Efficacy of Tigecycline Alone and in Combination with Gentamicin in the Treatment of Experimental Endocarditis Due to Linezolid-Resistant Enterococcus faecium

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We evaluated the efficacy of tigecycline in a rabbit model of experimental endocarditis caused by a linezolid-resistant clinical strain of Enterococcus faecium. Tigecycline-treated animals had a 2.8-log₁₀-CFU/g reduction in microbial counts in excised vegetations compared with controls. Addition of gentamicin caused a further arithmetical reduction in colony counts. The therapeutic effect was sustained 5 days after completion of treatment, as shown by relapse studies performed in treatment groups.

Optimal treatment of multidrug-resistant (MDR) Enterococcus faecium endocarditis is an unresolved issue (1). Such an infection occurs primarily in the hospital environment, where rates of resistance to linezolid might reach 11 to 16% (2, 3) although still being less than 1% in the community (4). We evaluated the efficacy of tigecycline and explored the synergistic potential with gentamicin in a rabbit model of endocarditis caused by a linezolid-resistant strain of E. faecium.

The bacterial isolate was collected during a vancomycin-resistant Enterococcus (VRE) outbreak (5). It harbored at least one copy of the G2576U mutation of 23S rRNA, which is linked to linezolid resistance. The isolate was esp gene positive as shown by PCR (5). MICs and minimal bactericidal concentrations (MBCs) were determined by broth microdilution, following CLSI guidelines (6). A time-kill assay was performed to evaluate the in vitro bactericidal effect of tigecycline and its combination with gentamicin against a high bacterial inoculum. CLSI definitions were used for interpretation of results (7).

The optimal pharmacokinetic/pharmacodynamic (PK/PD) indicator of tigecycline activity is the area under the concentration-time curve (AUC)/MIC ratio (8). Human pharmacokinetic studies have shown that AUC values after 50- to 100-mg intravenous (i.v.) injections in steady state are between 3 and 5 mg·h/liter (9). A previous study (10) has documented that tigecycline dosing in rabbits has a linear relationship with AUC and that a single 7-mg/kg-of-body-weight dose produces an AUC of 9.5 mg·h/liter. Thus, we predicted that a 4-mg/kg dose would be appropriate and performed a confirmatory pharmacokinetic study, using an agar diffusion assay, with Bacillus subtilis ATCC 6633 as the indicator of tigecycline concentration. Blood was drawn from 2 healthy animals at serial time points after a single i.v. infusion of 4 mg/kg tigecycline. The maximum concentration of drug in serum (Cmax) and AUC from 0 to 12 h (AUC0−12) were calculated.

The study was approved by the Veterinary Directorate of the Prefecture of Athens (Athens, Greece).

Endocarditis was induced to study animals at time zero as previously described (11). On day 1, rabbits were infected by intravenous administration of 10⁶ CFU of the enterococcal strain. On day 2, animals were assigned to the following treatment groups: A, control (n = 14); B, tigecycline (n = 15); C, tigecycline plus gentamicin (n = 15); D, tigecycline test of relapse (ToR) (n = 12); E, tigecycline plus gentamicin ToR (n = 13).

ToR groups consisted of animals receiving treatment for 5 days followed by an equal antibiotic-free period. Such a relapse study is advised for experimental endocarditis (12) and has not yet been performed for tigecycline use. The gentamicin dosage was 6 mg/kg given subcutaneously (s.c.) once daily (q.d.) in accordance with the findings of Gavaldá et al. (13).

At autopsy, proper positioning of the catheter was verified. Vegetations were excised, as were approximately 50-mg segments of lung, liver, spleen, and kidney. Vegetations were weighed, homogenized in NaCl (0.9%), and quantitatively cultured in duplicate on blood agar plates after seven serial dilutions in Mueller-Hinton broth. After incubation for 48 h at 35°C and 5% CO2, the colonies of E. faecium growing on agar were expressed as log₁₀ CFU per gram of vegetation. When no growth was visible on agar plates, the value of 1.4 log₁₀ CFU/g was assigned. Tissue samples were qualitatively cultured.

For all strains recovered from tigecycline-treated animals, MICs were tested via Etest strips. Vegetations’ bacterial load was expressed by its mean value ± standard error (SE), and comparisons between groups were performed using one-way analysis of variance (ANOVA) with Bonferroni’s adjustment. Rates of sterile cultures were compared using Fisher’s exact test. P values below 0.05 were considered significant.

The MICs/MBCs of the infecting strain were as follows: ampicillin, 128/>512 mg/liter; gentamicin, 64/>128 mg/liter; vancomycin, 512/>512 mg/liter; linezolid, 12/>512 mg/liter; tigecycline, 4/>8 mg/liter; and gentamicin, 4/>8 mg/liter.
cline, 0.06–2 mg/liter; daptomycin, 0.5/4 mg/liter. The time-kill curves are depicted in Fig. 1. Mean $C_{\text{max}}$ and AUC$_{0-12}$ values were 2.7 mg/liter and 4.25 mg · h/liter, respectively.

