Activity of Tigecycline (GAR-936), a Novel Glycylcycline, against Enterococci in the Mouse Peritonitis Model

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A novel glycylcycline agent, tigecycline (GAR-936), was evaluated in vivo in the mouse model of peritonitis against three *Enterococcus faecalis* and four *Enterococcus faecium* isolates with different susceptibilities to vancomycin and tetracyclines, all of which were inhibited by ≤ 0.125 μ g of tigecycline/ml. Using a single subcutaneous dose, tigecycline displayed a protective effect (50% protective dose, ≤ 5.7 mg/kg of body weight) against all strains tested, including two with Tn925 (from the Tn916 family), which contains the Tet(M) tetracycline resistance determinant, as well as VanA and VanB strains. As expected, tetracycline and minocycline were ineffective against the isolates carrying Tn925.

Enterococci have long been known to cause endocarditis, urinary tract infections, intraabdominal and pelvic infections, intravascular catheter-related bacteremia, bacteremia from unknown origin, soft tissue infections, and neonatal sepsis and meningitis (4, 8). The interest in this organism has increased in recent years, particularly because of the rising incidence of vancomycin-resistant enterococci (VRE), with a major impact in nosocomial infections, where enterococci have been ranked as the third most common cause of bloodstream infections (5). Among enterococcal strains causing nosocomial infections in intensive care units during the year 2000, 26.3% were VRE, representing a 31% increase in the vancomycin resistance rate compared with the mean rate of vancomycin resistance observed in the previous 5 years, as reported by the Centers for Disease Control and Prevention (1). This situation has led to a search for antienterococcal agents with new mechanisms of action or to reformulation of older ones.

The efficacy of tetracyclines has been impaired by the widespread existence of genes conferring resistance to them, probably secondary to the long history of tetracycline use in humans, in field crops and fruit trees, and as food additives for growth promotion in animals (14). In 1988, a new tetracycline with modifications in the minocycline hydrophobic domain conferring activity against tetracycline-resistant organisms was developed, and this eventually led to the synthesis of novel structures called glycylcyclines. The glycylcycline tigecycline (GAR-936) is a 9-*t*-butylglycylamido derivative of minocycline; this new agent inhibits protein synthesis even in the presence of Tet(M)-protected ribosomes, probably due to its tight binding (11). Tigecycline has shown in vitro activity against many gram-positive organisms, including vancomycin-susceptible and vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*, regardless of the susceptibility pattern to tetracy-

clines (3). The aim of this study was to evaluate the in vivo activity of this new agent against various *E. faecalis* and *E. faecium* strains, including tetracycline- and vancomycin-resistant ones, in the mouse peritonitis model.

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MATERIALS AND METHODS

Organisms. Three *E. faecalis* isolates used in this study were OG1RF (ATCC 47077), a well-known plasmid-free isolate (9); V583, a *vanB* and *erm*(B) clinical isolate (15); and INY1200 (OG1RF::Tn925) (Tn925 is a conjugative transposon from the Tn916 family of transposons [13]) which carries the *tet*(M) gene encoding tetracycline-minocycline resistance (18). The four *E. faecium* isolates were TX5034, a VanA type of VRE strain resistant also to erythromycin and streptomycin; TX5037 (TX5034::Tn925), a transconjugant of TX5034 and INY1200, carrying the Tet(M) determinant; TX0016 (DO), a tetracycline- and erythromycin-resistant endocarditis isolate (2) (partial sequence available at <http://www.hgsc.bcm.tmc.edu/microbial/Efaecium/index.html>); and TX2465, another clinical isolate carrying the *vanA* gene cluster, from an oncologic patient with bacteremia.

Antibiotics. Tigecycline in powder form was obtained from Wyeth Research, Pearl River, N.Y. Other antibiotics used were tetracycline hydrochloride, minocycline, vancomycin hydrochloride, erythromycin, and ampicillin sodium salt and were obtained from Sigma, St. Louis, Mo. Quinupristin-dalfopristin was obtained from Aventis Pharma, Croix-de-Berny, France.

In vitro susceptibility testing. The MICs of each antimicrobial agent were determined by the agar dilution method using Mueller-Hilton agar II (Becton Dickinson and Company, Cockeysville, Md.) and following the National Committee for Clinical Laboratory Standards guidelines (10). *E. faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 29213 strains were used for quality control.

