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Activities of Clarithromycin against Eight Slowly Growing Species of Nontuberculous Mycobacteria, Determined by Using a Broth Microdilution MIC System

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MICs of clarithromycin against 324 clinical isolates belonging to eight species of slowly growing nontuberculous mycobacteria were determined by using a broth microdilution system. Isolates were inoculated into twofold drug dilutions in Middlebrook 7H9 broth (pH corrected to 7.4) and then incubated at 30°C for 7 days for *Mycobacterium marinum* and for 14 days for all other species. The MIC for 90% of the strains (MIC_{90}) was ≤0.5 μg/ml for isolates of *Mycobacterium gordonae* (6 strains), *Mycobacterium scrofulaceum* (5 strains), *Mycobacterium szulgai* (6 strains), and *Mycobacterium kansasii* (35 strains). MICs for *M. marinum* (25 strains) and *Mycobacterium avium* complex (237 strains) were higher, but 100% and 89% of the strains, respectively, were susceptible to ≤4 μg/ml. In contrast, MICs for five of six *M. simiae* strains were >8 μg/ml, and the range of MICs for *Mycobacterium nonchromogenicum* varied from ≤0.125 to 8 μg/ml. For the 237 isolates of *M. avium* complex, the MIC_{50} was 2 μg/ml and the MIC_{90} was 8 μg/ml. MICs for most isolates (77%) were in the 1- to 4-μg/ml range. For the 80 isolates in this group known to be from AIDS patients, the MIC_{50} was 4 μg/ml and the MIC_{90} was 8 μg/ml. These MIC studies combined with preliminary clinical trials suggest that clarithromycin may be useful for drug therapy of most species of the slowly growing nontuberculous mycobacteria except *M. simiae*.

Slowly growing nontuberculous mycobacteria, including *Mycobacterium kansasii*, *M. marinum*, *M. simiae*, *M. scrofulaceum*, *M. szulgai*, *M. gordonae*, *M. nonchromogenicum*, and *M. avium-M. intracellulare* complex, have been incriminated in various types of human diseases, including skin and soft-tissue infections (20, 22, 26, 28–31), pulmonary disease (1, 18, 20, 28, 29, 31, 32), and disseminated disease in nonimmunocompromised patients and in immunocompromised patients, including those with AIDS (8, 9, 17, 18). Susceptibility patterns of the slowly growing nontuberculous mycobacteria can be divided into two groups. Isolates of *M. marinum*, *M. gordonae*, *M. kansasii*, and *M. nonchromogenicum* are usually susceptible to ethambutol, rifampin, sulfonamides, and streptomycin (2, 24, 27, 28). A second, more resistant group includes isolates of *M. avium* complex, *M. scrofulaceum*, *M. simiae*, and *M. szulgai*. These isolates are not usually susceptible to first- or second-line antimycobacterial agents and may require combination antibiotic therapy and/or surgical intervention (28, 29, 31, 32).

Drug therapy in these patients is complex and involves use of drugs with greater incidences of side effects and more toxicity (28). Thus, newer, less toxic antimicrobial agents are needed for those cases of mycobacterial disease resistant to standard antibiotic regimens. In this study, we tested a large number of *M. avium-M. intracellulare* complex isolates and seven other slowly growing nontuberculous mycobacterial species against a new macrolide, clarithromycin (6).

(A portion of this research was presented at the 92nd General Meeting of the American Society for Microbiology, New Orleans, La. [6].)

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MATERIALS AND METHODS

Isolates. Three hundred twenty-four clinical isolates of *M. kansasii*, *M. marinum*, *M. simiae*, *M. scrofulaceum*, *M. szulgai*, *M. gordonae*, *M. nonchromogenicum*, and *M. avium-M. intracellulare* complex submitted to the Mycobacteria/Nocardia Research Laboratory at The University of Texas Health Center at Tyler between 1984 and 1992 were evaluated. Most or all of the isolates in each species were from patients. Organisms from 18 states were tested; together Texas, Florida, and Oklahoma contributed 85% of the isolates, with the greatest number (58%) from Texas. Isolates were identified by standard methods and susceptibility patterns (23, 24, 27). No effort was made to separate *M. avium* from *M. intracellulare*. The majority of the 324 isolates used in testing (51%) were from respiratory samples. An additional 22% were from bone marrow and blood, 19% were from skin and soft tissue, and 8% were from stool, urine, cerebrospinal fluid, pleural fluid, and environmental sources. One percent of the samples had no source given.

