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Comparative In Vitro Activities of MDL 473, Rifampin, and Ansamycin against *Mycobacterium intracellulare*

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The susceptibilities of 20 clinical isolates of *Mycobacterium intracellulare* to rifampin, MDL 473, and ansamycin were determined by broth and agar dilution methods. Ansamycin was more active than MDL 473, which in turn was usually more active than rifampin by either method. The MICs for 90% of the strains tested by agar dilution were 1, 2, and 4 μg of ansamycin, MDL 473, and rifampin per ml, respectively.

Mycobacterium intracellulare is a frequent cause of nontuberculous pulmonary mycobacterial infection. Pulmonary infections have been treated with various multidrug regimens (3, 8), the efficacy of which is unclear, in part because of an inadequate understanding of the natural history of this disease process. These organisms have infrequently been implicated in extrapulmonary disease (11). Disseminated disease due to *M. intracellulare* has recently been reported in patients with the acquired immunodeficiency syndrome (4).

These organisms are usually resistant in vitro to the standard antituberculous drugs (2, 4). There is evidence that the resistance of *M. intracellulare* to currently available antimicrobials is based on impermeability to these agents (5, 7, 12). Rifampin and the related compounds MDL 473 and ansamycin have been reported to have in vitro activity against these organisms (9, 13–15). This study was undertaken to evaluate the comparative in vitro activities of these compounds against clinical isolates of *M. intracellulare*.

Clinical isolates of *M. intracellulare* were obtained from the following: Nancy Warren, Division of Consolidated Laboratory Services, Department of General Services, Richmond, Va.; Barbara Body, University of Virginia Medical Center, Charlottesville, Va.; and from patients with the acquired immunodeficiency syndrome cared for at Upstate Medical Center, Syracuse, N.Y.

The antibiotics evaluated were obtained as standard powders: rifampin (potency, 1,007 μg/ml) from CIBA-GEIGY Corp., Summit, N.J.; MDL 473 (potency, 1,000 μg/ml) from Merrell Dow Pharmaceuticals, Cincinnati, Ohio; and ansamycin (potency, 1,000 μg/ml) from Farmitalia Carlo Erba, Milan, Italy. Stock solutions of each antibiotic were prepared by hydrating a known weight of drug in ethanol with subsequent dilution in distilled water and sterilization by filtration through a GA-6 0.22-μm membrane filter (Gelman Sciences, Inc., Ann Arbor, Mich.). The final concentration of ethanol was 2% for 16 μg of rifampin per ml and 0.2% for comparable levels of MDL 473 and ansamycin. The antibiotic and ethanol were serially twofold diluted. Ethanol was not inhibitory to mycobacteria at these concentrations.

The mycobacteria were grown for several days in Middlebrook and Cohn 7H10 broth (10) with Middlebrook OADC enrichment (Difco Laboratories, Detroit, Mich.) and 0.05% Tween 80. Cultures were then diluted in 7H10 broth to yield 1 Klett unit per ml (Klett-Summerson colorimeter; Klett Manufacturing, Brooklyn, N.Y.). The titers of the mycobacteria were determined in duplicate on 7H10 agar plates (BBL Microbiology Systems, Cockeysville, Md.) with OADC enrichment to determine the size of the final inoculum. The smooth, transparent phenotype was the only morphologic variant present on the titer plates.

Quadrant plates for the agar dilution method were prepared with 7H10 agar containing OADC enrichment and using serial twofold dilutions from 16 to 0.062 μg of the various antibiotics per ml. Amounts (10 μl) of the 1-Klett-unit (approximately 5×10^6 CFU/ml) and of the 0.1-Klett-unit suspensions were spotted in duplicate onto the plates containing antibiotics. A control quadrant with no drug was run for each strain. The CFU per spot for the 1-Klett-unit suspensions ranged from 1×10^4 to 1.3×10^5. The plates were incubated at 37°C for 12 days. The MIC was defined as the lowest concentration of antibiotic that produced a 99% (2 log) inhibition of growth.

For the broth dilution method, serial twofold dilutions of the antibiotics were done in 7H10 broth to yield final concentrations of 8 to 0.062, 4 to 0.031, and 4 to 0.015 μg of rifampin, MDL 473, and ansamycin per ml, respectively. These tubes and a growth control tube containing no drug were inoculated with a suspension of bacteria to yield a final concentration of approximately 10^5 CFU/ml (range, 2.5×10^4 to 4.2×10^5 CFU/ml).

