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

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Received 11 July 2002/Returned for modification 17 September 2002/Accepted 10 November 2002

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 \( \leq 0.125 \) \( \mu \)g of tigecycline/ml. Using a single subcutaneous dose, tigecycline displayed a protective effect (50% protective dose, \( \leq 5.7 \) mg/kg of body weight) against all strains tested, including two with \( \text{Tn}925 \) (from the \( \text{Tn}916 \) 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 \( \text{Tn}925 \).

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 presence 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 9,4-butylglyclalamido 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 tetracyclines (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.

This work was presented in part at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 16 to 19 December 2001.

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 ert(B) clinical isolate (15); and INY1200 (OG1RF::Tn925) (Tn925 is a conjugative transposon from the Tet16 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-Berry, 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).
The E. faecalis and E. faecium strains used in this study were all inhibited by ≤0.125 μg 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 PD₅₀ after a single subcutaneous dose of several antibiotics for three E. faecalis and four E. faecium strains.

The E. faecalis OG1RF (ATCC 47077) and INY1200 (OG1RF::Tn925) (Fig. 1A), although the PD₅₀ value for tigecycline was approximately 10 times lower than the PD₅₀ 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 PD₅₀ 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. faecalis isolates tested, tigecycline and minocycline were effective against the VanA-type strain TX0016. Mice infected with the derivative TX0037 (TX0034::Tn925), carrying the Tet(M) resistance determinant, were effectively protected by tigecycline (PD₅₀, 3.6 mg/kg), while tetracycline was inactive (PD₅₀ >200 mg/kg). When the tetracycline-resistant E. faecium clinical isolate TX0016 was tested, minocycline showed no protective effect, vancomycin was moderately effective (PD₅₀ = 16 mg/kg), and tigecycline displayed the lowest PD₅₀ value (5.7 mg/kg). Against the E. faecium strain TX2465, another VanA-type isolate, tigecycline displayed a slightly lower PD₅₀ value (5.5 mg/kg) than that obtained with minocycline (9.5 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 μg/ml and 0.12 μg/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.

The results of the tigecycline MICs for E. faecalis OG1RF and TX2465 are shown in Table 1. The PD₅₀ values obtained were not influenced by the tetracycline or vancomycin susceptibility pattern of the specific isolate. Tigecycline was inactive (PD₅₀, 5.5 mg/kg) against the tetracycline-susceptible strain OG1RF (ATCC 47077) (Fig. 1A), although the PD₅₀ value for tigecycline was approximately 10 times lower than the PD₅₀ 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 PD₅₀ 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. faecalis isolates tested, tigecycline and minocycline were effective against the VanA-type strain TX0016. Mice infected with the derivative TX0037 (TX0034::Tn925), carrying the Tet(M) resistance determinant, were effectively protected by tigecycline (PD₅₀, 3.6 mg/kg), while tetracycline was inactive (PD₅₀ >200 mg/kg). When the tetracycline-resistant E. faecium clinical isolate TX0016 was tested, minocycline showed no protective effect, vancomycin was moderately effective (PD₅₀ = 16 mg/kg), and tigecycline displayed the lowest PD₅₀ value (5.7 mg/kg). Against the E. faecium strain TX2465, another VanA-type isolate, tigecycline displayed a slightly lower PD₅₀ value (5.5 mg/kg) than that obtained with minocycline (9.5 mg/kg).

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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.

In summary, the new glycylcycline 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.

**ACKNOWLEDGMENT**

This study was supported by a grant from Wyeth Research.

**REFERENCES**