Susceptibility testing. Broth microdilution plates were prepared, and clarithromycin was dispensed with the Mini-Quick Spense II reagent dispenser (Dynatech Laboratories, Chantilly, Va.).

Serial twofold dilutions of clarithromycin were added to Middlebrook 7H9 broth (Difco Laboratories, Detroit, Mich.) with 5% oleic acid-albumin-dextrose enrichment to achieve final concentrations of 0.125 to 8 μg/ml. The Middlebrook 7H9 broth was adjusted to pH 7.4 with 1 M potassium hydroxide. The antibiotic concentrations were then dispensed into the wells of microdilution plates at 0.1 ml per well. The plates were inoculated with disposable inoculators to yield a final inoculum size of 10^4 CFU/ml (27).

The plates were covered, sealed in plastic bags, and incubated in room air at 35°C for 14 days, with the exception

TABLE 1. Cumulative percentage of isolates for which MICs of clarithromycin against eight species of slowly growing mycobacteria are ≤0.125 or >8 μg/ml

Organism	No. of isolates tested	MIC (μg/ml)[a]		Cumulative % of isolates for which MIC is (μg/ml):							
		50%	90%	≤0.125	0.25	0.5	1	2	4	8	>8
M. avium-M. intracellulare complex											
AIDS isolates	80	4	8				6	41	81	96	100
Non-AIDS isolates	157	2	4	3	11	18	39	71	92	97	100
Total	237	2	4	3	8	12	28	61	89	97	100
M. gordonae	6	≤0.125	0.25	50	83	100					
M. marinum	25	1	2	12	28	44	80	96	100		
M. kansasii	35	≤0.125	0.25	74	100						
M. scrofulaceum	5	≤0.125	≤0.125	100							
M. simiae	6	>8	>8				17				100
M. szulgai	6	≤0.125	0.5	50	83	100					
M. nonchromogenicum	5	1	8	20			60	80		100	

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

of *M. marinum*, which was incubated for 7 days at 30°C. The MIC was defined as the lowest concentration that completely inhibited macroscopic growth.

Quality control was performed with *M. marinum* ATCC 927. The currently recommended control strain for susceptibility testing of clarithromycin is *Enterococcus faecalis* ATCC 29212, which will not grow in 7H9 broth. The *M. mariunum* strain, for which the MIC range was 0.25 to 1.0 μg/ml by this technique, was the fastest-growing readily available reference strain that we found for which the MIC of clarithromycin was in the approximate middle of the test concentrations. *Staphylococcus aureus* ATCC 29213 would not grow in 7H9 broth, the MIC for *Escherichia coli* ATCC 25922 was too high, and *Mycobacterium fortuitum* ATCC 6841 gave inconsistent results. The MICs of clarithromycin in the 7H9 broth (pH 7.4) were the same as those in Mueller-Hinton broth with 5% oleic acid-albumin-dextrose for *M. marinum* ATCC 927 (data not shown).

RESULTS

A summary of MIC results of clarithromycin against the eight species of slowly growing mycobacteria is given in Table 1. By using the manufacturer's suggested breakpoint of 4 μg/ml for clarithromycin, 89% of the isolates of *M. avium-M. intracellulare* complex were inhibited by ≤4 μg/ml, with 61% of the isolates susceptible to ≤2 μg/ml. Of the 237 *M. avium-M. intracellulare* complex isolates, 80 (34%) were from patients with AIDS. Forty-one percent and 81% of the latter isolates were inhibited at 2 and 4 μg/ml, respectively. For only 6%, MICs of clarithromycin were <2 μg/ml. For 96% and 80%, respectively, of the isolates of *M. marinum* and *M. nonchromogenicum* tested MICs of clarithromycin were ≤2 μg/ml, and for 100% of the isolates of *M. gordonae*, *M. kansasii*, *M. szulgai*, and *M. scrofulaceum* MICs were ≤0.5 μg/ml. In contrast, for 83% of the *M. simiae* tested with clarithromycin MICs were >8 μg/ml.

Multiple isolates of *M. avium* complex from the same patient were tested and compared for 20 patients. For the 17 patients with two isolates tested, MICs for isolates from 15 (88%) were within 1 dilution of each other. For the two patients with three isolates and one patient with five isolates, the repeat MICs were also within 1 dilution. Overall, MICs for 23 of 25 (92%) of repeat isolates were within 1 dilution of the first value.

