Tigecycline Potentiates Clarithromycin Activity against *Mycobacterium avium* In Vitro

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The *in vitro* activities of clarithromycin and tigecycline alone and in combination against *Mycobacterium avium* were assessed. The activity of clarithromycin was time dependent, highly variable, and often resulted in clarithromycin resistance. Tigecycline showed concentration-dependent activity, and mycobacterial killing could only be achieved at high concentrations. Tigecycline enhanced clarithromycin activity against *M. avium* and prevented clarithromycin resistance. Whether there is clinical usefulness of tigecycline in the treatment of *M. avium* infections needs further study.

Although the introduction of macrolides improved the success of treatment of *Mycobacterium avium* infections, the overall prognosis is still poor (1). Therefore, new powerful treatment strategies are needed. Tigecycline has been shown to have good *in vitro* activity against rapidly growing nontuberculous mycobacteria (NTM) (2–4), and success in clinical use has been reported when the drug is combined with macrolides (5, 6). In contrast, little is known about tigecycline activity against slowly growing NTM, including *M. avium*, and *in vitro* activity has not been demonstrated so far (7). In the present study, the bactericidal activities of clarithromycin and tigecycline alone and in combination against *M. avium* were assessed.

Suspensions of the *M. avium* complex (MAC) 101 strain (kindly provided by L. S. Young, Kuzell Institute for Arthritis and Infectious Diseases, San Francisco, CA) were cultured in Middlebrook 7H9 broth (Difco Laboratories), supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC; Baltimore Biological Laboratories, Baltimore, MD), 0.5% glycerol (Scharlau Chemie SA, Sentmenat, Spain), and 0.02% Tween 20 (Sigma Chemical Co., St. Louis, MO) under shaking conditions at 96 rpm at 37°C. Cultures on solid medium were grown on Middlebrook 7H10 agar (Difco), supplemented with 10% OADC and 0.5% glycerol, for 14 days at 37°C with 5% CO2. The susceptibility of the *M. avium* strain in terms of MICs determined according to the guidelines of the Clinical and Laboratory Standards Institutes (CLSI) (8) was 2 mg/liter for clarithromycin and 16 mg/liter for tigecycline. The concentration- and time-dependent killing capacities of clarithromycin and tigecycline were determined as previously described (9, 10). Briefly, *M. avium* cultures were exposed to antimicrobial drugs at 4-fold increasing concentrations for 21 days at 37°C under shaking conditions. At days 1, 3, 7, 10, and 21 during exposure, samples were collected, centrifuged at 14,000 × g, and subcultured onto antibiotic-free solid medium. Subculture plates were incubated for 14 days at 37°C with 5% CO2 to determine the number of CFU. The lower limit of quantification was 5 CFU/ml (log_{10} 2). To assess selection of clarithromycin-resistant *M. avium*, subcultures were also performed on solid medium containing 32 mg/liter clarithromycin. The stabilities of clarithromycin and tigecycline in mycobacterium-free Middlebrook 7H9 broth at 37°C were determined using two test concentrations of clarithromycin (CLR) and tigecycline (TGC) toward *M. avium*. The drug concentrations are milligrams per liter.

A slightly different format is used for the references, with the authors listed first, followed by the year of publication, journal name, and DOI.

**FIG 1** (A) Concentration- and time-dependent bactericidal activity of clarithromycin (CLR) toward *M. avium*. (B) Concentration- and time-dependent bactericidal activity of tigecycline (TGC) toward *M. avium*. The drug concentrations are milligrams per liter.
TABLE 1 Synergistic activity and prevention of selection of clarithromycin resistance in M. avium after 21 days of drug exposurea

<table>
<thead>
<tr>
<th>Clarithromycin (mg/liter)</th>
<th>0 mg/liter</th>
<th>0.125 mg/liter</th>
<th>0.5 mg/liter</th>
<th>2 mg/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ClrC</td>
<td>Synergy</td>
<td>ClrC</td>
<td>Synergy</td>
</tr>
<tr>
<td>0</td>
<td>1.45e−7*</td>
<td>−</td>
<td>1.12e−7*</td>
<td>−</td>
</tr>
<tr>
<td>0.125</td>
<td>0.31%</td>
<td>−</td>
<td>1.1e−7*</td>
<td>+</td>
</tr>
<tr>
<td>0.5</td>
<td>0.06%</td>
<td>−</td>
<td>1.0e−7*</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>80%</td>
<td>+</td>
<td>ND</td>
<td>+ (E)</td>
</tr>
<tr>
<td>8</td>
<td>54%</td>
<td>+ (E)</td>
<td>0%</td>
<td>+</td>
</tr>
</tbody>
</table>

a ClrC, clarithromycin resistance; *, spontaneous mutation frequency; +, synergy; −, no synergy; E, elimination (limit of quantification = <5 CFU/ml); ND, not determined.
antibacterial drug behavior that cannot be detected when static susceptibility assays such as MICs are used. This is in line with our previous studies (10, 16) as well as with another recent study assessing the activities of several antibacterial drugs against Mycobacterium abscessus (17). The results of our in vitro study underline the need for potentiation of clarithromycin activity against M. avium. Whether tigecycline might be clinically useful when added to clarithromycin-based regimens needs further study.

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REFERENCES