In Vitro Susceptibility of Mycobacterium kansasii to Clarithromycin

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Received 27 April 1992/Accepted 19 June 1992

The MICs of the macrolide clarithromycin for 31 clinical isolates of Mycobacterium kansasii were determined by three different methods. The methods employed were the proportion resistance method on 7H10 agar, the radiometric (BACTEC) method, and the T100 method of datum analysis. All methods gave similar results. The MICs were in a narrow range from 0.16 to 0.50 μg/ml with the MICs for 90% of isolates tested of 0.50 μg/ml for the agar dilution and radiometric methods and 0.37 μg/ml for the T100 method. The MBCs were determined for nine representative isolates by the radiometric broth method. The MBCs were equal to the MICs for four isolates, and the MBCs were twofold higher than the MICs for five isolates. Killing of 99.9% of the bacterial population was achieved at a clarithromycin concentration of 2.0 μg/ml for all nine isolates tested.

Pulmonary infections caused by Mycobacterium kansasii usually occur in patients with underlying pulmonary disease, especially smoking-related chronic obstructive pulmonary disease. Disseminated disease in immunocompetent individuals occurs infrequently, with fewer than 40 cases described in the literature (14). The advent of AIDS in 1983 created yet another category of patients susceptible to opportunistic infections by this organism. The current approach to chemotherapy of infections caused by M. kansasii is a multiple-drug regimen, usually including isoniazid, rifampin, and ethambutol for 18 to 24 months (1, 4, 18). Treatment failures with the standard regimen are rare for M. kansasii infections, as are adverse reactions to individual drugs. However, when the responses of patients to therapy are less than expected, when in vitro susceptibility tests indicate resistance to one or more drugs, or when adverse reactions occur, alternative therapeutic agents must be considered.

Recently, new macrolides have been synthesized which include clarithromycin, roxithromycin, and azithromycin. The in vitro evaluations of these new macrolides as potential therapeutic agents of mycobacterial infections have shown that each has different levels of activity against isolates of Mycobacterium tuberculosis, Mycobacterium avium complex, and other nontuberculous mycobacteria causing infections in humans (3, 6-8, 16, 19). The purpose of this study was to determine the in vitro activity of clarithromycin against M. kansasii in order to assess its potential usefulness as an alternative or additional therapeutic agent in treatment regimens.

Clarithromycin was generously provided by Abbott Laboratories (Abbott Park, Ill.). Middlebrook 7H10 medium (pH 6.6) (BBL Microbiology Systems, Cockeysville, Md.) supplemented with oleic acid, albumin, glucose, and catalase (BBL Microbiology Systems) was used for agar dilution susceptibility testing and for subculture of BACTEC 12B vials during performance of bactericidal tests. The radiometric medium was BACTEC 7H12B TB medium (pH 6.8) (Johnston Laboratories, Towson, Md.). Isolated colonies were chosen and suspended in 0.5 ml of sterile distilled water and adjusted to approximate the optical density of a McFarland standard 1. This standard suspension served as the starting point for all in vitro susceptibility testing methods. The 31 isolates of M. kansasii used in this study were isolated from patients with clinically significant infections in local hospitals and obtained from the respective microbiology laboratories. Organisms were identified by use of the GenProbe ACCUPROBE DNA probes (Gen-Probe Inc., San Diego, Calif.) specific for M. kansasii, with the results confirmed by conventional biochemical techniques.

The proportion resistance method was used to test the susceptibilities of all isolates to clarithromycin (13). Two-fold-higher concentrations of clarithromycin (0.25, 0.5, 1.0, and 2.0 μg/ml) were incorporated into M7H10 agar and dispensed into quadrant petri dishes. The concentrations tested were derived from results obtained previously by Berlin et al. (2). According to the principles of the proportion method, the organisms were considered to be susceptible to the drug at the concentration present in the medium when the number of colonies present on a drug-containing quadrant was less than 1% of the number of colonies present on the drug-free quadrant. The lowest concentration of drug that inhibited more than 99% of the bacterial population was considered to be the MIC. Clarithromycin MICs were determined for all isolates by the BACTEC radiometric method. Two-fold-higher concentrations of clarithromycin (0.125 to 2.0 μg/ml) were added to the vials and tested. The MIC was defined as the lowest concentration of drug that produced a daily growth index (GI) increase and a final GI reading lower than that of the 1:100 control vial (11). MICs were also determined by the T100 method of analysis (12). In this method, a defined value, termed T100, is a measure of the effect of an antimicrobial agent on the growth of M. kansasii in BACTEC culture vials. The T100 value is the time (in hours) necessary for the culture to produce a cumulative GI of 100, i.e., the sum of individual GI readings measured at 24-h intervals. Since this is a kinetic relationship, measurement of the production of 14CO2 at carefully timed intervals is necessary. Culture vials were inoculated in the same manner as for the radiometric method, and daily GI readings were recorded. The MIC was determined by extrapolation from the points of intersection between the growth response curve and the T100 values for the inocula diluted 1:100 (1% control).

