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Comparative In Vitro Activities of Clarithromycin, Azithromycin, and Erythromycin against *Borrelia burgdorferi*

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The in vitro activities of the macrolide antibiotics clarithromycin, 14-hydroxy-clarithromycin, azithromycin, and erythromycin against 19 isolates of *Borrelia burgdorferi* were investigated. MICs ranged from 0.003 to 0.03 μg of clarithromycin per ml, 0.007 to 0.03 μg of 14-hydroxyclarithromycin per ml, 0.003 to 0.03 μg of azithromycin per ml, and 0.007 to 0.06 μg of erythromycin per ml. Time-kill studies using the B31 strain of *B. burgdorferi* demonstrated a ≥3-$\log_{10}$-unit killing after 72 h with each of the macrolide antibiotics tested in concentrations representing twice the respective MICs.

The variability of the clinical course of Lyme disease, a multisystemic illness caused by the spirochete *Borrelia burgdorferi*, has made evaluation of the effectiveness of antimicrobial therapy difficult. Although *B. burgdorferi* has been reported to be susceptible to a number of antibiotics, including penicillin, amoxicillin, tetracycline, doxycycline, and erythromycin (2, 4), clinical experience has not always correlated with in vitro results (6). For example, erythromycin is extremely active against *B. burgdorferi* in vitro, but it has been clinically less effective than tetracycline, doxycycline, or penicillin in the treatment of early Lyme disease (16). Preac-Mursic and colleagues have previously reported excellent in vitro activity with the newer macrolide antibiotics clarithromycin, azithromycin, and roxithromycin against limited numbers of *B. burgdorferi* isolates (11). The newer macrolide antibiotics are more rapidly absorbed, have a longer elimination half-life, and appear to be better tolerated than erythromycin (3, 10). In addition, they penetrate tissue well and maintain high levels there (3, 5, 15). These features suggest that clarithromycin and azithromycin may be more useful than erythromycin in the treatment of Lyme disease.

In this study we used broth microdilution MICs, MBCs determined by subsurface plating, and time-kill studies to determine the susceptibility of *B. burgdorferi* to macrolide antibiotics in vitro. The drugs tested were erythromycin and azithromycin (Pfizer, Groton, Conn.) and clarithromycin and the major human metabolite of clarithromycin, 14-hydroxy-clarithromycin (Abbott Laboratories, North Chicago, Ill.). Antibiotics were reconstituted in the diluents recommended by their manufacturers. Nineteen human, tick, and mouse isolates of *B. burgdorferi* from North America, Europe, and Russia were studied. Included in this group were low-passage (<10 passages in vitro) and high-passage isolates of the same strains and two mutant isolates that lacked the major outer membrane proteins of *B. burgdorferi* (Table 1). One isolate of *Borrelia hermsii*, an agent of tick-borne relapsing fever, was also studied.

MICs were determined by a broth microdilution method previously described (2). Briefly, antibiotics were diluted twofold in BSK II medium (1) and 100 μl of each concentration to be tested was dispensed into microtiter tray wells in triplicate rows. In addition, 100 μl of BSK II medium was dispensed into wells of each microtiter tray for growth controls of each strain tested and for negative controls. The ranges of antibiotic concentrations tested were 0.0018 to 0.5 μg/ml. Clarithromycin and 14-hydroxyclarithromycin were tested in combination at a fixed 4:1 ratio in an effort to stimulate the ratio observed in human serum following administration of clarithromycin. All wells except negative control wells were inoculated with 10 μl of actively growing cultures of borrelias propagated in BSK II medium and adjusted to yield a final inoculum of ca. 10^6 cells per ml as determined by enumeration with a Petroff-Hausser chamber and phase-contrast microscopy. Microdilution trays were sealed with sterile plastic adhesive and incubated for 72 h at 34°C. The lowest concentration of antibiotic that showed inhibition of visual turbidity and lack of color change from pink to yellow of the BSK II medium indicator as compared with the growth control was interpreted as the MIC. Color discrimination, particularly with low-passage strains, was improved after MIC plates had been held at 4°C for 2 to 3 h (14).

MBCs of clarithromycin, 14-hydroxyclarithromycin, azithromycin, and erythromycin were determined in duplicate by a subsurface plating method previously described (2). The medium for subsurface plating consisted of a 2× concentrate of BSK II medium without gelatin, a 3% bottom agarose, and a 2% top agarose. Briefly, spirochete suspensions were prepared in 2× BSK II medium and 100 μl was added to aliquots of molten agarose and 2× BSK II. After being vortexed gently, suspensions were poured immediately onto the surface of 2× BSK II agar. Plates were rotated gently to spread the suspension evenly, the agarose was allowed to solidify, and plates were incubated in a candle jar at 34°C. Plates were examined visually and colonies were counted after 10 to 12 days of incubation.

