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Comparison of the In Vitro Activity of the Glycylcycline Tigecycline (Formerly GAR-936) with Those of Tetracycline, Minocycline, and Doxycycline against Isolates of Nontuberculous Mycobacteria

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We compared the in vitro activity of the glycylcycline tigecycline (formerly GAR-936) with those of tetracycline, doxycycline, and minocycline by broth microdilution against 76 isolates belonging to seven species of rapidly growing mycobacteria (RGM) and 45 isolates belonging to five species of slowly growing nontuberculous mycobacteria (NTM). By using a resistance breakpoint of >4 μg/ml for tigecycline and >8 μg/ml for tetracycline, all RGM were highly susceptible to tigecycline, with inhibition of 50% of isolates at ≤0.12 μg/ml and inhibition of 90% of isolates at 0.25 μg/ml for *Mycobacterium abscessus* and inhibition of both 50 and 90% of isolates at ≤0.12 μg/ml for *M. chelonae* and the *M. fortuitum* group. The MICs of tigecycline were the same for tetracycline-resistant and -susceptible strains, and RGM isolates were 4- to 11-fold more susceptible to tigecycline than to the tetracyclines. In contrast, no slowly growing NTM were susceptible to tigecycline, and isolates of *M. marinum* and *M. kansasii* were less susceptible to this agent than to minocycline. This new antimicrobial offers exciting therapeutic potential for the RGM, especially for isolates of the *M. chelonae-M. abscessus* group, against which the activities of the currently available drugs are limited.

Treatment of infections due to rapidly growing mycobacteria (RGM) remains difficult, in part because of resistance to the first-line antituberculous agents and also in part because of resistance to almost all antibacterial agents (3, 21). The only drugs with activity against all three major pathogenic species (*Mycobacterium chelonae*, *M. abscessus*, and *M. fortuitum*) are amikacin (13) and clarithromycin (4). In vitro studies with prior glycylcyclines (*N*,*N*-dimethylglycylamido-minocycline [DMG-MINO] and *N*,*N*-dimethylglycylamido-6-demethyl-6-deoxytetracycline [DMG-DMDOT]) have shown this class of agents to be extremely active against this group of organisms, including tetracycline-resistant strains (1, 3). The latest glycylcycline, tigecycline (GAR-936), has shown excellent activity against many tetracycline-resistant bacterial species (1, 8, 17; R. N. Jones, A. C. Gales, L. M. Deshpande, D. M. Johnson, and D. J. Biedenbach, poster 407, 39th Intersci. Conf. Antimicrob. Agents Chemother., 1999). With this in mind, we undertook a comparative study of the in vitro susceptibilities of multiple species of nontuberculous mycobacteria (NTM) to tetracycline, minocycline, doxycycline, and tigecycline.

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MATERIALS AND METHODS

Organisms. Clinical mycobacterial isolates submitted to the Mycobacterial/Nocardia Laboratory at The University of Texas Health Center for susceptibility testing and selected reference strains were chosen for testing. Clinical isolates were tested upon receipt in the laboratory, while the reference strains were taken from frozen stocks stored at −70°C in Trypticase soy broth with 15% glycerol. This included 76 clinical isolates of RGM of the *M. fortuitum* group ($n = 26$), *M. abscessus* ($n = 20$), *M. chelonae* ($n = 26$), *M. immunogenum* ($n = 1$), and the *M. smegmatis* group ($n = 1$ isolate each of *M. smegmatis* sensu stricto, *M. wolinskyi*, and *M. goodii*). American Type Culture Collection (ATCC) reference or type strains of RGM included *M. fortuitum* ATCC 6841^T, *M. peregrinum* ATCC 14467^T, the current NCCLS-recommended susceptibility control strain, *M. peregrinum* ATCC 700686 (10), *M. fortuitum* third biovariant sorbitol-positive strain ATCC 49403 (proposed species *M. neworleansense*), and *M. fortuitum* third biovariant sorbitol-negative strain ATCC 49404 (proposed as *M. bonickei*) (M. F. Schinsky, M. P. Douglas, A. G. Steigerwalt, R. W. Wilson, M. M. Floyd, M. I. Daneshvar, B. A. Brown-Elliott, R. J. Wallace, Jr., M. M. McNeil, D. J. Brenner, and J. M. Brown, unpublished data).

