Efficacy of an Evernimicin (SCH27899) In Vitro and in an Animal Model of Lyme Disease

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The MICs of evernimicin at which 90% of Borrelia burgdorferi patient isolates were inhibited ranged from 0.1 to 0.5 mg/ml. Evernimicin was as effective as ceftriaxone against B. burgdorferi in a murine model of experimental Lyme disease. As assessed by culturing the urinary bladders of infected C3H mice, no live Borrelia isolates were recoverable following antibiotic treatment.

Microculture techniques have recently been described and are especially useful for assessment of the growth of the Lyme disease spirochete in the presence of certain potentially inhibitory substances such as immune sera and antibiotics (1, 4–9). In this regard, our laboratory has previously reported results that have demonstrated the in vitro borreliacidal activities of penicillin-type and various cephalosporin-type antibiotics (6, 7), as well as of sera from Lyme disease patients (8). This report describes a follow-up study of the effects of a novel antibiotic, evernimicin, on the growth of a large number of North American isolates of Borrelia burgdorferi. Evernimicin is an oligosaccharide antimicrobial agent produced by Microsarcina carbonacea (11). It had recently been under renewed clinical investigation (2) due primarily to the general emergence of antibiotic-resistant, nonspirochetal microorganisms (3).

MICs and minimal bactericidal concentrations (MBCs) for 27 clinical isolates (12, 13) and 3 reference strains (strains B31, CA287, and 297) of B. burgdorferi were determined by a modified microplate dilution assay (12). Stock and maintenance cultures of all 30 isolates were obtained by growing organisms in Barbour-Stoenner-Kelly (BSK) medium. Triplicate wells contained 5 × 10^5 B. burgdorferi organisms in BSK medium with and without diluted evernimicin (Schering-Plough Research Institute, Kenilworth, N.J.), penicillin (Sigma, St. Louis, Mo.), or ceftriaxone (Roche, Nutley, N.J.). After incubation for 24 to 48 h, the wells were examined by dark-field or phase-contrast microscopy, and the surviving Borrelia were enumerated as described previously (7, 8). The in vitro activity of evernimicin was nearly equivalent to that of ceftriaxone and was slightly superior to that of penicillin (Table 1). In addition, the selected B. burgdorferi isolates were uniformly susceptible to evernimicin. Along with a lack of motility, dying or nonviable organisms often appeared as very thin, delicate, and shortened degenerating forms, with some organisms having blebs, yet they still retained their characteristic spiral shape. Three isolates with antibiotic susceptibilities representative of the group were chosen for in vivo study.

Separate groups of 4- to 5-week-old female C3H mice (Charles River Laboratories, Wilmington, Mass.) were infected intradermally in the abdominal area with 0.1 ml of BSK medium containing 100,000 B. burgdorferi organisms with a tuberculin syringe. The inoculated organisms were obtained from diluted, low-passage cultures (≤10 in vitro passages in BSK medium). Then, 7 to 10 days later, the mice were given a single daily dose of evernimicin (25 mg/kg of body weight), ceftriaxone (50 mg/kg of body weight) or the excipient diluent control (placebo) for 5 consecutive days.

### Table 1. Susceptibilities of 3 reference strains and 27 patient isolates of B. burgdorferi

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Range of MICs (μg/ml) for:</th>
<th>Reference strains</th>
<th>Patient isolates</th>
<th>Range of MBCs (μg/ml) for:</th>
<th>Reference strains</th>
<th>Patient isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50%</td>
<td>90%</td>
<td>50%</td>
<td>90%</td>
<td>50%</td>
<td>90%</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.2–0.4</td>
<td>0.5–2.0</td>
<td>0.2–0.4</td>
<td>0.5–2.0</td>
<td>2.0–4.0</td>
<td>1.0–4.0</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.05–0.08</td>
<td>0.1–0.25</td>
<td>0.05–0.08</td>
<td>0.1–0.25</td>
<td>0.2–0.5</td>
<td>0.2–0.5</td>
</tr>
<tr>
<td>Evernimicin</td>
<td>0.05–0.08</td>
<td>0.1–0.25</td>
<td>0.05–0.10</td>
<td>0.1–0.50</td>
<td>0.2–0.5</td>
<td>0.2–0.5</td>
</tr>
</tbody>
</table>

*The MICs were defined as the lowest concentrations of antibiotic causing either 50 or 90% growth inhibition. The MBCs were defined as the lowest concentration causing 100% killing of B. burgdorferi.

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TABLE 2. Efficacy of evernimicin against *B. burgdorferi* infection in C3H mice

<table>
<thead>
<tr>
<th><em>B. burgdorferi</em> strain</th>
<th>No. of mice infected</th>
<th>No. of mice treated with:</th>
<th>No. of cured mice/No. of mice given:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Evernimicin</td>
<td>Ceftriaxone</td>
</tr>
<tr>
<td>B193</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>B296</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>B322</td>
<td>9</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Combined totals</td>
<td>27</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

*Patient isolates (from erythema migrans rashes).*  
*b* Based on cultured extracts of urinary bladder in BSK medium yielding live *Borrelia*.

treatments were administered by the intramuscular (i.m.) route on days 1, 3, and 5 and by the intraperitoneal route on days 2 and 4. Ceftriaxone was injected via the i.m. route only. Two days after the last dose of antibiotic or placebo was given, the mice were killed and cultures of their urinary bladders were established in BSK medium (8). These were examined weekly for up to 6 weeks for motile spirochetes based on phase-contrast and fluorescence microscopy. As shown in Table 2, evernimicin was as effective as ceftriaxone in eliminating borrelial infection, based on the failure to culture live spirochetes from the urinary bladders of 100% of the mice treated with either antibiotic. Also absent, based on microscopic examination, from cultures of the bladders of antibiotic-treated mice, were any remnants of nonviable, disintegrating spirochetal forms.

In this study, it was shown that evernimicin possesses excellent in vitro and in vivo activities against a wide variety of borrelial isolates, and these results correlated well with the MIC and MBC results reported by others (1). However, our study may have examined the in vitro susceptibilities of the largest number of North American *B. burgdorferi* isolates to date; these isolates were derived mostly from the skin and blood of patients with early Lyme disease (12, 13). We also found that evernimicin’s inhibitory effects were comparable to or slightly better than those attributable to two other antibiotics (penicillin and ceftriaxone) which are commonly used for the treatment of Lyme disease (14). The dosages of evernimicin studied in vivo were comparable to those successfully used to treat CD1 mice successfully against lethal pneumonia caused by a penicillin-resistant strain of *Streptococcus pneumoniae* (10). Important limitations of our in vivo studies were that the evaluation of drug efficacy was based solely on the ability to culture *B. burgdorferi* from the urinary bladder of infected mice and that evernimicin’s effectiveness was tested against only three patient isolates.

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REFERENCES


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