ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2004, p. 344–347 Vol. 48, No. 1
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.1.344–347.2004

Comparison of In Vitro Activities of Ketolides, Macrolides, and an Azalide against the Spirochete *Borrelia burgdorferi*

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Received 13 March 2003/Returned for modification 3 June 2003/Accepted 2 October 2003

Two ketolides, three macrolides, and one azalide were tested in vitro against 17 isolates of the *B. burgdorferi* s.l. complex. As measured in micrograms per milliliter, activity was highest for cethromycin (MIC at which 90% of the tested isolates were inhibited [MIC_{90}], 0.0019 μg/ml) and telithromycin (MIC_{90}, 0.0078 μg/ml). Electron-microscope analysis and time-kill studies also supported enhanced effectiveness of both ketolides.

Borrelia burgdorferi is susceptible to macrolides in vitro (5, 8, 9, 18, 20), and important clinical indications for macrolides in the therapy of acute Lyme diseases (LD) include pregnancy, β-lactam allergy, and treatment of children <14 years of age (17, 23, 24, 26). Recently new clarithromycin derivatives, the ketolides, proved highly active in vitro against atypical microorganisms, such as *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella* species (1, 3, 6, 22). If effective in vitro and in vivo against borreliae, ketolides should be considered for treatment of LD as well. Here, we investigated under standardized conditions the in vitro activities of ketolides in comparison to those of macrolides and one azalide against 17 isolates of the *B. burgdorferi* complex, including all three genospecies pathogenic for humans, in addition to one *Borrelia valaisiana* and one *Borrelia bissettii* tick isolate.

The clinical, geographic, and genotypic characteristics of the strains tested (Table 1) have been published elsewhere (8, 10, 12). Except for reference strain B31 (ATCC 35210), low-passage isolates (10 to 20 passages) were tested using microtiter trays carrying lyophilized antimicrobial agents (Merlin-Diagnostika GmbH, Bornheim-Hersel, Germany). The test ranges appear in Table 1. Ceftriaxone and apramycin served as controls with known high activity and no activity, respectively, against borreliae (12, 13). MICs were determined after 72 h using a colorimetric assay, as recently described in detail (9, 13). Minimal borreliacidal concentrations (MBCs) were determined under stringent conditions (100% killing in liquid medium) at 72 h. Aliquots (20 μl) from all vials without detectable growth were diluted (1:1,000) below the MIC in Barbour-Stoenner-Kelly medium (BSK) and inspected for regrowth after 3 weeks of subculture (9, 10, 12). For each isolate and substance, independent experiments were performed on different days, with MICs and MBCs reported as the median of all three experiments. Moreover, time-kill studies with *B. burgdorferi* strain PKa-1 and *Borrelia afzelii* strain FEM1 exposed to telithromycin, cethromycin, and erythromycin for 120 h and electron-microscope analysis of *B. garinii* PSth cultures in the log phase of growth treated with 0.0312 μg of cethromycin/ml for 72 h were performed as described elsewhere (10, 13, 19). To detect possible differences in MIC and MBC data for the borrelial genospecies, the Kruskall-Wallis test was applied using BIAS, version 5.03 (Epsilon Verlag, Hochheim, Germany), for statistical calculation. Finally, possible antibiotic-medium interactions were investigated after 24 h of preincubation of the antibiotic-BSK preparation followed by conventional MIC determination for another fastidious organism, *S. pneumoniae* ATCC 49619 (Table 1). Testing was performed in triplicate, following NCCLS protocols (15) except for use of a preincubated antibiotic-BSK preparation.

