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In Vitro Susceptibility of *Borrelia burgdorferi* to 11 Antimicrobial Agents

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The in vitro susceptibility of *Borrelia burgdorferi* to 11 antimicrobial agents was investigated. The antimicrobial agents evaluated included ceftizoxime, FK037, cefotaxime, dirithromycin, clarithromycin and its metabolite 14-hydroxy-clarithromycin, erythromycin, doxycycline, amoxicillin, ciprofloxacin, and ofloxacin. Isolates of *B. burgdorferi* tested included two reference strains (B31 and ATCC 53899), six isolates from the midwestern United States, and three from Europe. A broth macrodilution method was used to determine MICs and MBCs. *B. burgdorferi* was inhibited by ≤0.5 μg of each of the agents except the quinolones per ml. The MBCs for 90% of strains tested of ceftizoxime, FK037, clarithromycin, 14-OH clarithromycin, and dirithromycin (≤1.0 μg of each per ml) were superior to those of amoxicillin (2.0 μg/ml) and doxycycline (4.0 μg/ml). Further in vivo studies are warranted to determine whether these agents may be efficacious in the treatment of Lyme borreliosis.

Lyme borreliosis is the most common tick-borne disease in the United States (7). The etiologic agent, *Borrelia burgdorferi*, is transmitted by the bite of an *Ixodes* species tick (5, 12, 22). The disease induced by *B. burgdorferi* infection is a multisystem disorder with early and late clinical manifestations (21). Antimicrobial treatment has been shown to be effective during both early and late disease (20).

In vitro antimicrobial susceptibility studies have demonstrated that the penicillins, tetracyclines, and macrolides possess activity against *B. burgdorferi* (10). Several cephalosporins, including cefuroxime, cefotaxime, and ceftriaxone, have also been shown to be active agents (1, 18). Animal studies parallel in vitro results with the exception that the activity of some of the macrolides in vivo appears to be less than that expected on the basis of in vitro data (11, 18).

We report the in vitro susceptibilities of *B. burgdorferi* to several new macrolides, clarithromycin, its 14-OH metabolite, and dirithromycin. Additionally, two new cephalosporins, ceftizoxime and FK037, were studied. The activities of these newer agents were compared with those documented for several older antimicrobial agents.

MATERIALS AND METHODS

Origin and cultivation of borrelia isolates. Eight isolates of *B. burgdorferi* from the United States were evaluated. These included two American Type Culture Collection reference strains, B31, obtained from a Shelter Island tick (New York), and ATCC 53899, obtained from human spinal fluid. Also included was IS-17, obtained from a Wisconsin mouse and recently characterized (16). Five additional isolates, one recovered from an Illinois field mouse, *Peromyscus leucopus*, and four recovered from *Ixodes dammini* ticks collected from regions of endemicity in northwestern Illinois (14), were also evaluated. Three European (Slovenian) strains recently isolated in our laboratory from tissue biopsy samples obtained from patients with erythema migrans were also studied. All strains were third- or fourth-passage organisms.

Organisms were maintained in Barbour-Stoenner-Kelly medium modified as described by Anderson et al. (2). Media used in our study did not contain rifampin.

Antimicrobial agents. The antibiotics evaluated included amoxicillin, doxycycline, and erythromycin (Sigma Chemical Co., St. Louis, Mo.), cefotaxime (Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J.), clarithromycin and 14-OH-clarithromycin (Abbott Laboratories, Abbott Park, Ill.), dirithromycin (Eli Lilly, Indianapolis, Ind.), ceftizoxime (SmithKline Beecham, Philadelphia, Pa.), and FK037 (R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J.). Drugs were prepared according to the guidelines of Anhalt and Washington (3).

In vitro antimicrobial susceptibility procedures. A broth macrodilution procedure similar to a previously published method (9) was used to determine MICs and MBCs. The concentrations of antimicrobial agents used ranged from 0.03 to 64 μg/ml. Cryovials containing 2 ml of Barbour-Stoenner-Kelly medium were inoculated with 10^5 spirochetes per ml. Spirochetes were counted by using a Petroff-Hausser counting chamber (Hausser Scientific Partnership, Horsham, Pa.) (1). After 1 week of incubation at 34°C, the MICs were determined. Medium from each cryovial was examined by dark-field microscopy. Five to ten high-power fields (400×) were examined for the presence or absence of motile organisms. The concentration at which no motile spirochetes were observed was determined to be the MIC for that agent (1). To determine the MBC, an aliquot of 0.1 ml was removed from all vials with no demonstrable motile spirochetes, inoculated into 0.9 ml of antibiotic-free Barbour-Stoenner-Kelly medium, and incubated for 3 weeks at 34°C. The MBC was defined as the lowest concentration of antibiotic at which no spirochetes were subcultured (9).

