Susceptibility of Mycobacterium kansasii to Ofloxacin, Sparfloxacin, Clarithromycin, Azithromycin, and Fusidic Acid

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The MICs of ofloxacin, sparfloxacin, clarithromycin, azithromycin, and fusidic acid for clinical isolates of Mycobacterium kansasii were determined by the radiometric (BACTEC) method. All drugs except azithromycin elicited MICs for 90% of the strains tested that were lower than previously reported achievable maximum concentrations in serum. Ofloxacin, sparfloxacin, and clarithromycin had the largest maximum concentration in serum/MIC for 90% of strains ratio of the drugs tested.

Mycobacterium kansasii is a photoschromogenic mycobacterium whose presence in humans is usually associated with disease (23). This manifests most often as a pneumonitis that mimics pulmonary tuberculosis, but disseminated infections have been reported, usually in immunocompromised individuals (23) and AIDS patients (27). Accepted treatment regimens for M. kansasii have included rifampin, isoniazid, ethambutol, and/or streptomycin; generally patients have done well, and resistance has been minimal (1, 15). Patients do better on a rifampin-containing regimen (15) and fare poorly without treatment (9). However, patients with drug reactions, resistant isolates, or suboptimal response require alternative treatment regimens.

Laboratory-guided therapy utilizing in vitro susceptibility testing of M. kansasii correlates better with clinical response than does that of any of the other nontuberculous mycobacteria (20). Testing in broth medium rather than agar may produce more-accurate results for drug susceptibility testing of the nontuberculous mycobacteria (20). A recent study comparing three different methods (proportion resistance method, radiometric [BACTEC] method, and T100 datum analysis method) of determining MICs of clarithromycin for M. kansasii revealed equivalent results for each method (4).

Quinolones, macrolides, and fusidic acid show promise against various mycobacteria, including Mycobacterium tuberculosis and Mycobacterium leprae, in preclinical studies (6, 10-12, 17, 30). The purpose of this study was to determine the effect of the 4-quinolones ofloxacin and sparfloxacin, the 14-membered macrolide clarithromycin, the 15-membered macrolide azithromycin, and the tetracyclic tripterpenoid fusidic acid on M. kansasii by the BACTEC 460 system.

The 19 isolates of M. kansasii used for this study were obtained from patients treated at hospitals in the greater New Orleans, La., area, and subcultures on Lowenstein-Jensen media were provided by the mycobacteriology laboratory at Charity Hospital in New Orleans. Isolates were identified by traditional mycobacterial biochemical techniques (2), and identifications were confirmed by an M. kansasii-specific DNA probe (ACCUPROBE; Genprobe Inc., San Diego, Calif.). Established antimycobacterial drug susceptibilities of M. kansasii were no different from those previously reported, i.e., with some isoniazid resistance in all isolates and variable ethambutol and rifampin susceptibilities (1, 23).

Antibiotic reference powders were generously donated by the following: clarithromycin by Abbott Laboratories (Abbott Park, Ill.), azithromycin by Pfizer Central Research Division (Groton, Conn.), ofloxacin by Ortho Pharmaceutical (Raritan, N.J.), and sparfloxacin by Parke-Davis Pharmaceutical (Ann Arbor, Mich.). Fusidic acid was obtained from Sigma Chemical Co. (St. Louis, Mo.). Drugs were solubilized according to the manufacturers’ recommendations, and dilutions were prepared in Middlebrook 7H9 broth (BBL Microbiology Systems, Cockeysville, Md.). Final twofold concentrations in BACTEC 12B (Johnston Laboratories, Towson, Md.) of ofloxacin were 0.25 to 8.0 μg/ml, of sparfloxacin and clarithromycin were 0.0312 to 1.0 μg/ml, of azithromycin were 0.5 to 16 μg/ml, and of fusidic acid were 2 to 64 μg/ml.

Isolates were subcultured in BACTEC 12B (pH 6.8), and the growth index (GI) was measured daily until it reached 999. At that point, samples were diluted 1:4 in 7H9 with 0.5% bovine albumin, and 1-ml aliquots were stored in freezer vials at −80°C. This inoculum was found to be suitable for determining MICs within an 8- to 10-day incubation.