Apramycin was ineffective against the 17 borrelial strains. Table 1 summarizes the in vitro activities of the other antimicrobial agents. MICs and MBCs of each antimicrobial agent for the same isolate spanned a maximum range of ±1 $\log_2$ unit dilution around the median only. The ketolides were the most potent against borrelial isolates on a micrograms-per-milliliter basis. For all agents except cethromycin and telithromycin, the MIC at which 90% of isolates were inhibited (MIC_{90}) and the MBC at which 90% of the isolates were killed were ≥0.01 μg/ml and >0.25 μg/ml, respectively. Statistical analysis, including all measured MICs and MBCs (n = 816), did not show significant differences in the tested genospecies. In our time-kill experiments (Fig. 1A to C), exposure to the ketolides for PKa-1 and FEM1 at three $\log_2$ unit dilutions above the MIC led to a >3 $\log_{10}$ unit (99.9%) reduction of morphologically intact motile cells after 48 to 120 h. Reduction was more pronounced for FEM1 than for PKa-1. Erythromycin clearly was less effective than the ketolides. Electron-microscopic analysis of strain PSth exposed to cethromycin at 0.0312 μg/ml (4 $\log_2$ unit dilutions above the MIC_{90}) further substantiated in vitro activity by showing spheroblast formation and severe cell disintegration (data not shown).

Test results for *S. pneumoniae* ATCC 49619 in BSK appear in Table 1. The conventional MICs for apramycin (data not shown), erythromycin, and ceftriaxone were in the range published for these agents by the NCCLS (15, 16). The MICs for azithromycin, clarithromycin, telithromycin, and cethromycin, however, were at the lower range limits or were one to two $\log_2$ unit dilutions below the ranges specified by the NCCLS (15)

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TABLE 1. Antibiotic susceptibility of 17 *B. burgdorferi* isolates and medium control organism to macrolides, one azalide, ketolides, and ceftriaxone as determined in BSK[a]

Isolate or parameter	Value (μg/ml) for antimicrobial agent[b]													
	Erythromycin		Roxithromycin		Clarithromycin		Azithromycin		Telithromycin		Cethromycin		Ceftriaxone	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B. burgdorferi														
B31 (ATCC 35210)	0.0156	>0.5	0.0156	0.5	0.0039	0.5	0.0019	0.12	≤0.0002	0.06	≤0.0002	0.06	0.0625	1
297	0.0039	>0.5	0.0019	0.25	0.0019	0.25	≤0.0002	0.5	≤0.0002	0.12	≤0.0002	0.03	0.0625	0.5
LW2	0.0312	>0.5	0.0156	0.5	0.0039	>0.5	0.0009	0.5	≤0.0002	0.12	≤0.0002	0.03	0.0312	2
Z25	0.0078	>0.5	0.0039	0.5	0.0019	0.5	0.0009	0.5	≤0.0002	0.12	≤0.0002	0.12	≤0.0156	2
PKa-I	0.0156	>0.5	0.0078	>0.5	0.0156	>0.5	0.0004	0.5	≤0.0002	0.06	≤0.0002	0.06	≤0.0156	2
B. garinii														
PTrob	0.0078	>0.5	0.0078	>0.5	0.0039	0.25	0.0009	0.5	0.0004	0.12	≤0.0002	0.03	0.0312	2
JP2	0.0156	>0.5	0.0156	>0.5	0.0039	0.5	0.0019	0.25	≤0.0002	0.06	≤0.0002	0.03	≤0.0156	0.5
A87SB	0.0078	>0.5	0.0039	0.25	0.0019	0.12	0.0004	0.03	≤0.0002	0.25	≤0.0002	0.12	≤0.0156	1
ZQ1	0.0156	>0.5	0.0078	>0.5	0.0039	0.5	0.0009	0.25	0.0009	0.12	≤0.0002	0.03	≤0.0156	2
PSth	0.0625	>0.5	0.0625	>0.5	0.0312	>0.5	0.0156	0.5	0.0078	0.25	0.0019	0.12	0.0625	2
B. afzelii														
EB1	0.0625	>0.5	0.0625	>0.5	0.0312	>0.5	0.0156	0.5	0.0078	0.25	0.0078	0.25	0.0625	2
EB2	0.0156	>0.5	0.0156	>0.5	0.0078	0.5	0.0039	0.25	0.0009	0.12	0.0004	0.12	≤0.0156	1
FEM1	0.0156	>0.5	0.0078	0.12	0.0039	0.12	0.0019	0.12	≤0.0002	0.03	≤0.0002	0.03	≤0.0156	1
VS461	0.0078	>0.5	0.0039	>0.5	0.0019	0.5	0.0004	0.25	≤0.0002	0.12	≤0.0002	0.12	0.0625	1
FAC-1	0.0312	>0.5	0.0156	>0.5	0.0078	0.12	0.0019	0.06	≤0.0002	0.12	≤0.0002	0.12	≤0.0156	0.5
B. valaisiana														
VS116	0.0156	>0.5	0.0078	0.5	0.0039	>0.5	0.0009	0.06	0.0004	0.12	≤0.0002	0.06	0.0312	2
B. bissettii														
25015	0.0312	>0.5	0.0312	0.5	0.0078	0.5	0.0039	0.03	0.0009	0.5	≤0.0002	0.06	0.0312	2
Range	0.0039–0.0625	0.25–>0.5	0.0009–0.0625	0.125–>0.5	0.0009–0.0312	0.12–>0.5	≤0.0002–0.0156	0.0156–0.5	≤0.0002–0.0078	0.0156–0.5	≤0.0002–0.0078	0.0156–0.25	≤0.0156–0.0625	0.25–2
MIC_{90} or MBC_{90}[c]	0.0625	>0.5	0.0625	>0.5	0.0312	>0.5	0.0156	0.5	0.0078	0.25	0.0019	0.125	0.0625	2
Medium control, *S. pneumoniae* (ATCC 49619)[d]														
Median MIC in BSK	0.0625		0.0312		0.0156		0.0156		0.0019		0.0019		0.0625	
MIC Range in BSK	0.0312–0.0625		0.0156–0.0625		0.0078–0.0156		0.0078–0.0156		0.0019–0.0039		0.0009–0.0019		0.0625	
NCCLS range	0.0312–0.012		NA		0.0312–0.12		0.0625–0.25		0.0039–0.312[e]		0.0039–0.0156[f]		0.0312–0.12	

