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In Vitro Activity of Rokitamycin, a New Macrolide, against *Borrelia burgdorferi*

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The activity of rokitamycin, a new macrolide with a 16-member ring, was tested against *Borrelia burgdorferi* in vitro. The antibiotic had a lower MIC at which 50% of the isolates are inhibited than erythromycin, the parent 14-member macrolide, but the same MIC at which 50% of the isolates are inhibited as the other recent 14- and 15-member macrolides, like clarithromycin and azithromycin. The MBC was equal to the MIC at which 50% of the isolates are inhibited, so rokitamycin can be considered bactericidal against *B. burgdorferi*. The sensitivity of the *Borrelia* strains tested was not correlated with the particular species *Burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* or with the number of subcultures of the isolates.

Lyme borreliosis is a very common tick-borne infection that is caused by a spirochete, *Borrelia burgdorferi*. The variability of the clinical course of the disease has made it difficult to assess the effectiveness of antimicrobial therapy. Although the spirochete has been reported to be susceptible in vitro to a number of antibiotics, including penicillin, tetracycline, doxycycline, ceftriaxone, erythromycin, and josamycin (4, 5, 7, 16), the efficacies of the agents in animal studies in vivo did not always parallel the in vitro efficacies of some of them, such as erythromycin (8, 13). Although erythromycin is less effective than expected, the newer macrolides like clarithromycin, azithromycin, and roxithromycin were extensively assayed against *B. burgdorferi* in vitro in view of their longer elimination half-life and better penetration of tissues, where they maintain a high concentration (9, 14).

Rokitamycin is a new, semisynthetic macrolide with a 16-member ring which is reported to have some more interesting properties than the previous antibiotics of the same series; these properties include stronger binding to ribosomes, more active cell transport, and bactericidal activity close to the MIC (1, 6, 10).

In this study, we assayed the susceptibility to rokitamycin in vitro of *Borrelia* strains belonging to the three recently defined species, *B. burgdorferi* sensu stricto, *B. garinii*, and *B. afzelii* (2), employing recent isolates as high-passage-adapted strains.

The following *B. burgdorferi* strains were studied: B31, Myo1, HB6, Alcaide, and Emilia, belonging to the species *B. burgdorferi* sensu stricto; BL3 and Nancy, belonging to *B. afzelii*; and BITS, B45, and N34, belonging to *B. garinii* (3). Strains BL3, Emilia, and Myo1 were subcultured not more than three times, so they were considered low-passage isolates. Strains were grown in BSK II medium at 34°C.

The antibiotics used were rokitamycin, provided by Formenti (Milan, Italy), and erythromycin (Sigma Chemical Co., St. Louis, Mo.). Stock solutions were prepared (1 mg/ml) and stored at −20°C for not more than 1 month. A broth microdilution method was used to determine the MIC; 100 μl of BSK II medium was dispensed into each well of the microtiter tray in duplicate rows. The antibiotic was diluted to the appropriate concentration, and 100 μl was dispensed into the first well for each set of experiments, carefully mixed by repeated pipetting, and diluted twofold in each well, skipping one for growth control. The concentrations ranged from 0.00008 to 2.5 μg/ml. A culture growing in the log phase was used; density was determined with a Thoma standard counting chamber (Hawksley, England) and dark-field microscopy. Ten microliters of each culture was inoculated into each well, yielding a final concentration of 10^6 borreliae per ml. Microtiter trays were sealed with sterile polyester adhesive film (Sarstedt) and incubated at 34°C. After a week, the wells were examined by dark-field microscopy and the lowest concentration of antibiotic showing no visible growth was taken as the MIC. The MBC was determined for strains Nancy and BITS by removing 20 μl from all wells with no visible growth (and from two growing wells as positive controls), inoculating this in duplicate in 2 ml of fresh, antibiotic-free BSK II medium, and then incubating this for 2 weeks at 34°C. The MBC was defined as the lowest concentration of antibiotic at which no spirochetes were subcultured. Each determination was performed in duplicate. Data were evaluated by Student's *t* test (with 95% confidence intervals) with GraphPad Software (San Diego, Calif.).

MICs and MBCs of rokitamycin against each *Borrelia* strain are reported in Table 1, together with erythromycin activity. The MICs at which 50 and 90% of the isolates are inhibited calculated from the MICs were 0.0097 and 0.056 μg/ml, respectively. These antibiotics achieved excellent inhibition of *B. burgdorferi* compared with erythromycin, which had corresponding values of 0.031 and 0.062 μg/ml, respectively. This sensitivity was comparable to or even better than that shown in previous studies with other macrolides such as clarithromycin, 14-OH-clarithromycin, and azithromycin, which showed MICs at which 50% of the isolates are inhibited of 0.007, 0.015, and 0.007 μg/ml, respectively (5, 11). MICs varied for the different *Borrelia* strains tested but not significantly compared with the MIC at which 50% of the isolates are inhibited ($P = 0.0709$). This indicated that neither the number of passages of the isolates nor their species appeared to affect rokitamycin's activity. The latter finding is in contrast with that of a recent report (12) in which strains belonging to the species *B. garinii* showed stronger susceptibility to antibiotics than strains belonging to the species *B. burgdorferi* sensu stricto and *B. afzelii*. In that study, however, different classes of antibiotics with different mechanisms of activity were assayed. Furthermore,

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TABLE 1. Sensitivity to rokitamycin and erythromycin of various strains of *B. burgdorferi* sensu stricto and other *Borrelia* species

Species	Strain	MIC (μg/ml)		MBC (μg/ml)	
		Rokitamycin	Erythromycin	Rokitamycin	Erythromycin
B. burgdorferi sensu stricto	B31	0.0048	0.031	ND[a]	ND
	HB6	0.0048	0.0075	ND	ND
	Alcaide	0.0097	0.015	ND	ND
	Myo1[b]	0.047	0.015	ND	ND
	Emilia[b]	0.115	0.062	ND	ND
B. garinii	BITS	0.0072	0.031	0.0097	0.062
	N34	0.023	0.015	ND	ND
	B45	0.056	0.031	ND	ND
B. afzelii	Nancy	0.0097	0.031	0.0097	0.062
	BL3[b]	0.047	0.062	ND	ND

[a] ND, not determined.
[b] Low-passage isolates.

few *Borrelia* strains were employed, including only one representative of *B. burgdorferi* sensu stricto, two representatives of *B. garinii*, and two representatives of *B. afzelii*. The MBCs of rokitamycin calculated for strains BITS and Nancy were the same as the MICs, suggesting that the antibiotic was bactericidal. The bactericidal effect of rokitamycin already documented for other microorganisms could possibly be due to the new characteristics of this macrolide, such as its high level of ribosome affinity and better penetration of cells. On the basis of its favorable in vitro activity, rokitamycin could offer a new choice for the treatment of Lyme borreliosis. Preliminary clinical experimentation (15) with one group of patients indicated that rokitamycin was very effective, because it completely eliminated the erythema migrans and systemic symptoms and also prevented late Lyme borreliosis pathology.

In conclusion, this new macrolide could offer a new choice for the treatment of early Lyme borreliosis. The results cast light on the efficacy of the new macrolides, which are the only antibiotics currently in use that are able to reach borreliae in the cells and consequently prevent their survival within tissue.

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