The radiometric (BACTEC) method (16) was used to determine susceptibilities of isolates to the various test drugs. Frozen M. kansasii culture (0.1 ml) was inoculated into BACTEC 12B broth vials containing serial drug concentrations. A drug-free control vial and a 1:100-diluted control vial were included. Cultures were incubated at 37°C, and the GI was read daily. The MIC was defined as the lowest concentration of the drug that generated a daily GI increase and final GI lower than the 1:100-diluted control vial readings on the day when the 1:100 GI was ≥30.

Accepted maximum concentrations in serum (C_max) for clinical dosage used for the drugs were as follows: after one 400-mg oral dose of ofloxacin, 8.6 μg/ml (24); after one 200-mg dose of sparfloxacin, 0.74 μg/ml (21); after one 500-mg oral dose of clarithromycin, 2.4 μg/ml (5, 24); after one 500-mg oral dose of azithromycin, 0.4 μg/ml (8); and after one 500-mg oral dose of fusidic acid, 33 μg/ml (28).

The MICs for 90% of the strains tested (MIC_90) of both ofloxacin and sparfloxacin were significantly below the C_max of the respective drugs (Table 1). Ofloxacin results were similar to previously published MICs by a variety of
methods (6, 7, 14, 22, 29, 30). The clarithromycin MIC90 matched the results of the previous reports (3, 4) and was much lower than that of azithromycin, which had not been previously tested. The fusidic acid MIC90 appeared high at 32 μg/ml, but results for all isolates were within the Cmax of the drug after one 500-mg oral dose. Also, fusidic acid is known to accumulate during a course of treatment, and levels in serum greater than 100 μg/ml can be attained (13). The potential therapeutic value of each drug was calculated by determining the Cmax/MIC90 ratio. Cmax/MIC90 ratios of the study drugs in descending order were as follows: ofloxacin, 17.2; sparfloxacin, 11.8; clarithromycin, 4.8; fusidic acid, 1.03; and azithromycin, 0.05.

Macrolides have shown much greater potency against a variety of bacteria at more-Alkaline pHs (25, 26). A recent report tested one strain of M. kansasi with clarithromycin at pH 6.0, 6.8, and 7.4. The MICs were identical (0.2 μg/ml) for testing at both pH 6.8 and pH 7.4, while the MIC at pH 6.0 was four times higher, 0.8 μg/ml (25). Our testing was done at pH 6.8, so improved results at pHs other than the physiologic pH have not yet been proven. Mycobacteria would normally be attacked in the more-acidic environment of the macrophage, so any in vitro pH advantage translating to an in vivo advantage is speculative.

Both quinolones and macrolides are known to produce higher intracellular concentrations than concentrations in serum (18). The significance of this fact with respect to M. kansasi is unknown, at least until drug activity has been evaluated in cultured M. kansasi-infected macrophages and/or evaluated in vivo. Further in vitro susceptibility testing, including combination drug testing, macrophage studies, and estimating the emergence of resistant organisms (determining the MBCs), has been recommended before the initiation of any clinical trials involving M. kansasi (19).

In summary, M. kansasi was tested with two quinolones, two macrolides, and fusidic acid. This is the first reported in vitro testing of one of the quinolones (sparfloxacin), one of the macrolides (azithromycin), and fusidic acid. Ofloxacin had the greatest Cmax/MIC90 Ratio, followed by sparfloxacin and clarithromycin. Despite a low Cmax/MIC90 ratio, the peculiar accumulative effects of fusidic acid would result in a higher ratio in a full treatment course. The most promising candidates for further testing against M. kansasi appear to be sparfloxacin, ofloxacin, and clarithromycin.

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table 1. Antimycobacterial activity against 19 clinical isolates of M. kansasi

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (μg/ml)*</th>
<th>50%</th>
<th>90%</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25-8.0</td>
<td></td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>0.0625</td>
<td>0.0625</td>
<td>0.03-1.0</td>
<td></td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>0.25</td>
<td>0.5</td>
<td>0.03-1.0</td>
<td></td>
</tr>
<tr>
<td>Azithromycin</td>
<td>8.0</td>
<td>8.0</td>
<td>0.5-16.0</td>
<td></td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>32.0</td>
<td>32.0</td>
<td>2.0-64.0</td>
<td></td>
</tr>
</tbody>
</table>

*50% and 90%, MIC90 and MIC50, respectively.

REFERENCES

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