The mean vegetation bacterial load was significantly reduced in groups B and C versus results for the control. Addition of gentamicin induced a further, non-statistically significant reduction.

Concerning relapse study, tigecycline-treated animals 5 days after completion of treatment had arithmetically fewer bacteria than their end-of-therapy counterparts. All strains recovered from vegetations exhibited a stable MIC to tigecycline. Vegetation and peripheral tissue culture results are depicted in Table 1.

Despite belonging to a bacteriostatic class of antimicrobials, tigecycline proved bactericidal in vitro and induced reductions in the vegetation bacterial load that were comparable to those observed by others in models of endocarditis treated with bactericidal regimens (13). Similar results were reproduced in other studies examining the efficacy of tigecycline in experimental enterococcal endocarditis both in rabbits (10) and in rats (14). Moreover, successful use of tigecycline combinations has been described for clinical enterococcal endocarditis (15, 16). A possible explanation for the discrepancy between tigecycline’s bacteriostatic nature and these promising data would be the low MICs of Gram-positive cocci that allow achievement of high AUC/MIC values.

The therapeutic effect of daptomycin, another potential agent in this setting, against MDR E. faecium endocarditis in rabbits has been previously tested (17). Caron et al., using identical methodology, studied the activity of daptomycin against a glycopeptide-resistant strain and found comparable sterilization rates and reductions in bacterial load. In rats, however, Vouillamoz et al. (18) were able to show a 5-log$_{10}$-CFU/g decrease in bacterial counts when daptomycin was used in MDR enterococcal endocarditis.

Data on tigecycline’s potential for combination with other antimicrobials are lacking (19). Both in vitro and in vivo, the addition of gentamicin induced a trend for acceleration of the inhibitory effect. These results are in the same direction as data provided by McConeghy and LaPlante (20) showing tigecycline-gentamicin synergy against biofilm-producing Staphylococcus aureus in an in vitro pharmacodynamic model.

Antimicrobial dosage in animal studies is crucial in order for the results to be clinically useful. In the unique previous study in the field (10), tigecycline was found to be potent in decreasing vegetation bacterial counts but at an AUC that would probably be toxic (8), since it was several times higher than the one achieved in humans.

![Fig 1](http://aac.asm.org/)

**Fig 1** Time-kill curves of linezolid-resistant E. faecium against treatment regimens. Filled circle (●), control; filled square (■), tigecycline at 0.8 mg/liter; open square (□), tigecycline at 0.8 mg/liter plus gentamicin at 16 mg/liter. Antimicrobial concentrations were chosen in accordance with peak levels achieved in humans.

<table>
<thead>
<tr>
<th>Regimen</th>
<th>No. alive/total</th>
<th>No. infected/total</th>
<th>Log$_{10}$ CFU/g</th>
<th>Lung</th>
<th>Liver</th>
<th>Spleen</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11/14</td>
<td>14/14</td>
<td>8.02</td>
<td>6/14</td>
<td>9/14</td>
<td>9/14</td>
<td>6/14</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>14/15</td>
<td>15/15</td>
<td>3.21$^{a}$</td>
<td>1/15</td>
<td>1/15</td>
<td>1/15</td>
<td>0/15</td>
</tr>
<tr>
<td>Tigecycline ToR</td>
<td>11/12</td>
<td>10/12</td>
<td>3.86$^{b}$</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Tigecycline + Gentamicin</td>
<td>13/15</td>
<td>13/15</td>
<td>3.93$^{b}$</td>
<td>1/15</td>
<td>0/15</td>
<td>0/15</td>
<td>0/15</td>
</tr>
<tr>
<td>Tigecycline + Gentamicin ToR</td>
<td>13/13</td>
<td>9/13</td>
<td>3.77$^{b,c}$</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

* $P < 0.05$ versus control group.
* $P = 0.12$ versus tigecycline group.
* $P = 0.88$ versus tigecycline-plus-gentamicin group.
* ToR, test of relapse; ND, not done.
humans. Nevertheless, our results provide some evidence that tigecycline could be efficient at feasible AUC/MIC ratios.

To conclude, in this model of endocarditis caused by a linezolid-resistant \textit{E. faecium} strain, tigecycline monotherapy and combination with gentamicin produced a sustainable antimicrobial effect. Should these results be confirmed in additional studies, tigecycline could be an option for the medical management of the rare patient with linezolid-resistant enterococcal endocarditis.

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We have no conflict of interest to declare.

REFERENCES