In vivo efficacy model. Female, 4- to 6-week-old, outbred ICR mice (Harlan Sprague Dawley, Houston, Tex.) with a mean weight between 20 and 25 g were used as previously described (16, 17). Each dosing group was composed of six animals. For the bacterial inoculation, the different strains of enterococci were grown on brain heart infusion (BHI) agar (Difco Laboratories, Detroit, Mich.) plates, then inoculated in BHI broth, and incubated at 37°C overnight. Mice were injected intraperitoneally with 1 ml of the solution containing the infecting enterococcal strain in 12.5% sterile rat fecal extract, resulting in an inoculum of about 10 times the minimum lethal dose (16). Antibiotics were given subcutaneously as a single dose immediately after the intraperitoneal injection. Based on previous observation of the time course of disease, mice infected with *E. faecalis* were monitored for 96 h, and those infected with *E. faecium* were monitored for 120 h. The 50% lethal dose and the 50% protective dose (PD₅₀) were determined by the method described by Reed and Muench (12). Preapproved guidelines of

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the Animal Welfare Committee of the University of Texas Health Science Center at Houston were followed throughout the animal experiments. Bacteria were recovered from the spleens of dead mice and saved for phenotypic comparison and/or pulsed-field gel electrophoresis.

RESULTS AND DISCUSSION

The *E. faecalis* and *E. faecium* strains used in this study were all inhibited by ≤ 0.125 μg of tigecycline/ml (Table 1); the MIC values obtained were not influenced by the tetracycline or vancomycin susceptibility pattern of the specific isolate.

Table 1 displays the MICs and PD_{50} s for the three *E. faecalis* and four *E. faecium* isolates tested, and Fig. 1 shows two dose-response curves. PD_{50} values from previous experiments done in our laboratory under different conditions were included for ampicillin and vancomycin (single subcutaneous dose) against OG1RF and TX2465 and for quinupristin-dalfopristin (single intravenous dose) against TX0016 and TX2465, which were previously reported (17). None of the mice in the drug control groups (where only the antibiotic is given) died, and none of the mice in the infection control groups (where mice are infected and no antimicrobial agent is given) survived.

Both tetracycline and tigecycline had protective effects ($\text{PD}_{50} \leq 6$ mg/kg) against the tetracycline-susceptible *E. faecalis* strain OG1RF (ATCC 47077) (Fig. 1A), although the PD_{50} value for tigecycline was approximately 10 times lower than the PD_{50} obtained with tetracycline. Against the *E. faecalis* strain INY1200 (OG1RF::Tn925) (Fig. 1B), tetracycline and minocycline did not decrease mouse lethality, even when doses up to 200 mg/kg of body weight were administered; tigecycline, on the other hand, had a low PD_{50} value (1.9 mg/kg), similar to that obtained against the parental tetracycline-susceptible strain OG1RF. For *E. faecalis* V583, tigecycline and minocycline displayed protection against this strain. Among the *E. faecium* isolates tested, tigecycline and minocycline were effective against the VanA-type strain TX5034. Mice infected with the derivative TX5037 (TX5034::Tn925), carrying the Tet(M) resistance determinant, were effectively protected by tigecycline (PD_{50} , 3.6 mg/kg), while tetracycline was inactive (PD_{50} , >200 mg/kg). When the tetracycline-resistant *E. faecium* clinical isolate TX0016 was tested, minocycline showed no protective effect, vancomycin was moderately effective ($\text{PD}_{50} = 16$ mg/kg), and tigecycline displayed the lowest PD_{50} value (5.7 mg/kg). Against the *E. faecium* strain TX2465, another VanA type of isolate, tigecycline displayed a slightly lower PD_{50} value (5.5 mg/kg) than that obtained with minocycline (9.3 mg/kg).

