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In Vitro Activities of New Macrolides and Rifapentine against *Brucella* spp.

J. A. GARCÍA-RODRÍGUEZ,* J. L. MUÑOZ BELLIDO, M. J. FRESNADILLO, AND I. TRUJILLANO

Department of Microbiology, Hospital Clínico Universitario, Paseo de San Vicente 108, 37007 Salamanca, Spain

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We have tested the in vitro activities of streptomycin, rifampin, tetracyclines, trimethoprim-sulfamethoxazole, erythromycin, four new macrolides (roxithromycin, azithromycin, clarithromycin, and dirithromycin), and rifapentine against 62 strains of *Brucella* spp. Azithromycin and clarithromycin were, respectively, eight- and twofold more active than erythromycins (MIC for 90% of strains = 2, 8, and 16 μg/ml, respectively). The activity of rifapentine was similar to that of rifampin (MIC for 90% of strains = 1 μg/ml).

Brucellosis remains a public health problem in several areas, such as Mediterranean countries (6). Current treatments of acute brucellosis are tetracyclines plus streptomycin or tetracyclines plus rifampin. Both combinations show good results (3, 4, 14, 19, 22). Relapse rates for tetracyclines plus streptomycin range between 0 and 8.4% (3, 4, 14, 19, 22). Relapse rates for tetracyclines plus rifampin are unacceptable when the period of treatment is 30 days (38.8%) (1), but they are similar to those of tetracyclines plus streptomycin when the period of treatment is 6 weeks (0% to 13.4%) (4). Nevertheless, these regimens have disadvantages. Besides the possibility of side effects, streptomycin is administered intramuscularly for 2 weeks, streptomycin and tetracyclines must be avoided by pregnant women, and tetracyclines are also contraindicated for children. Rifampin alone, the usual treatment in pregnant women, has shown a high proportion of relapses and might select resistant strains (16, 21). Furthermore, since the incidence of tuberculosis in some areas in the world is increasing, the use of rifampin in nontuberculous patients in these areas should be avoided. Thus, new antimicrobial agents should be tested against *Brucella* spp. to improve the alternatives in the treatment of human brucellosis. We have tested the in vitro activities of five macrolides and a new rifamycin, rifapentine, in comparison with streptomycin, tetracycline, rifampin, and trimethoprim-sulfamethoxazole against 62 strains of *Brucella* spp. (19 type strains of *Brucella* spp. and 43 clinical strains of *Brucella melitensis*).

The antibiotics used (streptomycin, tetracycline, trimethoprim-sulfamethoxazole, rifampin, rifapentine, erythromycin, roxithromycin, azithromycin, clarithromycin, and dirithromycin) were kindly provided by their respective manufacturers.

The 62 strains of *Brucella* spp. included 19 type strains (*B. melitensis* ATCC 23456, 23457, and 23458; *B. abortus* ATCC 23448, 23449, 23450, 23451, 23452, 23453, and 23455 and NCTC 8038 and 11363; *B. suis* ATCC 23444, 23445, 23446, and 23447; *B. neotomae* ATCC 23459; *B. ovis* ATCC 25840; and *B. canis* 23365) and 43 clinical isolates of *Brucella melitensis* (biotype undetermined), all of them obtained from blood cultures of patients with acute brucellosis. The strains were stored in skim milk at −70°C and twice subcultured before starting the study.

The in vitro activities of the antimicrobial agents tested were determined by the agar dilution method, by using previously described methods (7). Breakpoints utilized and results obtained are shown in Table 1. The most active macrolide was azithromycin, whose activity was four- to eightfold higher than that of erythromycin. Clarithromycin was also more active than erythromycin, but roxithromycin and dirithromycin were not significantly more active than erythromycin against *Brucella* spp. Rifapentine showed activity similar to that of rifampin. There was not any significant difference in susceptibility to antimicrobial agents either between the different species tested or between the type and clinical strains in *B. melitensis*. Recent studies in Spain, on 358 strains (15) of *B. melitensis*, show MICs of azithromycin slightly lower than MICs found in this study (1 twofold dilution for the MIC for 50% of the strains [MIC_{50}] and 1 to 2 twofold dilutions for the MIC_{90}); the difference might result from differences in methods or be related to slight differences of sensitivity in our area, but it probably has no therapeutic repercussions. MIC_{90}s of tetracycline were 0.25 μg/ml both in our study and in the study previously described (15). These results show a remarkable uniformity in the susceptibility of *Brucella* spp., since results on more than 400 strains in different studies performed in different geographic areas show the same MIC_{90}s of tetracycline and MIC_{90}s of azithromycin ranging between 0.5 and 2 μg/ml.

