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 (log0.7). 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 and tigecycline (8 and 32 mg/liter). Antimicrobial activity over time was assessed using the standard large-plate agar diffusion assay as previously described (11). In contrast to clarithromycin (showing no decline during 21 days of...
To our knowledge, this is the first study showing that the addition of tigecycline to clarithromycin increased bactericidal activity against *M. avium* and prevented the selection of clarithromycin resistance. Recently, Huang et al. showed that tigecycline could potentiate clarithromycin activity against rapidly growing mycobacteria (RGM) *in vitro* (2), supporting the use of this combination in the treatment of infections caused by RGM. In humans, the steady-state maximum serum concentration at clinical dosages of tigecycline is around 0.6 mg/liter (14). Although this concentration is far below the tigecycline concentrations needed to achieve mycobacterial killing in our *in vitro* study, it approximates the concentrations effecting synergy in combination with clarithromycin in our study. Moreover, tigecycline has been shown to accumulate in tissues and human macrophages (14, 15). Further studies, including macrophage-infection and pharmacodynamic/pharmacokinetic models, and *in vivo* models are needed to establish to what extent tigecycline can contribute to killing and elimination of *M. avium* and whether tigecycline can indeed effect synergy in combination with clarithromycin as we have shown in the present study. Although clarithromycin is considered the cornerstone agent in the treatment of *M. avium* infections, consistent mycobacterial killing was only seen at the highest concentration tested (32 mg/liter). Importantly, at clinically relevant concentrations, clarithromycin activity against *M. avium* was highly variable between experiments, alternating mycobacterial killing and mycobacterial regrowth. These results might explain the fact that despite macrolide-containing regimens, the overall success of treatment of *M. avium* infections is still unpredictable and disappointing (1). The results of our study also illustrate that the dynamic time-kill kinetics assay can detect important differences in exposure), tigecycline concentrations fell to 20% of the original concentrations within the first 24 h, and thus 80% of the original tigecycline concentrations was added daily. The two endpoints of this study were drug synergy and the prevention of the emergence of clarithromycin resistance. Synergistic activity was defined as a ≥100-fold (2-log<sub>10</sub>) increase in mycobacterial killing with the combination compared to the most active single drug or when the drug combination achieved elimination of *M. avium* after 21 days of drug exposure, which was not achieved during single-drug exposure (12, 13).

Clarithromycin showed time-dependent bactericidal activity toward *M. avium* (Fig. 1A). At the intermediate concentrations (2 and 8 mg/liter), high interexperimental variability was observed showing mycobacterial killing in 2 out of 4 experiments and mycobacterial regrowth in the other 2 experiments, which was associated with the selection of clarithromycin resistance. Only at the highest concentration tested (32 mg/liter) was ≥99% killing consistently observed. Tigecycline showed concentration-dependent bactericidal activity toward *M. avium* (Fig. 1B). At tigecycline concentrations of ≥8 mg/liter, ≥99% killing was observed, and mycobacterial elimination was achieved only at the highest concentration tested (32 mg/liter).

A concentration-dependent enhancement of clarithromycin activity by tigecycline was observed (Fig. 2 and Table 1). Whereas a low concentration of tigecycline (0.125 mg/liter) effected synergy in combination with clarithromycin at ≥2 mg/liter, tigecycline at 0.5 mg/liter effected synergy with clarithromycin at ≥0.5 mg/liter, and tigecycline at 2 mg/liter effected synergy with clarithromycin at ≥0.125 mg/liter, except with clarithromycin at 2 mg/liter. All tested tigecycline concentrations prevented the emergence of clarithromycin resistance when combined with clarithromycin at 0.125 to 8 mg/liter.

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<th>Clarithromycin (mg/liter)</th>
<th>Tigecycline</th>
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<th>0.125 mg/liter</th>
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<sup>a</sup> Clr<sup>a</sup>, clarithromycin resistance; <sup>*</sup>, spontaneous mutation frequency; +, synergy; −, no synergy; E, elimination (limit of quantification = <5 CFU/ml); ND, not determined.

FIG 2 (A) Concentration- and time-dependent bactericidal activity of clarithromycin (CLR) at 2 mg/liter combined with tigecycline (TGC) at various concentrations (milligrams per liter) toward *M. avium*. (B) Concentration- and time-dependent bactericidal activity of clarithromycin at 8 mg/liter combined with tigecycline at various concentrations (milligrams per liter) toward *M. avium*.
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