Failure of Topical Antibiotics to Prevent Disseminated *Borrelia burgdorferi* Infection Following a Tick Bite in C3H/HeJ Mice

Gary P. Wormser,[1] Thomas J. Daniels,[2,5] Susan Bittker,[1] Denise Cooper,[1] Guiqing Wang,[3,4] and Charles S. Pavia[1,6]

[1]Division of Infectious Diseases, Department of Medicine, [2]Department of Family and Community Medicine, [3]Department of Microbiology and Immunology, and [4]Department of Pathology, New York Medical College, Valhalla; [5]Department of Biological Sciences, Fordham University, Bronx, New York; and [6]Department of Biomedical Sciences, New York College of Osteopathic Medicine of New York Institute of Technology, Old Westbury

A prior study in mice has shown that the timely application of topical antibiotics to the skin at the tick bite site could eradicate *Borrelia burgdorferi* infection. That study, however, did not evaluate antibiotic preparations that are considered suitable for use in humans. In this murine study, topical application of 2% erythromycin and 3% tetracycline preparations that are acceptable for use in humans was found to be ineffective in eliminating *B. burgdorferi* from the tick bite site or in preventing dissemination to other tissues. Reasons for the discrepant findings are discussed.

Prior reports demonstrated that *Borrelia burgdorferi*, the bacterium that causes Lyme disease, remains at the site of deposition in the skin of CD-1 mice after an *Ixodes scapularis* tick bite for ≥2 days [1] and that timely application of topical formulations of active antibiotics at the tick bite site could eliminate the infection [2]. Unfortunately, none of the antibiotic formulations that proved successful is commercially available and all but one of the antibiotics were dissolved in dimethyl sulfoxide (DMSO) for improved absorption, a compound that is unsatisfactory for routine use in humans.

In this study, we evaluated the efficacy of a commercially available 2% erythromycin ointment licensed for human use, as well as a preparation of 3% tetracycline that is also considered safe for human use, for topical therapy of *B. burgdorferi* infection following a tick bite in C3H/HeJmice.

Received 31 January 2011; accepted 12 April 2011; electronically published 19 September 2011.

Correspondence: Gary P. Wormser, MD, Division of Infectious Diseases, Departments of Medicine, New York Medical College, Munger Pavilion, Rm 245, Valhalla, NY 10595 (gary_wormser@nymc.edu).

The Journal of Infectious Diseases 2012;205:991–4

DOI: 10.1093/infdis/jir382

METHODS

Mice

Groups of 10–12 eight-week-old female C3H/HeJ mice (Charles River Laboratories, Wilmington, MA) were housed in a filtered-air environment maintained at 20 ± 2° C. Antibiotic treated and non-treated (control) mice were kept in individual cages. All animal experiment protocols were approved by the Institutional Animal Care and Use Committee of New York Medical College.

Ticks

The nymphal stage *I. scapularis* ticks, provided by Durland Fish, Ph.D. at Yale University, had been infected during the larval stage with the BL206 strain of *B. burgdorferi*.

Experimental Infection of Mice

The hair on the back of each mouse, between the shoulder blades, was gently clipped (without producing abrasions) with an electric clipper (Oster Professional Products, McMinnville, Tenn.) fitted with a size 40 blade to expose the skin. A Nalgene capsule (1 cm diameter × 1 cm high) (Thermo Fisher, St. Louis, MO) was glued to the skin [3], and a single infected *I. scapularis* nymph was introduced into the capsule. The capsule opening was covered by a piece of nylon mesh and sealed with a screw-cap into which a small hole had been bored; this permitted air exchange through the mesh while preventing the tick from escaping. Mice were checked the following day to confirm tick attachment, then daily until ticks had fed to repletion, detached, and were removed from the capsule. Only those mice on which an infected tick had fed to repletion were further studied. Replete ticks were stored individually in high humidity chambers until molting to adults.

Topical Antibiotic Preparations Utilized

In the first experiment a 2% topical erythromycin ointment (Akne-mycin, Coria Laboratories, LTD, Fort Worth, Texas), FDA-approved for the topical control of acne vulgaris, was used. In the second experiment a 3% topical gel formulation of tetracycline hydrochloride, obtained from a compounding pharmacy (Bryce Laboratories, Stamford, CT), was studied.

Treatment of Mice

The topical antibiotic preparation was applied to the feeding site using a sterile cotton-tipped applicator over an approximate

radius of 0.75 cm twice daily for three consecutive days, beginning approximately 24–48 hours after tick detachment (the 24–48 hours range is stated since the exact time of tick detachment was not precisely known). The site of application was covered with a non-occlusive dressing or with a capsule to prevent the mice from ingesting the drug preparation. Two other groups of mice on which infected ticks had fed served as controls: one of the groups was treated with petrolatum (the usual vehicle in ointments) and the other group received no treatment at all.