Nine representative isolates were chosen for determination of the MBCs of clarithromycin (11). Drug concentra-
were twofold with the they by evidenced concentration of 2.0 T100 0.125-2.0 concentration of 0.25 killing inoculum concentrations of 0.50 and dilution scheme. The concentration of 0.25 from (45%). The isolates (56%). Results of the MIC determinations are shown in Table 1. Agar dilution MICs by the proportion method were 0.50 µg/ml for all isolates. The MICs determined radiometrically were 0.25 µg/ml for 17 isolates (55%) and 0.50 µg/ml for 14 isolates (45%). The MICs determined by the T100 method ranged from 0.16 to 0.43 µg/ml. Exact agreement for MICs determined by the radiometric and T100 methods at the concentration of 0.25 µg/ml was observed with eight isolates. The remainder of the T100-determined MICs agreed with the radiometrically determined MICs in the twofold dilution scheme.

Results of the MIC determinations and calculation of the MBC/MIC ratios are shown in Table 2. The MBCs were equal to the MICs for four isolates (44%), and the MBCs were twofold higher than the MICs for the remaining five isolates (56%). An illustration of the clarithromycin killing curve for isolate 20 is shown in Fig. 1. The clarithromycin concentration of 0.25 µg/ml was unable to achieve 99% killing of the initial inoculum after 7 days of incubation, but concentrations of 0.50 and 1.0 µg/ml were able to kill 99% of the inoculum after 7 days of incubation. The clarithromycin concentration of 2.0 µg/ml, the highest concentration tested for this isolate, killed 99.9% of the inoculum in 7 days, as evidenced by the absence of growth on serially diluted aliquots removed from the BACTEC vial.

Current methods used to evaluate the in vitro potential of new antimicrobial agents against atypical mycobacteria focus on determining the MIC and MBC and comparing them to the achievable drug concentrations in serum and tissue, if they are known. By testing many wild-type strains of mycobacteria against an antimicrobial agent, one can determine the apparent critical concentration of drug to be used for determining the in vitro susceptibility or resistance. It should be noted that the critical concentration may not have a direct relationship to the peak serum drug level obtainable in patients. The critical proportion of mutants which is compatible with therapeutic success must be determined by use of both bacteriologic and clinical data (15). Applying these criteria to the results of our study, isolates requiring MICs of 0.25 µg/ml or less would be classified as susceptible, since the MIC is substantially less than the maximum concentration of drug in serum of 2.7 µg/ml and is equal to or lower than the apparent critical concentration of drug for this organism of 0.25 µg/ml. Isolates requiring MICs greater than 0.25 to 1.0 µg/ml would be classified as moderately susceptible. On the basis of the results of our study, we suggest testing twofold dilutions of clarithromycin in a range from 0.125 to 2.0 µg/ml.

### TABLE 1. In vitro susceptibilities of 31 isolates of *M. kansasii* to clarithromycin determined by three different methods

<table>
<thead>
<tr>
<th>Method of MIC determination</th>
<th>MIC (µg/ml)*</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agar (proportion)</td>
<td>0.25–2.0</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>BACTEC (radiometric)</td>
<td>0.125–2.0</td>
<td>0.25</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>T100</td>
<td>0.125–2.0</td>
<td>0.25</td>
<td>0.37</td>
<td></td>
</tr>
</tbody>
</table>

*Defined MIC as less than 1% growth of the inoculum. 50 and 90%, MICs for 50 and 90% of isolates tested, respectively.