Growth was monitored turbidimetrically with a spectrophotometer modernization system (model 252; Gilford Instrument Laboratories, Inc., Oberlin, Ohio) of a DU spectrophotometer (Beckman Instruments, Inc., Fullerton, Calif.). The optical density at 550 nm (OD_{550}) of the inoculated tubes was not measurably different from that of the medium blank. The cultures were incubated on a rotary shaker for 4 days at 37°C. The OD_{550} was measured for the control tubes and for those tubes that contained antibiotics and were not visually turbid. The tubes containing antibiotics were considered to be inhibited if the OD_{550} was ≤ 0.010 (1).

The MICs determined by broth and agar dilution are shown in Table 1. The study was performed in two batches with isolate LPR used as an internal control. Good agreement was found between the two runs for the MICs by both methods for this isolate. Ansamycin was more active than MDL 473 or rifampin by either the agar dilution or the broth dilution method. The MIC of antibiotic for 50 or 90% of the strains was generally 1 dilution lower by broth dilution compared with agar dilution susceptibility testing. Ansa-

TABLE 1. MICs for *M. intracellular* isolates determined by broth and agar dilution susceptibility testing

Isolate	OD_{550} (control tube)	Broth dilution MIC (μg/ml)			Agar dilution MIC (μg/ml)		
		Rifampin	MDL 473	Ansamycin	Rifampin	MDL 473	Ansamycin
SKV	0.186	1	0.25	0.125	2	0.5	0.062
2-9	0.326	1	0.5	0.5	4	2	1
2-12	0.314	0.25	0.25	0.125	2	0.5	0.25
2-15	0.379	1	0.5	1	2	0.5	0.25
2-16	0.376	0.25	0.25	0.25	2	0.5	0.25
LPR	0.402	1	0.5	0.5	2	0.5	0.062
2-23	0.426	0.5	0.125	0.5	2	0.5	0.25
1023	0.396	1	0.5	0.25	4	1	0.25
AMT	0.379	1	1	0.5	2	0.5	0.062
953	0.456	2	1	0.5	8	2	1
998	0.132	0.125	0.062	0.125	1	≤0.125	0.125
420	0.397	1	1	0.5	4	2	0.5
74	0.274	0.25	0.125	0.125	1	≤0.125	0.062
92	0.407	0.25	0.125	0.5	1	0.25	0.125
109	0.347	0.5	0.25	0.5	2	0.25	0.125
119	0.109	0.5	0.5	0.125	4	1	0.5
141	0.216	2	0.5	0.5	16	2	1
1296	0.340	1	0.25	0.5	4	0.5	0.125
SHE	0.185	2	0.25	0.25	16	2	1
LYN	0.515	1	0.5	0.5	2	2	0.5
LPR	0.456	1	0.5	0.25	2	0.5	0.062
		1[a]	0.25[a]	0.25[a]	2[a]	0.5[a]	0.25[a]
		2[b]	1[b]	0.5[b]	4[b]	2[b]	1[b]

[a] MIC for 50% of all isolates tested.
[b] MIC for 90% of all isolates tested.

mycin was usually 1 dilution more active than MDL 473, which was 1 dilution more active than rifampin.

The MICs for *M. intracellulare* were generally lower by broth dilution than by agar dilution. This difference was most apparent with rifampin and least apparent with ansamycin. The MIC for 90% of the strains tested was 1 dilution lower by the broth dilution method than by the agar dilution method. This difference may be due in part to the presence of Tween 80 in the broth and the absence of this compound in the agar (5). Rifampin was found to be more active against *M. intracellulare* than has been reported previously (9, 13–15). The activity of ansamycin was similar to that observed by other investigators (13).

The colonial morphology of the strains used in susceptibility testing appears to play an important role in the observed MIC (9). In vitro there are two predominant morphologic variants: opaque, dome-shaped variants and smooth, transparent variants (7). The former are more susceptible to antibiotics (6, 7, 9). Rastogi and co-workers (7) described the presence of an outer polysaccharide layer in transparent, colony-type variants that may account for the enhanced pathogenicity and drug resistance as compared with the opaque, dome-shaped variants. In this study, the transparent phenotype was the only morphologic variant present.

A need for improved antimicrobial therapy for infections caused by *M. intracellulare* is apparent. Further in vitro testing of these antibiotics on more isolates is necessary to evaluate their potential for in vivo use. Their relative in vivo activities in human clinical disease or in an animal model of *M. intracellulare* infection will depend on the pharmacokinetics of these agents.

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