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In Vitro Susceptibilities of *Borrelia burgdorferi* to Five Oral Cephalosporins and Ceftriaxone

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We determined the in vitro susceptibilities of eight *Borrelia burgdorferi* isolates to five oral cephalosporins. MICs for *B. burgdorferi* 297 were 23 μg/ml (cephalexin), 45 μg/ml (cefadroxil), 91 μg/ml (cefaclor), 0.13 μg/ml (cefuroxime), 0.8 μg/ml (cefixime), and 0.02 μg/ml (ceftriaxone). When *B. burgdorferi* isolates were exposed to concentrations twice the MIC of cefuroxime, cefixime, or ceftriaxone, at least 72 h of incubation was required to kill 99% of the organisms.

Human Lyme disease is a multisystem disorder transmitted by ticks of the *Ixodes ricinus* complex (3) infected with the spirochete *Borrelia burgdorferi* (5, 22). Infections with *B. burgdorferi*, currently hyperendemic in the upper Midwest and northeastern regions of the United States, have become the most common tick-associated illness in the country (8).

Treatment of early Lyme disease with oral antibacterial agents such as penicillin, tetracycline, and erythromycin is usually successful for resolving erythema migrans and the accompanying symptoms (23). However, no study has clearly defined the optimal treatment with these antibacterial agents, and treatment failures have been reported (4, 9, 23, 25). In addition, Dattwyler et al. (12) reported that patients treated with tetracycline, penicillin, and erythromycin during early disease may become seronegative and still develop late Lyme disease.

In contrast to that of early disease, treatment of late Lyme disease often requires high-dose, intravenous therapy with penicillin (11, 21, 24) or ceftriaxone (11). These treatments are often inconvenient and costly, and they can potentially cause iatrogenic diseases such as intravenous-line sepsis. As an alternative, prolonged courses of oral doxycycline have been used with some success for patients with mild peripheral neurologic disease (13). However, semisynthetic tetracyclines are not appropriate therapy for young children, and the ability of doxycycline to eradicate *B. burgdorferi* sequestered in tissues remains uncertain.

In this study, we determined the in vitro susceptibilities of eight *B. burgdorferi* isolates to five oral cephalosporins, cephalexin, cefadroxil, cefaclor, cefuroxime, and cefixime, and compared them with that of ceftriaxone, a proven effective antibacterial agent for treating late Lyme disease (11). In addition, we performed time-killing assays in order to determine the hours of incubation required for the most active compounds (cefuroxime axetil, cefixime, and ceftriaxone) to kill 99% of the *B. burgdorferi* organisms. These data were combined with recommended dosages and serum half-lives to predict the effectiveness of these oral cephalosporins for treating early and late Lyme disease.

MICs were determined by using a previously described macrodilution method (15). Briefly, the following antibacterial agents were obtained from their respective manufacturers: cephalexin and cefaclor (Lilly Research Laboratories, Indianapolis, Ind.), cefadroxil (Bristol Myers Co., Evansville, Ind.), cefuroxime axetil (Glaxo Pharmaceuticals, Research Triangle Park, N.C.), cefixime (Lederle Laboratories, Pearl River, N.Y.), and ceftriaxone (Roche Laboratories, Belvidere, N.J.). Stock solutions were prepared as recommended by Anhalt and Washington (1). Subsequently, antibacterial agents were serially diluted in culture tubes containing modified Barbour-Stoenner-Kelly (BSK) medium (2, 6).

B. burgdorferi isolates 297 (human spinal fluid), B-31 (*Ixodes dammini* tick, New York), HEM-W1 (human erythema migrans, Wisconsin), WIT (*I. dammini*, Wisconsin), C-1-11 (meadow vole, Illinois), IP (*Ixodes pacificus*, California), P/Bi (human skin, Germany), and SW (*I. ricinus*, Sweden) were cultured at 32°C in separate tubes containing modified BSK medium. After growth, *B. burgdorferi* isolates were added to BSK medium containing antibacterial agents to a final concentration of 10^5 organisms per ml. After being inoculated, the culture tubes were incubated in the dark at 32°C for 7 days. The MIC was the lowest concentration of antibacterial agent in which viable spirochetes could not be detected. Spirochetes which did not exhibit motility were considered nonviable (7). As a control of antibacterial activity, MICs were also determined by using *Escherichia coli* ATCC 25922 according to National Committee for Clinical Laboratory Standards standard procedures (19).

Minimal borrelial lethal concentrations (MBLCs) were determined by inoculating 0.6 ml of negative cultures (no detectable spirochetes) into 5.4 ml of fresh antibacterial agent-free BSK medium (10%, vol/vol) and incubating it in the dark for an additional 7 days at 32°C. The MBLC was the lowest concentration of antibacterial agent from which spirochetes could not be subcultured. All assays were performed in duplicate.