RESULTS

Ciprofloxacin (MIC, 0.25 to 2 μg/ml) and ofloxacin (MIC, 0.5 to 2 μg/ml) were the least active agents against *B.*

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TABLE 1. MICs of 11 antimicrobial agents for *B. burgdorferi*

Antibiotic	MIC (μg/ml)[a]		
	Range	50%	90%
FK037	≤0.03–0.125	≤0.03	0.125
Ceftizoxime	0.06–0.5	0.125	0.25
Cefotaxime	≤0.03	≤0.03	≤0.03
Erythromycin	≤0.03–0.06	≤0.03	0.06
Dirithromycin	≤0.03–0.06	≤0.03	≤0.03
Clarithromycin	≤0.03–0.06	≤0.03	≤0.03
14-OH-clarithromycin	≤0.03–0.06	≤0.06	≤0.06
Amoxicillin	≤0.03–0.06	≤0.03	≤0.03
Doxycycline	0.125–0.5	0.25	0.25
Ciprofloxacin	0.25–2.0	1.0	1.0
Ofloxacin	0.5–2.0	2.0	2.0

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

burgdorferi. Doxycycline (MIC, 0.125 to 0.5 μg/ml) and ceftizoxime (MIC, 0.06 to 0.5 μg/ml) demonstrated intermediate activity. Clarithromycin (MIC, ≤0.03 to 0.06 μg/ml), 14-hydroxy-clarithromycin (MIC, ≤0.03 to 0.06 μg/ml), FK037 (MIC, ≤0.03 to 0.06 μg/ml), and dirithromycin (MIC, ≤0.03 to 0.06 μg/ml) demonstrated activities similar to those of amoxicillin (MIC, ≤0.03 to 0.06 μg/ml), erythromycin (MIC, ≤0.03 to 0.06 μg/ml, and cefotaxime (MIC, ≤0.03 μg/ml) (Table 1).

Bactericidal activity for 90% of the strains tested was achieved at ≤1.0 μg/ml for each of the newer agents: ceftizoxime (1.0 μg/ml), dirithromycin (0.125 μg/ml), FK037 (0.25 μg/ml), clarithromycin (0.25 μg/ml), and 14-OH-clarithromycin (0.5 μg/ml) (Table 2). Of the other agents tested, cefotaxime (0.25 μg/ml) and erythromycin (0.25 μg/ml) possessed the greatest in vitro bactericidal activity. Doxycycline (4.0 μg/ml), ciprofloxacin (8.0 μg/ml), and ofloxacin (8.0 μg/ml) appeared to be less active.

DISCUSSION

Erythromycin may be considered an alternative oral agent in the treatment of early Lyme disease (20). In our study, erythromycin had excellent in vitro activity, confirming the results of previous reports. In human and animal studies, however, erythromycin has been associated with limited efficacy (9, 18). A related macrolide, clarithromycin, also exhibited excellent in vitro activity. In the only previously published report of the in vitro effect of clarithromycin against *B. burgdorferi*, Preac-Mursic et al. demonstrated a MIC for 90% of strains tested comparable to that shown in our study but found that clarithromycin was less effective than would have been expected when tested in a gerbil animal model of *B. burgdorferi* infection (19).

TABLE 2. MBCs of 11 antimicrobial agents for *B. burgdorferi*

Antibiotic	MBC (μg/ml)[a]		
	Range	50%	90%
FK037	≤0.03–0.25	≤0.06	0.25
Ceftizoxime	0.25–1.0	0.5	1.0
Cefotaxime	≤0.03–0.25	≤0.03	0.25
Erythromycin	0.06–0.5	0.125	0.25
Dirithromycin	0.06–0.125	0.06	0.125
Clarithromycin	0.06–0.25	0.125	0.25
14-OH-clarithromycin	0.06–0.5	0.125	0.5
Amoxicillin	≤0.03–2.0	0.06	2.0
Doxycycline	0.25–4.0	2.0	4.0
Ciprofloxacin	0.5–16	2.0	8.0
Ofloxacin	1.0–8.0	2.0	8.0

[a] 50% and 90%, MBCs for 50 and 90% of isolates tested, respectively.

The activity of dirithromycin and the 14-hydroxy metabolite of clarithromycin has not previously been evaluated against *B. burgdorferi*. We found them to have in vitro activities similar to those of erythromycin and clarithromycin. However, the pharmacokinetic properties of clarithromycin, 14 hydroxy-clarithromycin, and dirithromycin enhance their attractiveness for further evaluation. These three drugs produce prolonged tissue concentrations well above the minimum bactericidal levels we defined, exceeding those achievable with standard doses of erythromycin (6, 8, 17). Future in vitro studies should be directed toward the elucidation of possible synergistic interactions between parent compounds (clarithromycin and dirithromycin) and their metabolites (14-OH-clarithromycin and erythromycylamine).

The in vitro activities of cefotaxime, ampicillin, doxycycline, ciprofloxacin, and ofloxacin demonstrated in this study are similar to those described in previously published reports (10, 13, 18). The in vitro activities of the two cephalosporin agents, FK037 and ceftizoxime, against *B. burgdorferi* have not previously been evaluated. Our data indicate that these agents are very active in vitro. Animal studies indicate that a 20-mg dose of FK037 per kg of body weight, given intravenously, attains plasma levels at least 100-fold greater than the MBC of this agent against *B. burgdorferi* measured in our study (15). Whether this property applies to humans remains to be determined. Similarly, after a 1-g dose given intravenously, ceftizoxime levels are 50-fold greater than the MBC for *B. burgdorferi* (4).

We have demonstrated favorable in vitro activities of ceftizoxime, FK037, clarithromycin, 14-OH-clarithromycin, and dirithromycin against *B. burgdorferi*. On the basis of their favorable in vitro activity, further clinical studies of the effects of these five agents for the treatment of Lyme borreliosis may be warranted.

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