The problems with currently accepted therapeutic regimens for brucellosis make studies on new antibiotics against *Brucella* spp. desirable. Other antibiotics, such as fluorinated quinolones, initially had good promise, since preliminary studies showed MIC_{90}s usually lower than 1 μg/ml (8), and they penetrate leukocytes and macrophages (5). However, quinolones lack bactericidal activity against *Brucella* spp. (7) and development of resistance in *B. melitensis* during treatment with ciprofloxacin has been described (2). Moreover, relapse rates are higher than 80% when human brucellosis is treated with ciprofloxacin (23). Thus, fluorinated quinolones do not currently constitute adequate therapy for brucellosis.

During the past few years, new macrolide antibiotics have been developed. Macrolides (and especially erythromycin, the main antibiotic in this group) are generally perceived as safe and effective antibiotics, with good activity against other intracellular pathogens. Our results show that more than 50% of strains of *Brucella* spp. are resistant or intermediate against erythromycin. Dirithromycin and roxithro-

* Corresponding author.

TABLE 1. In vitro activities of 10 antimicrobial agents against *Brucella* spp.

Antimicrobial agent	MIC (μg/ml) (no. of strains)[a]		
	50	90	Range
Streptomycin	2 (53)	4 (62)	0.1–4
Tetracycline	0.2 (62)	0.2 (62)	0.01–0.2
Rifampin	0.5 (40)	1 (59)	0.1–4
Rifapentine	0.5 (38)	1 (58)	0.2–4
T/S[b]	4/76 (62)	4/76 (62)	0.1/1.9–4/76
Erythromycin	4 (41)	16 (62)	0.2–16
Dirithromycin	8 (42)	16 (62)	0.5–16
Roxithromycin	8 (44)	16 (60)	0.1–32
Clarithromycin	2 (44)	8 (62)	0.06–8
Azithromycin	1 (47)	2 (59)	0.1–4

[a] 50 and 90, MICs for 50 and 90% of strains. Numbers in parentheses are the numbers of strains inhibited. Breakpoints utilized were as follows: tetracycline, ≥16 μg/ml; rifampin, ≥4 μg/ml; T/S, ≥8/152 μg/ml; erythromycin, ≥8 μg/ml. Rifapentine, dirithromycin, roxithromycin, clarithromycin, and azithromycin breakpoints are not yet assigned.

[b] T/S, trimethoprim-sulfamethoxazole.

mycin showed MIC_{50}s and MIC_{90}s similar to those of erythromycin. Only clarithromycin, and mainly azithromycin, are significantly more active than erythromycin against *Brucella* spp., showing MIC_{90}s two- and eightfold lower than those of erythromycin, respectively. These results agree with general results on activity of these macrolides (11) that show azithromycin and clarithromycin as the most active macrolides among those tested in this study. All the macrolides tested show better pharmacokinetics than erythromycin, with improved peak concentrations in serum (mainly roxithromycin and clarithromycin) and/or tissue (mainly dirithromycin and azithromycin) (12). These pharmacokinetic characteristics may make these macrolides more active in vivo than erythromycin (in the case of macrolides with activity in vitro similar to that of erythromycin) or increase the differences of activity (in the case of macrolides more active than erythromycin in vitro). Azithromycin, the most active macrolide according to our results, adds a high rate of tissue penetration to the high in vitro activity against *Brucella* spp. (12). Concentrations in tissue exceed those in serum by as much as 100-fold after a single 500-mg oral dose (17). Moreover, high concentrations of drug are found in polymorphonuclear leukocytes, macrophages, and fibroblasts (17, 18), the intracellular/extracellular concentration ratio being approximately 40 after 1 h of incubation (18), and the drug is released slowly in the absence of extracellular drug (27% of the initial amount of azithromycin remains cell associated after 48 h [9]). These characteristics (high in vitro activity, a very high rate of tissue penetration, and high and lasting concentrations in leukocytes and macrophages) are potentially or theoretically very favorable for the treatment of intracellular infections, and they suggest that azithromycin might be a macrolide alternative for the treatment of brucellosis.

Rifapentine, a rifamycin analog, has shown greater intrinsic activity than rifampin against mycobacteria, both in vitro (10) and in animal models (13), and activity similar to that of rifampin against other microorganisms (25). Our results also show similar activities for rifampin and rifapentine against *Brucella* spp. As for some macrolides tested, the main advantages of rifapentine may be related to pharmacokinetic characteristics. Rifapentine is concentrated in macrophages to a greater degree than rifampin (20), its plasma elimination half-life is four- to fivefold longer than that of rifampin (24), and its activity is not adversely affected by lowering the pH to 5.0 (10). Since *Brucella* spp. are usually intracellular pathogens, the value of in vitro testing is limited, because the need for the antibiotic to penetrate the cell and act under unfavorable conditions within the phagosome may definitely limit its usefulness. Nevertheless, the pharmacokinetic characteristics of rifapentine and its efficacy in animal models of tuberculosis make it probable that the intracellular situation of brucellae does not impair significantly the activity of these antimicrobial agents against *Brucella* spp.

These results warrant the development of further studies on animal models to know the potential of these antimicrobial agents for the treatment of human brucellosis.

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