[a] Antimicrobial susceptibility was determined on three different days, and MIC and MBC values for each isolate were reported as the median of three experiments:
[b] The test ranges (in μg/ml) were as follows: for erythromycin, roxithromycin, clarithromycin, azithromycin, telithromycin, and cethromycin, 0.0002 to 0.5; for ceftriaxone, 0.0156 to 32.
[c] MBC_{90}, MBC required to kill 90% of the isolates.
[d] To investigate significant antibiotic-medium interaction, MICs for *S. pneumoniae* (ATCC 49619) were determined on three different days, referring to the NCCLS method (15) except for the use of BSK and pre-incubation of the antibiotic-medium test preparation for 24 h before testing. Results were reported as the median of all three experiments.
[e] Tentative NCCLS range.
[f] Tentative range provided by manufacturer.

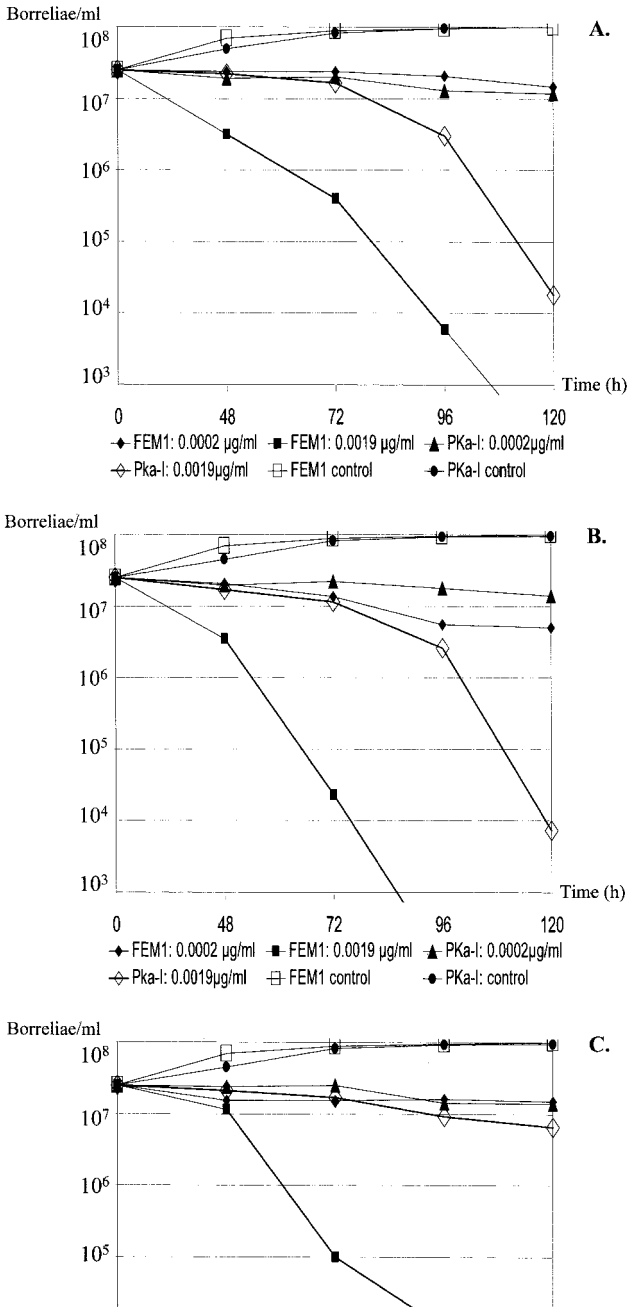

FIG. 1. (A to C) Time-kill curves for *B. burgdorferi* s.l. isolates PKa-1 and FEM1 with telithromycin (A) and cethromycin (B) at the MIC (0.0002 μg/ml) and eight times the MIC (0.0019 μg/ml), as well as with erythromycin (C) at the MIC (0.0156 μg/ml) and eight times the MIC (0.125 μg/ml). Experiments were performed on different days by investigation of growth using conventional cell counts, and data were reported as the means from two experiments.

and the tentative ranges indicated by the manufacturer for cethromycin.

In our study, the rank order of activity by classical macrolides and azalides against borreliae clearly corresponds to the effectiveness of these agents as revealed by in vitro susceptibility studies and clinical treatment trials to date (2, 4, 5, 7, 8, 9, 11, 23, 24), demonstrating higher in vitro effectiveness for azithromycin (MIC_{90}, 0.0156 μg/ml) than for erythromycin (MIC_{90}, 0.0625 μg/ml), roxitromycin (MIC_{90}, 0.0625 μg/ml), and clarithromycin (MIC_{90}, 0.0312 μg/ml). Median MICs of the different substances, however, tended to vary over a 10-fold range between individual strains, with