The MICs of the tested antibacterial agents ranged from 0.02 to 128 μg/ml (Table 1). The lowest MIC levels (0.02 μg/ml) were obtained by using ceftriaxone. Of the oral cephalosporins, cefuroxime and cefixime demonstrated the lowest MICs, 0.06 to 0.18 and 0.8 μg/ml, respectively. Cefaclor, cefadroxil, and cephalexin had the highest MIC levels (11 to 128 μg/ml) among the tested antibacterial agents. MBLCs were within 2 dilutions of the MICs for ceftriaxone, cefuroxime, and cefixime. MBLC levels were often greater than 2 dilutions higher for cefaclor, cefadroxil,

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TABLE 1. MICs and MBLCs of five oral cephalosporins and ceftriaxone against eight *B. burgdorferi* isolates

B. burgdorferi isolate	MICs (MBLCs)[a]					
	CEP	CFD	CFC	CFX	CFM	CTX
297	23 (181)	45 (≥128)	91 (256)	0.13 (0.35)	0.8 (1.6)	0.02 (0.04)
B-31	32 (≥256)	64 (≥128)	128 (≥256)	0.18 (0.75)	0.8 (1.6)	0.02 (0.06)
HEM-W1	32 (128)	32 (≥128)	91 (256)	0.18 (0.50)	0.8 (1.6)	0.02 (0.04)
WIT	32 (256)	64 (≥128)	91 (128)	0.18 (0.71)	0.8 (1.1)	0.02 (0.04)
C-1-11	16 (32)	32 (64)	32 (64)	0.13 (0.25)	0.8 (1.6)	0.02 (0.02)
IP	32 (64)	11 (32)	23 (128)	0.06 (0.35)	0.8 (1.1)	0.02 (0.03)
P/Bi	32 (64)	64 (64)	32 (64)	0.09 (0.25)	0.8 (0.8)	0.02 (0.04)
SW	32 (64)	128 (≥128)	64 (128)	0.13 (0.25)	0.8 (1.1)	0.02 (0.02)

[a] Geometric mean value of duplicate analyses. Values are micrograms per milliliter. CEP, cephalexin; CFD, cefadroxil; CFC, cefaclor; CFX, cefuroxime; CFM, cefixime; CTX, ceftriaxone.

and cephalexin. The MICs and MBLCs did not vary significantly among the eight *B. burgdorferi* isolates.

In order to more completely assess the potential efficacies of the most active antibacterial agents, we also determined the rates of *B. burgdorferi* killing by ceftriaxone, cefuroxime, and cefixime by using a modification of a previously described procedure (16). Briefly, *B. burgdorferi* isolates were added to modified BSK medium containing each antibacterial agent to a concentration of 10^7 organisms per ml. After 24, 48, 72, and 96 h of incubation at 32°C, the number of motile spirochetes was determined by using a Petroff-Hausser chamber, and the percentage of killing was calculated as follows: % viability = (number of motile spirochetes/motile organisms in initial inoculum) × 100. When *B. burgdorferi* isolate 297 was exposed to concentrations of an antibacterial agent twice the MIC, 72 h of exposure to ceftriaxone or cefuroxime and 96 h of exposure to cefixime were required to kill 99% of the spirochetes.

As expected and as reported by others (14–16), ceftriaxone demonstrated high in vitro activity. In addition, our results confirmed observations by Luft et al. (16) that prolonged exposure is necessary for effective killing of *B. burgdorferi*. Despite the 72 h of exposure necessary for 99% killing, its high in vitro activity in combination with its long serum half-life of approximately 8 h, its high attainable levels in serum, and its ability to penetrate joints (17) and the central nervous system (20) have made ceftriaxone effective for treating early and late Lyme disease (10, 11).

In this study, the oral cephalosporins cephalexin, cefadroxil, and cefaclor did not appear suitable for Lyme disease therapy because of their MICs, which were approximately equal to or significantly above concentrations in serum achieved with oral administration (approximately 23 to 39 μg/ml), and short serum half-lives (0.5 to 2 h). In contrast, cefuroxime (0.13 μg/ml) had sufficient in vitro activity against *B. burgdorferi* to indicate its potential value in treating Lyme disease. Johnson et al. (14) also reported similar in vitro activity, and additional in vivo studies showed that cefuroxime could prevent experimental *B. burgdorferi* infection in approximately 60% of the Syrian hamsters used (14). In addition, Nadelman et al. (18) recently reported clinical cure or improvement in 53 of 56 (95%) early Lyme disease patients with erythema migrans when they were treated with cefuroxime. Collectively, these results indicate that cefuroxime axetil may be a potential alternative oral therapy for early Lyme disease. The in vitro activity of cefixime (0.8 μg/ml) suggests that this oral cephalosporin may also be an effective potential therapy for early Lyme disease. However, clinical studies confirming these in vitro observations are necessary before cefuroxime or cefixime should be used as therapy for early Lyme disease.

Despite the adequate in vitro activities of cefuroxime and cefixime, these antibacterial agents have significantly shorter half-lives and lower peak levels in serum than ceftriaxone. These pharmacokinetic data and the prolonged serum levels necessary for effective killing leave the efficacies of both cefuroxime and cefixime for treating late Lyme disease in doubt.

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