At the time that microdilution trays were inoculated, the actual inoculum density of each MIC or MBC test was determined by subsurface plating as described previously (2). After 72 h of incubation and determination of MICs, 10 μl from each microdilution well determined to contain the MIC and above was mixed with 100 μl of 2× BSK II medium and subcultured by subsurface plating. After 10 to 12 days, plates were examined and colonies were counted. The

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TABLE 1. Isolates of *B. burgdorferi* and *B. hermsii* studied

Isolate(s)	Geographic origin	Source
B31-82[a] (ATCC 35210), B31-92, B31 (1p49⁻)[b]	New York	Tick (*Ixodes dammini*)
HB19-83,[a] HB19-92, HB19 (1p49⁻)[b]	Connecticut	Human blood
Ip90-88,[a] Ip90-92	Russia	Tick (*Ixodes persulcatus*)
G1-86,[a] G1-92	Germany	Human cerebrospinal fluid
BB[a]	Wisconsin	Mouse (*Peromyscus leucopus*)
ACA1[a]	Sweden	Human skin
DN127[a]	California	Tick (*Ixodes pacificus*)
Sh2-82[a]	New York	Tick (*Ixodes dammini*)
P/GAU[a]	Germany	Human skin
G25[a]	Sweden	Tick (*Ixodes ricinus*)
MAC13[a]	New York	Human skin
LV4[a]	Austria	Human cerebrospinal fluid
HV1[a]	Austria	Human heart
B. hermsii (HS1)	Washington	Tick (*Ornithodoros hermsi*)

[a] Low-passage isolate.
[b] Lacks the OspA and OspB proteins.

lowest concentration of antibiotic showing ≥99.9% killing of the final inoculum was designated the MBC (9).

The B31 strain of *B. burgdorferi* was used to determine generation times in BSK II medium and rates of killing by clarithromycin, 14-hydroxyclarithromycin, azithromycin, and erythromycin in concentrations representing two times the respective microdilution MIC. Polystyrene tubes containing 10 ml of BSK II medium with the antibiotic concentrations to be tested and one tube without antibiotics were inoculated with 100 μl of an actively growing culture adjusted to yield a final inoculum of ca. 10^6 cells per ml. Tubes were incubated at 34°C. At 0, 24, 48, and 72 h, tubes were gently vortexed and spirochete numbers for each tube were estimated with a Petroff-Hausser counting chamber. The estimated numbers were used to determine the dilutions needed to provide countable plates following subsurface plating. Plates were examined and colonies were counted after 10 to 12 days of incubation. A bactericidal effect was defined by a ≥3-$\log_{10}$-unit killing (99.9%) of the final inoculum (9).

MICs ranged from 0.003 to 0.03 μg of clarithromycin per ml, 0.007 to 0.03 μg of 14-hydroxyclarithromycin per ml, 0.003 to 0.03 μg of azithromycin per ml, and 0.007 to 0.06 μg of erythromycin per ml for the isolates of *B. burgdorferi* tested. The MICs for 50 and 90% of isolates tested and the MIC range of each antibiotic are shown in Table 2. Clarithromycin and 14-hydroxyclarithromycin tested in combination in a fixed 4:1 ratio showed no greater activity against the B31

TABLE 2. Activity of clarithromycin, 14-hydroxyclarithromycin, azithromycin, and erythromycin against 19 isolates of *B. burgdorferi*

Antibiotic	MIC[a] (μg/ml)		
	50%	90%	Range
Clarithromycin	0.007	0.015	0.003–0.03
14-Hydroxyclarithromycin	0.015	0.015	0.007–0.03
Azithromycin	0.007	0.03	0.003–0.03
Erythromycin	0.03	0.03	0.007–0.06