the *B. garinii* isolate PSth and the *B. afzelii* isolate EB1 showing the highest MICs for both the classical macrolides and the ketolides. In contrast to the recent findings of Sicklinger et al. (20), we could not show significant differences in MBCs for the different genospecies tested against macrolides or ketolides, possibly owing to differences in test methodology and inoculum. Instead, our in vitro findings point to interstrain variability of the in vitro susceptibilities of *B. burgdorferi* to macrolides rather than to intergenospecies-specific variations as observed for other antimicrobial agents (8, 9, 11, 13). Testing of *S. pneumoniae* ATCC 49619 clearly demonstrated increased activities of some macrolides in BSK. This side effect of BSK also was noted by other authors (2, 21). However, further investigations are necessary to assess possible consequences for in vitro susceptibility testing of these agents against *B. burgdorferi*.

Classical macrolides and azalides frequently fail in the therapy of early LD (7, 14, 17, 26), and clinical relapse has been observed following conclusion of treatment (14, 17, 26). Moreover, it has been speculated that resistance may develop in borreliae preexposed to erythromycin owing to resistant subpopulations (25). Based upon our findings, however, the ketolides were superior in vitro on a micrograms-per-milliliter basis when tested alongside classical macrolides under identical test conditions in BSK. This is further substantiated by our time-kill experiments (Fig. 1A to C) and by electron microscopy. Moreover, maximum concentrations of ketolides in plasma after regular oral dosage (1, 6, 27) are 90 to 270 times higher than the MIC_{90}s against borreliae in our study, and tissue concentrations exceed by 10-fold the maximum plasma concentrations of both drugs in controls (1, 6, 27). Therefore, the potential role of ketolides in the treatment of LD merits further evaluation.

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