We also tested 45 isolates of slowly growing NTM that included 11 *M. avium* complex, 10 *M. lentiflavum*, 11 *M. kansasii*, 11 *M. marinum*, 1 *M. xenopi*, and 1 *M. simiae* complex isolates for their susceptibilities to these agents.

Isolates of the *M. fortuitum* group, *M. chelonae*, and *M. abscessus* were identified to the species level by using drug susceptibility patterns (3, 11) or by PCR-restriction enzyme analysis (PRA) of a 439-bp sequence (Telenti fragment) of the 65-kDa *hsp* gene (12, 15). The remaining RGM species were identified only by PRA (2, 23). Isolates of slowly growing NTM were identified by use of a commercial DNA-RNA probe (Accu-Probe) (Gen-Probe, Inc) or PRA of the 65-kDa *hsp* gene (15).

Susceptibility testing. The isolates were tested for their susceptibilities to tetracycline, minocycline, doxycycline, and tigecycline (formerly GAR-936; Wyeth-Ayerst, Pearl River, N.Y.). MICs were determined by broth microdilution and a recently published tentative NCCLS standard method for mycobacterial susceptibility testing of RGM and slowly growing NTM (10). The resistance breakpoints were ≥16 μg/ml for tetracycline, doxycycline, and minocycline (9). For slowly growing NTM, the NCCLS document does not deal with breakpoints for tetracycline, and breakpoints for doxycycline and minocycline are given only for *M. marinum*. The breakpoints for the three tetracyclines used here are those for bacterial species that grow aerobically (9). The resistance breakpoint tentatively recommended by the manufacturer for tigecycline is ≥8 μg/ml.

Quality control. Quality control for the tetracyclines and tigecycline were performed by using *Staphylococcus aureus* ATCC 29213 and suggested quality control ranges recommended by the NCCLS for the tetracyclines or the manufacturer for tigecycline (9). Acceptable MIC ranges for *S. aureus* ATCC 29213 are 0.03 to 0.12 μg/ml for tigecycline, 0.12 to 0.5 μg/ml for doxycycline, 0.06 to 0.5 μg/ml for minocycline, and 0.25 to 1 μg/ml for tetracycline. All quality control results were within the acceptable ranges.

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TABLE 1. MIC ranges, concentrations that inhibits 50 and 90% of strains, and percentage of strains susceptible for tigecycline (GAR-936) and three tetracyclines for 72 isolates of RGM[a]

Species	MIC (μg/ml)			% Susceptible
	Range	50%	90%	
M. abscessus (20)[b]				
Tigecycline	≤0.06–1	≤0.12	0.25	100
Tetracycline	4–>128	64	>128	10
Minocycline	0.25–>64	>64	>64	5
Doxycycline	2–>128	>128	>128	5
M. chelonae (26)				
Tigecycline	≤0.06–≤0.25	≤0.06	<0.12	100
Tetracycline	1–>128	32	>128	15
Minocycline	≤0.12–>64	32	>64	38
Doxycycline	≤0.25–>128	>64	>128	15
M. fortuitum group (26)				
Tigecycline	≤0.06–≤0.25	≤0.06	<0.12	100
Tetracycline	≤0.25–64	4	32	60
Minocycline	≤0.12–>64	2	32	72
Doxycycline	≤0.25–>128	2	128	56

[a] The resistance breakpoints used were ≥8 μg/ml for tigecycline and ≥16 μg/ml for the three older tetracyclines.
[b] The values in parentheses are the number of isolates tested.

RESULTS

Results for the clinical isolates of the common pathogenic species of the RGM are shown in Tables 1 and 2. In general, the MICs of tigecycline for all isolates were very low, with comparable values for tetracycline-susceptible and tetracycline-resistant strains.

