Activity of Tigecycline (GAR-936) against *Acinetobacter baumannii* Strains, Including Those Resistant to Imipenem

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We determined the in vitro activities of tigecycline and imipenem against 49 isolates of *Acinetobacter baumannii*, including those resistant to imipenem. The MIC at which 50% of the isolates were inhibited (MIC₅₀) and the MIC₉₀ for tigecycline were 2 and 2 mg/liter and 32 and 128 mg/liter, respectively, with 92 and 20%, respectively, of the strains being susceptible. Tigecycline did not show bactericidal activity in the time-kill studies (n = 9 strains). Imipenem showed bactericidal activity against seven out of nine strains. These in vitro results show that tigecycline has good in vitro bacteriostatic activity against *A. baumannii*, including strains resistant to imipenem.

*Acinetobacter baumannii* is a nonfermentative gram-negative rod that causes nosocomial infections, especially in intensive care units (4, 5, 11, 13), with increasing frequency. This organism usually affects immunocompromised, ventilator-dependent, or debilitated patients, causing a great number of clinical conditions, including pneumonia, bacteremia, urinary tract infections, wound infections, endocarditis, and meningitis (1, 4). The mortality of nosocomial infections by *A. baumannii* is high, reaching 25 to 34% for bacteremia and 40 to 80% (6, 8) for nosocomial pneumonia.

Management of *A. baumannii* infections can be complicated due to the emergence of isolates with multiple-drug resistance (4, 6), including resistance to carbapenems (3). Therefore, it is necessary to evaluate new molecules that are potentially useful against *A. baumannii*.

Tigecycline, a derivate of minocycline, is a glycylcycline that exhibits potent activity against a broad spectrum of bacteria (7, 10, 12, 13, 15, 18, 20), including *Acinetobacter* spp. (2, 7, 9). In this study, we determined the in vitro activity of tigecycline against nosocomial *A. baumannii*, including strains resistant to imipenem.

*A. baumannii* clinical isolates were identified with MicroScan (Baxter H.C., West Sacramento, Calif.), the API 20NE system (Bio-Mérieux, Marcy l’Etoile, France), and the temperature growth test (44°C). The strains were stored frozen at −80°C in brucella broth containing 20% glycerol until they were tested for susceptibility. Fortynine strains from blood cultures corresponding to bacteremic patients were studied. Previously, these isolates were characterized molecularly by means of a repetitive extragenic palindromic sequence-based PCR method (14). The isolates were predominantly from the two quantitatively most important *A. baumannii* genotypes found in our institution (clones I and II).

For the determination of the MIC a broth microdilution method was used (cation-adjusted Mueller-Hinton broth; Bec-ton Dickinson, Cockeysville, Md.), in accordance with the NCCLS guidelines (16). Imipenem (Merck Sharp & Dohme Madrid, Spain) and tigecycline (Wyeth-Ayerst, Pearl River, N.Y.) were the drugs tested. MICs were interpreted according to NCCLS criteria for non-*Enterobacteriaceae* (16). Because there is no approved standard for considering *A. baumannii* susceptible or resistant to tigecycline, provisional MIC breakpoints used for this agent were ≥2, 4, and ≥8 μg/ml to designate susceptible, intermediate, and resistant strains, respectively (Wyeth Research, personal communication). *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used as quality control strains. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of drug that resulted in the killing of 99.9% of the original inoculum (16, 19).

Nine isolates with different susceptibilities to imipenem were chosen for time-kill studies: three imipenem-susceptible strains (MIC = 1 μg/ml), three with intermediate susceptibility to imipenem (MIC = 8 μg/ml for one strain and MIC = 16 μg/ml for two strains), and three resistant to imipenem (MIC = 32 μg/ml). Organisms were grown on Mueller-Hinton broth for 4 h (log phase of growth) and were further diluted in 20 ml of the same medium to yield a concentration of approximately 5 × 10⁵ CFU/ml, as verified by plate counts. Tigecycline powder was dissolved and prepared for in vitro testing according to instructions from the manufacturer and in compliance with NCCLS guidelines (16). Probe tubes contained imipenem or tigecycline at concentrations corresponding to the MIC and two and four times the MIC for each strain. Additional control tubes were inoculated with bacteria of each strain and without drugs. Tubes were incubated aerobically at 36°C for 24 h. Aliquots (0.1 ml of broth) were removed from each tube, and serial dilutions were plated onto blood-agar plates after 0, 2, 4, 8, and 24 h of incubation. Colony counts were performed after 24 h of incubation at 36°C. Bactericidal activity was defined as a ≥3 log₁₀ reduction compared with the initial inoculum (16, 19).