Four weeks after completion of treatment, the mice were euthanized by cervical dislocation and the tick bite site was again shaved. The mice were then placed into a beaker of 70% ethanol to disinfect the skin. Samples of urinary bladder, ear tissue, and skin tissue from the tick bite site each about 7 × 10 mm in size, were obtained and cultured for up to 4 weeks in BSK media (formulated without antibiotics) to ascertain infection status.

To attempt to document that the strain of *B. burgdorferi* used in this study had not already disseminated from the tick bite site within the 48 hours period following tick detachment, we cultured bladder and ear tissue of a group of untreated mice that were euthanized at 24–48 hours after tick feeding.

MIC DETERMINATIONS

Bacteria and Cultures

The patient-derived *B. burgdorferi* strain BL206 was maintained in low passage (< 10 passages) cultures using BSK media, as previously described [4]. Organisms used in these experiments were diluted to the appropriate concentration with the use of BSK media.

Antibiotics

Stock solutions of erythromycin (product #E5389) and tetracycline (product #T7660: tetracycline hydrochloride) (Sigma Chemical, St. Louis, MO) were made at 100 mg/mL and, from these, further working dilutions were made to the desired concentration (80–160 mcg/mL) using BSK media.

Growth Inhibition Experiments

Antibiotics were tested for inhibitory activity using a slight modification of a previously described method for measuring in vitro serum-mediated borreliacidal effects [5]. Each well of a flat-bottomed microculture plate (Costar, Cambridge, MA) contained 2.5×10^6 *B. burgdorferi* cells in a final volume of 250 μL with the desired concentration of antibiotic. Matching control wells contained *B. burgdorferi*, but no antibiotic. Cultures were kept airtight by putting the plates into sealed plastic bags followed by incubation at 33°C for 18–24 hours. At the end of the incubation period, the number of *B. burgdorferi* cells in each separate test well was counted microscopically. The percentage of inhibition of growth was calculated according to the formula: [1 − (number of motile *B. burgdorferi* cells in each diluted antibiotic suspension/number of motile *B. burgdorferi* cells in BSK media alone] × 100. The MIC was defined as 50%–60% inhibition, and the MBC as 100% inhibition.

Real-time PCR Analysis of *B. burgdorferi fla* Gene

Approximately 2 months after molting to the adult stage, ticks were preserved in individual vials containing 70% ethanol until DNA extraction. DNA was prepared from ticks by using a commercial DNA extraction kit (Qiagen QIAamp® DNA Mini Kit, Germantown, MD). Extracted DNA was resuspended in 50 μL of sterile water. Real-time PCR was performed in 96-well microplates in an ABI Prism 7900HT Sequence Detection System (Applied Biosystems Inc., Foster City, CA, USA). *B. burgdorferi*-specific chromosomally encoded *fla* was amplified and detected as described previously [6] with the exception that SYBR I was used as the reporter dye in the assay with 1x SYBR master mix (Applied Biosystems Inc.), and with 200 μM of each primer and 4 μL of extracted DNA for each sample.

Statistics

Proportions were compared using the Fisher's exact test, two-tailed. A P value of $< .05$ was considered significant.

RESULTS

The median MIC/MBC for the BL206 strain of *B. burgdorferi* was found to be 0.2/1.0 mcg/mL for erythromycin (5 replicates) and 1.0/4.0 mcg/mL for tetracycline (7 replicates). In the experiment to demonstrate whether dissemination of *B. burgdorferi* could already be demonstrated by culture of bladder or ear tissue within 48 hours of tick detachment, 0 of 20 evaluable mice had a positive culture of bladder or ear tissue (both the bladder and ear tissue cultures were contaminated with non-borrelial bacteria for 1 additional mouse). Ten mice, however, had a positive culture of the skin site where the tick bite occurred (the culture was contaminated for the other 11 mice).