For tetracycline-susceptible strains, tigecycline was approximately 4 dilutions more active than the older tetracyclines. For 15 of 16 tetracycline-susceptible *M. fortuitum* strains, the tetracycline MICs were 0.5 to 8.0 μg/ml, with the mode tetracycline MIC being 0.5 μg/ml. In contrast, for 15 of the 16 isolates the tigecycline MICs were ≤0.12 μg/ml, with the mode tigecycline MIC being ≤0.06 μg/ml (16-fold more active than tetracycline) (data not shown). Similar results were seen with *M. chelonae* and a single strain of *M. immunogenum*. For the four tetracycline-susceptible isolates tetracycline MICs were 1 to 4 μg/ml (mode, 4 μg/ml), while the tigecycline MICs for the same strains ranged from ≤0.06 to 0.25 μg/ml (mode, ≤0.06 μg/ml) (data not shown). Minocycline and doxycycline were more active against the tetracycline-susceptible strains, with modes of ≤0.12 and ≤0.25 μg/ml, respectively. For approximately 50% of the isolates, however, minocycline MICs were in the range of 0.25 to 4. 0 μg/ml, which were 3 to 6 dilutions higher than those of tigecycline.

Results for the five RGM reference strains are given in Table 3. Included are MICs of two glycylcyclines for these strains published previously (3). The activities of tigecycline against these strains were the same as those of DMG-DMDOT and comparable to those of DMG-MINO, with results similar to those achieved for the clinical isolates. This is in contrast to the results for staphylococcal species, against which minocycline is 1 to 4 dilutions more active than tigecycline (1).

All 33 strains of six species of slowly growing NTM were resistant to tigecycline (MICs, 16 to >32 μg/ml) (Table 4). Minocycline was more active than tigecycline against the closely related photochromogenic species *M. marinum* and *M. kansasii*. Single isolates each of *M. xenopi* and *M. simiae* were also resistant to tigecycline (MICs, 16 and >32 μg/ml, respectively) (data not shown in Table 4). Additional numbers of test isolates of the latter two species are needed to establish the apparent lack of activity of tigecycline.

TABLE 2. MIC ranges and concentrations of tetracycline, minocycline, and doxycycline and the new glycylcycline tigecycline (GAR-936) that inhibit 50 and 90% of 50 clinical tetracycline-resistant[a] isolates of RGM

Species	MIC (μg/ml)		
	Range	50%	90%
M. fortuitum group (10)[b]			
Tigecycline	≤0.06–≤0.12	≤0.06	≤0.12
Tetracycline	16–64	16	32
Minocycline	2–>64	32	32
Doxycycline	32–>128	64	128
M. abscessus (18)			
Tigecycline	≤0.06–1	≤0.12	0.25
Tetracycline	32–>128	128	>128
Minocycline	64–>64	>64	>64
Doxycycline	128–>128	>128	>128
M. chelonae (22)			
Tigecycline	≤0.06–≤0.12	≤0.06	≤0.12
Tetracycline	16–>128	32	>128
Minocycline	2–>64	16	>64
Doxycycline	16–>128	>64	>128

[a] Tetracycline resistance was defined as an MIC ≥16 μg/ml.
[b] The values in parentheses are the number of isolates tested.

DISCUSSION

The tetracyclines have been highly popular for antimicrobial therapy because of their activities against numerous bacterial organisms, although their use has declined dramatically in the past decade because of drug resistance. Some of the clinical isolates against which tetracyclines exhibit activities include some species of RGM such as *M. fortuitum*, *M. smegmatis*, and *M. mucogenicum* (6, 20, 21, 22), 50% or more clinical isolates of which are susceptible. However, at clinically achievable levels these agents are less effective against other common pathogenic species of RGM such as *M. chelonae* (26% of isolates are susceptible to doxycycline at 4 μg/ml) and *M. abscessus* (4% of isolates are susceptible to doxycycline at 4 μg/ml) (13, 18). Preliminary studies suggest that these species contain a number of resistance determinants that likely play a role in this resistance (11).