### TABLE 2. Bactericidal activities of clarithromycin against nine isolates of *M. kansasii*

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No. of organisms (CFU/ml)</th>
<th>MBC (µg/ml)</th>
<th>MIC (µg/ml)</th>
<th>MBC/MIC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.8 × 10⁴</td>
<td>5.0 × 10⁴</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2</td>
<td>8.0 × 10⁴</td>
<td>&lt;1.0 × 10⁴</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>2.1 × 10⁴</td>
<td>2.0 × 10⁴</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>4</td>
<td>9.0 × 10⁴</td>
<td>6.2 × 10⁴</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>2.3 × 10⁴</td>
<td>9.0 × 10⁴</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>11</td>
<td>2.6 × 10⁴</td>
<td>&lt;1.0 × 10⁴</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>20</td>
<td>1.4 × 10⁴</td>
<td>9.4 × 10³</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>24</td>
<td>1.6 × 10³</td>
<td>1.2 × 10²</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>27</td>
<td>1.0 × 10⁴</td>
<td>&lt;1.0 × 10⁴</td>
<td>0.5</td>
<td>0.25</td>
</tr>
</tbody>
</table>

### FIG. 1. Killing of isolate 20 of *M. kansasii* by various concentrations of clarithromycin. Clarithromycin (CLA) was added to the vials in the indicated concentrations (in micrograms per milliliter) on day 1 (arrow) after sampling and plating of 0.1-ml aliquots from the vials to establish the initial inoculum size. Dotted lines indicate the 1.0 and 0.1% survival levels.
The results of our study can be compared to only one other published report at this time. In 1987, Berlin and colleagues (2) reported the results of in vitro susceptibility testing of 10 strains of Mycobacterium kansasii to clarithromycin, using the modified disk susceptibility method in M7H11 agar. Their results indicated that the 10 strains were uniformly susceptible to 1.0 µg/ml, a result similar to those of our agar dilution procedure (MIC for 100% of isolates tested, 0.50 µg/ml). It has been stated previously that results of in vitro and in vivo tests with clarithromycin against Haemophilus influenzae suggest that standard methodologies and interpretation may underestimate the potential efficacy in humans (9). Several investigators have reported that the combination of parent drug and metabolite in vitro at clinically relevant concentrations is bactericidal and synergistic or additive against H. influenzae (5, 10, 17). Results from an experimental model of H. influenzae pulmonary infection indicated that low doses of 14-hydroxyclarithromycin potentiated the activity of clarithromycin in eradicating H. influenzae from the lungs of mice that did not produce the 14-hydroxy metabolite (21). Additional in vitro studies with the parent compound and 14-hydroxy metabolite against M. kansasii, as well as other species of mycobacteria, will be required in an attempt to duplicate and confirm the increased activity of clarithromycin in combination with 14-hydroxyclarithromycin. Recent attempts to test the effect of macrolides against mycobacteria at the physiologic pH of 7.4 by modifying Middlebrook 7H10 medium or by cultivating the organisms on Mueller-Hinton medium supplemented with oleic acid, albumin, glucose, and catalase and with a pH of 7.3 have yielded MICs that were approximately fourfold less than those obtained at a pH of 6.6 (20). Since the organisms reside within cells in which the pH may be significantly lower than 7.4, the clinical relevance of the observation that clarithromycin is more active at a physiologic pH is not entirely clear.

In summary, we found that clinical isolates of M. kansasii were uniformly susceptible to low levels of clarithromycin in vitro and that the drug has good bactericidal activity for this organism. All technical methods used for determining the MICs had excellent correlation, with MIC results from all methods being within one twofold dilution. These in vitro susceptibility results, in addition to the improved bioavailability and tolerance characteristics, appear to make clarithromycin an attractive alternative or additional therapeutic agent for treatment of M. kansasii infections, and clinical investigation employing clarithromycin in combination with standard antituberculous drugs may be warranted.

We thank Don Giger, Kathy Maxwell, Kathy Ubben, and Valerie Davis for kind assistance in supplying isolates included in this study. We also thank W. Eugene Sanders, Jr., for helpful comments.

No external financial support was obtained for this study.

REFERENCES