Results of susceptibility tests (MIC and MBC) for imipenem and tigecycline are shown in Table 1. Thirty-eight isolates were imipenem resistant (78%), 1 isolate showed intermediate susceptibility to imipenem (2%), and 10 isolates were imipenem
susceptible (20%). Conversely, 45 strains were tigecycline susceptible (92%), with a MIC range of 1 to 4 mg/liter.

In time-kill studies, imipenem showed bactericidal activity against 3, 7, and 6 strains at the MIC, twice the MIC, and four times the MIC, respectively, beginning after 8 h of incubation (Table 2). On the other hand, tigecycline was not bactericidal against any strain at any tested concentration; it produced decreases of 2.99 and 2.84 log10 CFU/ml for one imipenem-intermediate strain and one imipenem-resistant strain, respectively.

Our results show that 92% of the A. baumannii strains were tigecycline susceptible, by the provisional breakpoints previously detailed. Other in vitro studies have indicated that tigecycline is active against A. baumannii (2, 7, 9). One study (9) using the same provisional breakpoints for tigecycline showed that 91% of A. baumannii complex strains (n = 443 strains) were susceptible; however, only 2% of the strains included in this study had diminished susceptibility to imipenem (intermediate or resistant).

The MIC at which 90% of the isolates were inhibited (MIC90) of tigecycline in the present study was 2 μg/ml, equal to that found in other studies from the United Kingdom and the United States (9, 10) and lower than the MIC90 of 8 μg/ml found in the work by Betriu et al. from Spain (2). These results are valuable, taking into account that 78% of the strains in our study were imipenem resistant, with a MIC90 of imipenem of 128 μg/ml. In contrast, two other studies on the activity of tigecycline against A. baumannii found that only 28 and 0.4% of the strains were resistant to imipenem, with MIC90s of 128 and 0.5 μg/ml, respectively (2, 9).

Although tigecycline, like other derivatives of tetracycline, demonstrates bacteriostatic activity, it has been found to be bactericidal for a wide spectrum of gram-positive and gram-negative aerobic and anaerobic bacteria (18). Other studies have not included an evaluation of the bactericidal activity of tigecycline against A. baumannii (10, 12, 15, 17, 18). In the present study, tigecycline was not bactericidal against any strains, although for two of them the decrease in the count at 24 h was near 3 log10 CFU/ml.

In summary, tigecycline is active against A. baumannii strains, including those resistant to imipenem. The results of the time-kill studies show that tigecycline is bacteriostatic against A. baumannii.

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**TABLE 1.** Susceptibilities of 49 A. baumannii strains to imipenem and tigecycline

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (μg/ml)</th>
<th>MBC (μg/ml)</th>
<th>% of susceptibility</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range 50% 90%</td>
<td>Range 50% 90%</td>
<td>S I R</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1–128 32 128</td>
<td>1–128 32 128</td>
<td>20 2 78</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>1–4 2 2</td>
<td>2–&gt;8 8 &gt;8</td>
<td>92 8 0</td>
</tr>
</tbody>
</table>

* 50% and 90%, MIC90 and MIC50, respectively.

**TABLE 2.** Time-kill results of imipenem against nine A. baumannii strains with different susceptibilities to imipenem

<table>
<thead>
<tr>
<th>Concentration of imipenem</th>
<th>No. of strains against which imipenem showed bactericidal activity at different times of incubation (susceptibilities of strains to imipenem)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 h</td>
</tr>
<tr>
<td>MIC</td>
<td>0 0 1 (I)</td>
</tr>
<tr>
<td>2 × MIC</td>
<td>0 0 2 (I)</td>
</tr>
<tr>
<td>4 × MIC</td>
<td>0 0 2 (I)</td>
</tr>
</tbody>
</table>

* 2 × MIC, twice the MIC; 4 × MIC, four times the MIC.

**REFERENCES**


17. Petersen, P. J., P. A. Bradford, W. J. Weiss, T. M. Murphy, P. E. Sum, and...