In the topical erythromycin experiment, 21/25 (84.0%) of the evaluable mice that were treated with topical erythromycin had a positive culture of either bladder or ear tissue compared with 19/24 (79.2%) of mice treated with petrolatum only ($P = .73$) and 26/27 (96.3%) mice that received no topical therapy ($P = .18$) (Table 1). In addition, in the erythromycin experiment, 14/23 (60.9%) of the evaluable mice that were treated with topical erythromycin had a positive culture of the skin at the tick bite site compared with 16/24 (66.7%) of mice treated with petrolatum only ($P = .77$) and 24/26 (92.3%) mice that received no topical therapy ($P = .01$). The significant reduction in culture positivity at the tick bite site was apparently not due to the erythromycin per se, since a similar degree of efficacy was observed in the petrolatum-treated group ($P = .03$, for the

Table 1. Evaluation of Topical Antibiotics for Prevention of *Borrelia burgdorferi* Infection in C3H/HeJ Mice Following the Bite of an Infected *Ixodes scapularis* Tick

Treatment	Number of Mice With Positive Bladder and/or Ear Culture	Number of Mice With a Positive Skin Culture at Tick Bite Site
Experiment 1		
Erythromycin 2% ointment	21/25 (84.0%) [c]	14/23 (60.9%) [d]
Petrolatum	19/24 (79.2%)	16/24 (66.7%)
Untreated	26/27 (96.3%)	24/26 (92.3%) [e]
P values [a]	.73; .18	.77; .01
Experiment 2		
Tetracycline 3% gel	10/10 (100%)	10/10 (100%)
Petrolatum	10/10 (100%)	8/10 (80%)
Untreated	10/10 (100%)	9/9 (100%) [f]
P values [b]	1.0; 1.0	.47; 1.0

[a] The first listed *P* value is for the comparison of erythromycin versus petrolatum treatment; the second listed *P* value is for the comparison of erythromycin versus no treatment, each by the Fisher's exact test.

[b] The first listed *P* value is for the comparison of tetracycline versus petrolatum treatment; the second listed *P* value is for the comparison of tetracycline versus no treatment, each by the Fisher's exact test.

[c] Two mice considered unevaluable because both bladder and ear cultures were contaminated.

[d] Four mice considered unevaluable because the culture of skin from the tick bite site was contaminated.

[e] One mouse considered unevaluable because the culture of skin from the tick bite site was contaminated.

[f] One mouse considered unevaluable because the culture of skin from the tick bite site was contaminated.

comparison of efficacy of petrolatum [16/24] versus no treatment [24/26]). The detached ticks in this experiment were tested by PCR and over 95% had detectable *B. burgdorferi* DNA (all non-template controls were PCR negative). Mice were considered unevaluable if the pertinent cultures were contaminated (eg, if both the ear and bladder were contaminated, then dissemination could not be assessed) or if bitten by an uninfected tick based on PCR testing.

Topical tetracycline was also ineffective in eliminating infection at the tick bite site or in preventing dissemination (Table 1).

Table 2. Comparison of Study Methods

Methods	Shih and Spielman [2]	Current Study
Age and strain of mice	3–4 week-old CD-1	8 week-old C3H/HeJ
Gender of mice	Not stated	Female
Ticks	Nymphal *Ixodes scapularis*	Nymphal *Ixodes scapularis*
Strain of Bb	JD1 (RST 3, OspC C)	BL206 (RST 1, OspC A)
MICs for strain Bb	Not reported	See text
Tick placement on mouse	Ear	Back
Use of capsule to enclose tick	No	Yes
Detection of Bb infection at tick bite site	Not done	Culture of tissue at site
Detection of Bb infection at site distant from tick bite site	Xenodiagnosis	Culture of bladder and ear tissue
Tetracycline preparation	Various concentrations of tetracycline hydrochloride dissolved in dimethyl sulfoxide	3% tetracycline gel
Erythromycin preparation	100 mg/mL of erythromycin dissolved in 70% ethanol	2% erythromycin ointment
Timing of application of antibiotic	1–5 days after tick detachment for tetracycline and 1 day after detachment for erythromycin	1–2 days after tick detachment
Frequency and duration of application of tetracycline	Twice daily for 1–7 days	Twice daily for 3 days
Frequency and duration of application of erythromycin	Twice daily for 3 days	Twice daily for 3 days

Abbreviation: Bb, *Borrelia burgdorferi*.

Samples of bladder tissue and skin from the back and ear from 3 control mice that had not been bitten by a tick were uniformly culture negative for *B. burgdorferi.*

DISCUSSION

Topical 2% erythromycin and topical 3% tetracycline preparations that are acceptable for use in humans were ineffective in eradicating *B. burgdorferi* from the tick bite site or in preventing dissemination to other murine tissues under the above described experimental conditions in a C3H/HeJ mouse model of tick-transmitted infection. These results are in sharp contrast to those of Shih and Spielman [2]. There are numerous methodologic differences between their study and ours that may explain the discrepant findings (Table 2).