In a recent study (6), tigecycline MICs were determined against 1,203 clinical isolates by broth microdilution methods. The tigecycline MICs at which 90% of isolates were inhibited were ≤ 0.25 $\mu\text{g}/\text{ml}$ and 0.12 $\mu\text{g}/\text{ml}$ for 100 vancomycin-susceptible and vancomycin-resistant *E. faecalis* and *E. faecium* strains and for 10 *Enterococcus casseliflavus* and *Enterococcus gallinarum* (genotype *vanC*) strains, respectively. In the same study, all minocycline-resistant enterococci (4 *E. faecalis*, 13 *E. faecium*, and 1 *E. hirae* isolate) had tigecycline MICs of ≤ 0.25 $\mu\text{g}/\text{ml}$. Another study (3) compared the in vitro activities of tigecycline with those of doxycycline, minocycline, and tetracycline against 527 gram-positive clinical isolates. One third of the enterococci (including several strains of *E. faecalis* and *E.*

TABLE 1. MICs and PD_{50} s after a single subcutaneous dose of several antibiotics for three *E. faecalis* and four *E. faecium* strains

Strain and antibiotic	MIC ($\mu\text{g}/\text{ml}$)	PD_{50} (mg/kg)
<i>E. faecalis</i> OG1RF (ATCC 47077)		
Tigecycline	0.125	0.6
Tetracycline	1	6
Minocycline	<0.5	— ^c
Ampicillin	2	11.3 ^a
Vancomycin	0.5	29.5 ^a
<i>E. faecalis</i> INY1200 [<i>tet</i> (M)] (OG1RF::Tn925)		
Tigecycline	0.125	1.9
Tetracycline	32	>200
Minocycline	32	>200
Ampicillin	2	—
Vancomycin	4	—
<i>E. faecalis</i> V583 [<i>vanB</i> and <i>erm</i> (B)]		
Tigecycline	0.125	1.8
Tetracycline	1	—
Minocycline	2	2.8
Ampicillin	4	35.3
Vancomycin	128	—
<i>E. faecium</i> TX5034 (<i>vanA</i>)		
Tigecycline	<0.06	2.1
Tetracycline	<0.25	—
Minocycline	<0.5	3.8
Ampicillin	>128	—
Vancomycin	>256	—
Quinupristin-dalfopristin	1	—
<i>E. faecium</i> TX5037 [<i>tet</i> (M)] (TX5034::Tn925)		
Tigecycline	<0.06	3.6
Tetracycline	32	>200
Minocycline	32	—
Ampicillin	64	—
Vancomycin	>256	—
Quinupristin-dalfopristin	<0.25	—
<i>E. faecium</i> TX0016 (Tet ^r)		
Tigecycline	0.125	5.7
Tetracycline	>64	—
Minocycline	32	>200
Ampicillin	16	—
Vancomycin	0.5	16
Erythromycin	>256	—
Quinupristin-dalfopristin	<0.25	32.1 ^b
<i>E. faecium</i> TX2465 (<i>vanA</i>)		
Tigecycline	<0.06	5.5
Tetracycline	>64	—
Minocycline	8	9.3
Ampicillin	>128	$>200^a$
Vancomycin	>256	$>200^a$
Quinupristin-dalfopristin	<0.25	10.2 ^b

^a Values obtained from experiments done previously under a different batch of sterile rat fecal extract.

^b Previously reported results (17) using intravenous single dose administration.

^c —, not tested.

faecium with VanA and VanB types of glycopeptide resistance) were resistant to minocycline and doxycycline, and 44% were resistant to tetracycline; however, all were inhibited by tigecycline at ≤ 2 $\mu\text{g}/\text{ml}$ demonstrating, again, that tigecycline retains its activity against enterococci resistant to tetracyclines as a class. In other studies in animals, tigecycline produced a sta-

FIG. 1. Survival and dose-response curves for mice intraperitoneally infected with wild-type OG1RF and a tetracycline-resistant derivative. Therapy was given subcutaneously immediately after intraperitoneal injection of bacteria. (A) OG1RF, *E. faecalis* strain sensitive to tetracycline; (B) INY1200 (OG1RF::Tn925), an OG1RF derivative carrying the Tet(M) tetracycline resistance determinant.

tistically significant bacterial count reduction compared to untreated animals against vancomycin-susceptible and vancomycin-resistant *E. faecalis* isolates and a VanA-type *E. faecium* strain in a rat and rabbit model of endocarditis, respectively (7) (A. Lefort, M. Lafaurie, A. Saleh-Mghir, L. Garry, M. C. Peker, D. Le Guludec, C. Carbon, and B. Fantin, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 415, 1999).

In summary, the new glycylicycline tigecycline showed good potency in vitro and in vivo in a mouse peritonitis model against several *E. faecalis* and *E. faecium* strains, including tetracycline-resistant strains, suggesting that this may be an attractive agent for the treatment of enterococcal infections and that further studies in humans may be warranted.

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