The introduction of the glycylcyclines represents a significant advance in the tetracyclines, as the glycylcyclines are unaffected by the major genetic mechanisms (efflux, ribosomal protection) responsible for tetracycline resistance among bacterial species (14, 16). Previous studies with two early glycylcyclines, DMG-MINO and DMG-DMDOT, showed that they have excellent in vitro activities against all species of RGM including the members of the *M. fortuitum* group, *M. chelonae*, *M. abscessus*, and *M. mucogenicum* (3). These results were comparable to the results obtained in the present study with

TABLE 3. MICs of tetracycline, minocycline, doxycycline DMG-DMDOT, DMG-MINO, and tigecycline for reference strains of RGM

Strain	MIC (μg/ml)					
	Tetracycline	Minocycline	Doxycycline	Tigecycline	DMG-DMDOT[a]	DMG-MINO[a]
M. fortuitum group						
M. fortuitum ATCC 6841^T	0.5	≤0.125	≤0.25	≤0.06	NT[b]	NT
M. peregrinum ATCC 14467^T	16	16	128	≤0.06	≤0.063	≤0.25
M. peregrinum ATCC 700686	1	≤0.125	≤0.25	0.12	NT	NT
M. fortuitum third biovariant						
Sorbitol-positive strain ATCC 49403	16	16	64	0.12	0.125	≤0.25
Sorbitol-negative strain ATCC 49404	16	4	32	≤0.06	≤0.063	≤0.25
M. abscessus ATCC 19977^T	64	>64	>128	0.12	0.25	≤0.25
M. chelonae ATCC 35752^T	4	4	4	≤0.06	NT	NT

[a] Previously published values obtained by same MIC method (3).
[b] NT, not tested.

tigecycline (3). The MIC at which 50% of isolates are inhibited (MIC_{50}) and the MIC_{90} of DMG-DMDOT for the 30 isolates of the *M. fortuitum* group were ≤0.06 and 0.125 μg/ml, respectively, values which are the same as those for the 26 isolates tested in the present study against tigecycline. The MICs of the three glycylcyclines for the *M. fortuitum* reference strains (Table 3) are also similar or identical. For 60 isolates of *M. abscessus*, the MIC_{50} and MIC_{90} of DMG-DOT were 0.125 and 0.25 μg/ml, respectively (3), values which are also the same as those of tigecycline for the 20 isolates tested in the present study.

The activity of tigecycline could be highly useful clinically, especially against RGM species causing serious disease, before susceptibilities are available. Amikacin is the only agent with activity against all RGM species, but it has major renal and auditory toxicities. Tigecycline also has much anticipated usefulness against *M. abscessus* isolates causing chronic lung disease. The latter disease is incurable with currently available drugs (19). It is increasingly problematic in patients with cystic fibrosis, in whom subsequent transplantation with its associated immune suppression can result in disseminated disease (5, 7).

In contrast, tigecycline appears to have no in vitro activity against any of the species of slowly growing NTM studied, including those species (*M. marinum*, *M. kansasii*) that were minocycline susceptible at the currently suggested resistance breakpoints. Experience with MIC testing and specific MIC breakpoints is limited for many slowly growing species, however, especially less commonly encountered species such as *M. xenopi* and *M. simiae*. This shortcoming will require additional future studies, an observation recognized in the recent NCCLS document on mycobacterial susceptibility testing (10).

TABLE 4. MIC ranges, concentrations that inhibits 50 and 90% of strains tested, and percentage of strains susceptible for tigecycline (GAR-936) and three tetracyclines against 43 isolates of three species of slowing growing mycobacteria[a]

Species	MIC (μg/ml)			% Susceptible
	Range	50%	90%	
M. avium complex (11)[b]				
Tigecycline	32–>32	>32	>32	0
Tetracycline	64–>128	>128	>128	0
Minocycline	8–>64	64	>64	9
Doxycycline	8–>128	32	>128	9
M. lentiflavum (10)				
Tigecycline	32–>32	>32	>32	0
Tetracycline	16–>128	128	>128	0
Minocycline	16–>64	>64	>64	0
Doxycycline	2–>128	128	>128	0
M. marinum (11)				
Tigecycline	16	16	16	0
Tetracycline	4–16	16	16	45
Minocycline	2–8	4	4	100
Doxycycline	2–16	4	16	82
M. kansasii (11)				
Tigecycline	8–32	16	32	0
Tetracycline	16–128	32	128	0
Minocycline	2–8	8	8	100
Doxycycline	4–32	16	16	36

[a] The resistance breakpoints used were (≥8 μg/ml) for tigecycline and ≥16 μg/ml for the three older tetracyclines.
[b] The values in parentheses are the number of isolates tested.

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