Although some strains of *B. burgdorferi* are said to be resistant to erythromycin in vitro, the BL206 strain is regarded as susceptible [7], and resistance to tetracyclines has not been reported [8]. Shih and Spielman [2] did not report the MICs for the borrelial strain they used for any of the six antibiotics that were studied. The concentration of the alcohol-based solution of erythromycin they used was 5 times greater than what we evaluated, however, which may explain higher efficacy. On the other hand, it can be inferred from their report (meaning the information was not explicitly stated) that a lower concentration of topical tetracycline (1%) than was used in our study was also at least moderately effective (dissemination did not occur in 50% of 10 treated mice, whereas dissemination was demonstrated in all 10 of the control mice [$P = .03$]) [2]. The better results in this instance were probably attributable to the use of DMSO in the tetracycline preparation to enhance transdermal penetration of the antibiotic. It is also plausible that the skin of a mouse's pinna, the site of the tick bite in the Shih and Spielman study [2], may be thinner than that on the back, where the tick bite occurred in our study, allowing for better antibiotic penetration.

It is also possible that the BL206 strain of *B. burgdorferi* (this is a highly invasive RST 1, OspC type A strain [9]) in our study was able to disseminate more rapidly than the JD1 strain (which is a RST 3, OspC type C strain) employed by Shih and Spielman [2]. Another relevant factor may be the particular susceptibility of the C3H mouse to spirochetal dissemination compared with other mouse strains [10]. Although we did not find evidence of dissemination by culture of bladder or ear tissue of untreated mice 2 days after tick detachment, some level of dissemination cannot be excluded, given the uncertainty of the sensitivity of culture at this early time point.

We could recover *B. burgdorferi* from the site from which the tick had detached over 30 days earlier in over 50% of mice, regardless of treatment assignment. This finding contrasts with that of Shih et al [10] who were no longer able to recover the JD1 strain of *B. burgdorferi* from ear tissue of *untreated* C3H/HeJ mice at the site of a bite from an infected tick at 10 days after tick detachment. The disparity in results may be due to different times of sampling, different borrelial strains, or to differences between the skin tissues of the ear versus the back of C3H/HeJ mice.

We conclude that topical application of the erythromycin or tetracycline preparations studied here, both compatible with use in humans, was ineffective for prevention of dissemination of *B. burgdorferi* in mice after a tick bite under the experimental conditions employed in this study.

Notes

Acknowledgments. The authors thank Dr. Richard Falco and Dr. Ira Schwartz for advice and Lisa Giarratano and Mary Cox for assistance. The authors thank Marc Dolan of the Centers for Disease Control and Prevention for providing the modified recipe to glue capsules on the mice.

Financial support. This work was supported by the National Institutes of Health (NIH; RO3 AI067315). The contents of the manuscript are solely the responsibility of the authors and do not necessarily represent the official view of the NIH.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Shih CM, Pollack RJ, Telford SR 3rd, Spielman A. Delayed dissemination of Lyme disease spirochetes from the site of deposition in the skin of mice. J Infect Dis **1992**; 166:827–31.
2. Shih C-M, Spielman A. Topical prophylaxis for Lyme disease after tick bite in a rodent model. J Infect Dis **1993**; 168:1042–5.
3. Mbow ML, Christe M, Rutti B, Brossard M. Absence of acquired resistance to nymphal *Ixodes ricinus* ticks in Balb/C mice developing cutaneous reactions. J Parasitol **1994**; 80:81–7.
4. Pavia CS, Kissel V, Bittker S, Cabello F, Levine S. Antiborrelial activity of serum from rats injected with the Lyme disease spirochete. J Infect Dis **1991**; 163:656–9.
5. Pavia CS, Wormser GP, Norman GL. Activity of sera from patients with Lyme disease against *Borrelia burgdorferi*. Clin Infect Dis **1997**; 25(Suppl 1):S25–30.
6. Wang G, Ma Y, Buyuk A, McClain S, Weis JJ, Schwartz I. Impaired host defense to infection and Toll-like receptor 2-independent killing of *Borrelia burgdorferi* clinical isolates in TLR2-deficient C3H/HeJ mice. FEMS Microbiol Lett **2004**; 231:219–25.
7. Terekhova D, Sartakova ML, Wormser GP, Schwartz I, Cabello FC. Erythromycin resistance in *Borrelia burgdorferi*. Antimicrob Agents Chemother **2002**; 46:3637–40.
8. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. Clin Infect Dis **2006**; 43: 1089–134.
9. Wang G, Ojaimi C, Iyer R, et al. Impact of genotypic variation of *Borrelia burgdorferi* sensu stricto on kinetics of dissemination and severity of disease in C3H/HeJ mice. Infect Immun **2001**; 69: 4303–12.
10. Shih CM, Telford SR 3rd, Pollack RJ, Spielman A. Rapid dissemination by the agent of Lyme disease in hosts that permit fulminating infections. Infect Immun **1993**; 61:2396–9.