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

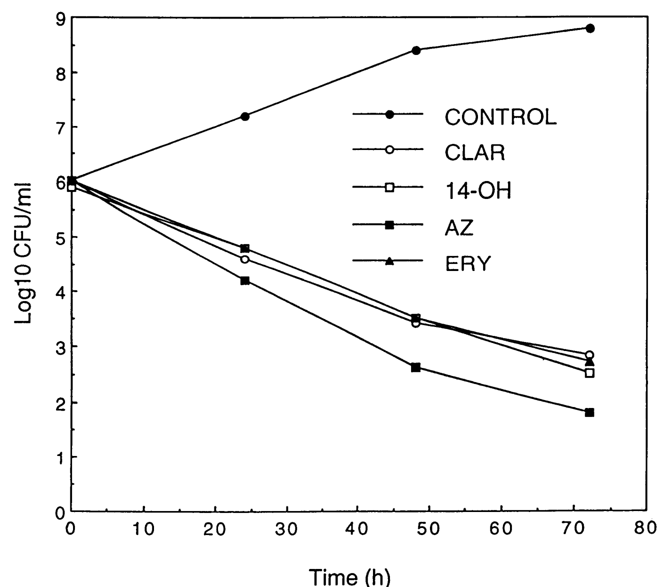

FIG. 1. Time-kill curves with the B31 strain of *B. burgdorferi*. Concentrations of clarithromycin (0.015 μg/ml), 14-hydroxyclarithromycin (0.03 μg/ml), azithromycin (0.015 μg/ml), and erythromycin (0.06 μg/ml) represent two times the respective MIC. CLAR, clarithromycin; 14-OH, 14-hydroxyclarithromycin; AZ, azithromycin; ERY, erythromycin.

strain of *B. burgdorferi* than the separate agents tested alone. The modal MICs for *B. hermsii* were 0.015 μg of clarithromycin per ml, 0.015 μg of 14-hydroxyclarithromycin per ml, 0.03 μg of azithromycin per ml, and 0.03 μg of erythromycin per ml. MBCs for the B31 strain of *B. burgdorferi* determined by the subsurface plating method described above were 0.015 μg of clarithromycin per ml, 0.007 μg of azithromycin per ml, and 0.06 μg of erythromycin per ml.

Time-kill studies with clarithromycin, 14-hydroxyclarithromycin, azithromycin, and erythromycin in concentrations representing two times the respective MIC demonstrated a ≥3-$\log_{10}$-unit killing (99.9%) of the final inoculum (Fig. 1). Azithromycin provided the greatest reduction in CFU after 72 h. The generation time of B31 determined from growth controls was approximately 8 to 9 h.

In this study, clarithromycin, 14-hydroxyclarithromycin, azithromycin, and erythromycin demonstrated excellent in vitro inhibitory activity against multiple isolates of *B. burgdorferi* from diverse geographic origins. In addition, clarithromycin, azithromycin, and erythromycin were bactericidal against the B31 strain of *B. burgdorferi*, as demonstrated by MBCs and time-kill studies.

MICs for low- and high-passage isolates of the same strain of *B. burgdorferi* were within 2-$\log_2$-unit dilutions. It is reasonable therefore to use high-passage strains in further in vitro investigations of macrolide antibiotics, since they offer the advantage of more-rapid growth and pose less of a laboratory hazard. The MICs for the mutant isolates of *B. burgdorferi* which lacked outer surface proteins A and B were not significantly different from those for isolates of the same strain which had both membrane proteins present (MICs were within 1 dilution of a modal value). The absence of an effect on MICs in mutants lacking OspA and OspB suggests that these proteins are probably not involved in transport of macrolides into the cell. In other words, OspA

and OspB do not seem to be functioning as porins. The loss of outer membrane proteins also does not appear to render the spirochetes supersusceptible to the hydrophobic macrolide antibiotics, as is the case with outer membrane-defective mutants of *Escherichia coli* and *Salmonella typhimurium* (17). Because there is evidence that the OspA and OspB proteins are not required for viability (13), this study indicates that even if *B. burgdorferi* variants lacking Osp proteins occur in vivo, there is no reason to expect antibiotic failures of macrolides on this basis.

Despite excellent in vitro activity against *B. burgdorferi*, erythromycin is regarded by most authors as only an alternative antibiotic for the treatment of early Lyme disease (7, 12). Conflicting data have been reported on the efficacy of azithromycin in the treatment of Lyme disease. Massarotti and colleagues reported comparable efficacy with azithromycin, amoxicillin plus probenecid, and doxycycline when given for 10 days for the treatment of early Lyme disease (8). However, Luft et al. reported that azithromycin given daily for 1 week was significantly less effective than amoxicillin given for 3 weeks in the treatment of erythema migrans (7). Clinical trials of clarithromycin in the treatment of Lyme disease are in progress. While the newer macrolide antibiotics show promise for the treatment of Lyme disease, definition of their role awaits more extensive clinical studies.

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