DISCUSSION

In the continuing search for antibiotics with predictable pharmacokinetic properties, less toxicity, greater achievable levels in blood or tissue, and greater antibacterial activity against species of nontuberculous mycobacteria, a new macrolide, clarithromycin, has emerged (3, 4, 7, 10–14, 25). Studies with clarithromycin have shown that it has better absorption, higher levels in serum, and a longer half-life in serum than does erythromycin (11, 13, 14).

Most previous in vitro studies of clarithromycin against mycobacteria have concentrated on *M. avium* complex by utilizing either agar dilutions in 7H10 or 7H11 medium (4, 12) or both agar dilutions and BACTEC methodology (15, 21). By these methods, MICs for approximately 90% of isolates were ≤4 μg/ml. As with other drugs and *M. avium* complex, MICs for isolates have generally been two- to fourfold lower in broth than in agar (15). As with erythromycin (19), clarithromycin was more active at pH 7.4 than at pH 6.8 or 5.0 (15). The present study confirmed the in vitro susceptibility of *M. avium* complex to clarithromycin by a broth microdilution technique in 7H9 broth at pH 7.4, with almost 90% of 237 strains inhibited by the tentative susceptible bacterial breakpoint of ≤4 μg/ml. This breakpoint may be problematic with mycobacteria, as MICs for approximately 10% of strains presumably never exposed to clarithromycin are 8 μg/ml. An even higher number is noted if a lower pH of medium is used (15). There are no data to show that the higher MICs for these isolates are consistently this high with repeat testing or that they will result in clinical failure with therapy.

Evaluation of the activity of the newer macrolides against nontuberculous mycobacteria other than *M. avium* complex is limited. Two previous studies have evaluated *M. kansasii*, a species which appears to be inhibited by very low concentrations of clarithromycin. Berlin and colleagues (4) utilized an agar dilution method with 7H11 medium (presumed pH 6.8) and reported 100% of 10 isolates of *M. kansasii* to be inhibited at clarithromycin concentrations of 1.0 μg/ml. Biehle and Cavalieri studied 31 strains of *M. kansasii* using both BACTEC methodology and agar dilutions in 7H11 medium (pH values unknown). The MICs of clarithromycin were 0.25 μg/ml for 16 strains and 0.5 μg/ml for the remaining 9 strains (5). In the present study all 35 of our *M. kansasii* isolates were inhibited by ≤0.25 μg/ml at pH 7.4. Some of these isolates were resistant to rifampin (MIC > 1.0 μg/ml).

As expected, there was no cross-resistance with clarithromycin.

Previous studies have shown clarithromycin and, to a lesser degree, other new macrolides to be highly active in vitro against rapidly growing mycobacteria (4), especially against *Mycobacterium chelonae* and *Mycobacterium abscessus* (7). Six additional species of slowly growing nontuberculous mycobacteria not previously evaluated were evaluated in the present study. Clarithromycin was found to be active at achievable levels in serum against most isolates of *M. gordonae*, *M. scrofulaceum*, *M. szulgai*, *M. nonchromogenicum*, and *M. marinum*. In contrast, isolates of *M. simiae* were not inhibited by clarithromycin (MIC > 8). Of the species tested in our study, *M. kansasii*, *M. szulgai*, *M. gordonae*, and *M. scrofulaceum* show promise for response to treatment with MICs of clarithromycin of ≤0.5 μg/ml.

Nontuberculous mycobacterial infections due to relatively drug-susceptible species such as *M. kansasii* and *M. marinum* can usually be managed favorably with proper antibiotic treatment. Although an acceptable 18- to 24-month drug regimen is available for *M. kansasii* (isoniazid, rifampin, and ethambutol) (28), an acceptable short-course regimen and a more effective regimen for use against rifampin-resistant strains is needed. Even prolonged and intensive multiple-drug regimens with currently available drugs may only partially control the more resistant types of disease caused by *M. avium* complex and *M. simiae*. Clearly, better drug treatment regimens are needed for infections caused by these organisms. Results from this study suggest that clarithromycin may provide an important additional agent for management of these diseases. Clinical trials are currently under way to assess the use of clarithromycin in the treatment of infections caused by these nontuberculous mycobacteria.

Metabolic studies have shown that clarithromycin in humans is metabolized in part to a 14-hydroxy metabolite. This metabolite is present in serum at a ratio of 2 to 4:1 (parent drug to metabolite) and is as active as the parent drug against some pathogens (e.g., *Haemophilus influenzae*) (16). In vitro studies thus far have not evaluated whether this major metabolite will or does contribute in any way to the activity of clarithromycin for mycobacterial pathogens. Combination studies should be included in future evaluations of this drug against the nontuberculous mycobacteria.

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