in preventing tick-transmitted *B. burgdorferi* infection as well as providing a cure for early established infection in a murine model of Lyme borreliosis. In addition, this doxycycline bait formulation, encompassing a bacteriostatic drug, demonstrated the unexpected result of clearing spirochete infection in feeding ticks. Additional experiments will be needed to determine optimal dosing and potential spirochete resistance with continuous exposure to doxycycline hyclate, as well as determining the ability of this formulation to protect against simultaneous transmission of *B. burgdorferi* and *A. phagocytophilum* infection. Ultimately, these laboratory studies may be evaluated in a field setting to determine the impact on enzootic transmission in nature.

We recognize that distributing doxycycline in an oral bait formulation for control of *B. burgdorferi* in animal reservoir populations could be considered controversial given that doxycycline is a drug currently used for treating several important zoonotic infections. Consequently, although these results demonstrate proof of concept, it may be appropriate to identify other antibiotics with similar anti-spirochetal activity that are not first-line drugs for treatment of Lyme disease and other zoonoses. Moreover, it may be possible to develop regimens for antibiotic use that would minimize the risk of antimicrobial resistance while at the same time achieve significant control levels.

Received January 16, 2008. Accepted for publication February 15, 2008.

Disclaimer: Author Richard M. Poché is an inventor on a United States Patent #5,932,437 "Control of Lyme Disease Spirochetes", related to this work. Doxycycline-treated bait used in these studies was provided by Genesis Laboratories, Inc., Wellington, CO 80549. The findings and conclusions of this study are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. Mention of a method or a product does not constitute an endorsement.

Animals were handled according to approved protocols on file with the Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases Animal Care and Use Committee.

Authors' addresses: Marc C. Dolan, Nordin S. Zeidner, Elizabeth Gabitzsch, Gabrielle Dietrich, and Joseph Piesman, Division of Vec-

tor-Borne Infectious Diseases, Centers for Disease Control and Prevention, 3150 Rampart Road, Fort Collins, CO 80521. Jeff N. Borchert and Rich M. Poché, Genesis Laboratories Incorporated, P.O. Box 1195, Wellington, CO 80549.

Reprint requests: Marc C. Dolan, Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention, 3150 Rampart Road, Fort Collins, CO 80521.

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