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,2,6 Susan Bittker,1 Denise Cooper,1 Guiqing Wang,3,4 and Charles S. Pavia1,6

1Division of Infectious Diseases, Department of Medicine, 2Department of Family and Community Medicine, 3Department of Microbiology and Immunology, and 4Department of Pathology, New York Medical College, Valhalla; 5Department of Biological Sciences, Fordham University, Bronx, New York; and 6Department 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/HeJ mice.

**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 × 10⁶ *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: 

\[
\text{Percentage of inhibition} = (1 - \frac{\text{number of motile } B.\ burgdorferi \text{ cells in each diluted antibiotic suspension/number of motile } B.\ burgdorferi \text{ cells in BSK media alone}}{100})
\]

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
comparison of efficacy of petrolatum [16/24] versus no treat-
ment [24/26]). The detached ticks in this experiment were
tested by PCR and over 95% had detectable
*Borrelia 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 contam-
inated, 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 1. Evaluation of Topical Antibiotics for Prevention of *Borrelia burgdorferi* Infection in C3H/HeJ Mice Following the Bite of an Infected *Ixodes scapularis* Tick

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

\(^a\) 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.

Table 2. Comparison of Study Methods

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

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* = 0.03]) [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**

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**References**