Acinetobacter baumannii bloodstream infection while receiving tigecycline: a cautionary report

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Objectives: Tigecycline has shown in vitro activity against Acinetobacter baumannii. Yet, published clinical experience with tigecycline use outside clinical trials is lacking. We describe, for the first time, bloodstream infection caused by tigecycline-non-susceptible A. baumannii occurring in patients receiving tigecycline for other indications. The possible mechanisms of resistance and pharmacokinetic limitations of the drug are addressed.

Methods: The clinical records of involved patients were systematically reviewed. Tigecycline susceptibility testing was initially performed using the Etest method and confirmed by agar dilution. Involved isolates underwent PFGE and exposure to phenyl-arginine-β-naphthylamide (PAβN), an efflux pump inhibitor.

Results: Two patients developed A. baumannii bloodstream infection while receiving tigecycline. Tigecycline was administered for other indications for 9 and 16 days, respectively, before the onset of A. baumannii infection. Patient 1 died of overwhelming A. baumannii infection and Patient 2 recovered after a change in antibiotic therapy. The MICs of tigecycline were 4 and 16 mg/L, respectively. Both isolates had a multidrug-resistant phenotype and were genotypically unrelated. After exposure to PAβN, the MICs reduced to 1 and 4 mg/L, respectively.

Conclusions: To our knowledge, this is the first clinical description of bloodstream infection caused by tigecycline-non-susceptible A. baumannii. Such resistance appears to be at least partly attributable to an efflux pump mechanism. Given the reported low serum tigecycline levels, we urge caution when using this drug for treatment of A. baumannii bloodstream infection.

Keywords: A. baumannii, glycylcycline, GAR-936, antibiotic resistance

Introduction

Acinetobacter baumannii has become an important hospital-acquired pathogen. Although most typically associated with hospital-acquired pneumonia, bloodstream infection is a significant problem in many institutions. Carbapenems have been the agents of choice for serious Acinetobacter infections, but unfortunately, outbreaks of A. baumannii resistant to all antimicrobials except the polymyxins, have now been reported.1 Tigecycline, a new, semi-synthetic tetracycline (glycylcycline) has provided hope for the treatment of infections caused by certain resistant Gram-negative organisms, including A. baumannii.2 The unique feature of tigecycline is its ability to evade the major determinants of tetracycline resistance, the tet(A-E) and tet(K) efflux pumps and the tet(M) and tet(O) determinants that provide ribosomal protection. Thus, tigecycline has a broad spectrum of in vitro activity, including susceptible and multidrug-resistant Gram-positive and -negative organisms, as well as,
Tigecycline-non-susceptible Acinetobacter

anaerobes and atypical organisms. Deficiencies in its spectrum include Proteaceae and Pseudomonas spp., with intrinsic resistance being mediated by chromosomally encoded multidrug efflux pumps from the resistance-nodulation-division (RND) family.\textsuperscript{3,4} Thus far, resistance to tigecycline in a clinical setting has rarely been described, with unpublished reports suggesting several instances of emerging resistance during the Phase III trials, including one \textit{A. baumannii} isolate.\textsuperscript{5} Also, several tigecycline non-susceptible \textit{A. baumannii} isolates (MIC 4–16 mg/L) are being investigated in the UK.\textsuperscript{6} Given the efficiency of \textit{Acinetobacter} at acquiring resistance determinants, its response to tigecycline exposure is awaited.

In this report, we describe two patients who developed bloodstream infection with \textit{A. baumannii} while receiving therapeutic doses of tigecycline and hypothesize the potential aetiology of this occurrence. Such cases raise important questions about the durability of this drug for the treatment of \textit{Acinetobacter} infection and the appropriateness of tigecycline in treating bloodstream infection caused by \textit{Acinetobacter}.

Patients and methods

Two patients were identified at the University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, USA, as having \textit{A. baumannii} bloodstream infection while receiving tigecycline for other indications. The patient’s records were systematically reviewed and available clinical isolates underwent further testing.

Tigecycline susceptibility testing was initially performed using the Etest method (AB Biodisk, Solna, Sweden). Confirmatory testing was performed by agar dilution on Mueller–Hinton II agar (Oxoid, Hampshire, England) as recommended by the CLSI. Fresh agar and tigecycline were prepared on the day of testing. Tigecycline powder was obtained from commercial sources and was prepared into solution using sterile water. Susceptibility testing for other antibiotics was initially performed by disc diffusion testing. Confirmatory testing was later performed by agar dilution according to CLSI guidelines. Molecular typing by PFGE was performed using the CHEF DR III system (Bio-Rad). The restriction endonuclease ApaI was used for \textit{in-situ} digestion of intact \textit{A. baumannii} genomic DNA. After staining with ethidium bromide, the fragments were visualized by using a Bio-Rad Gel Doc 2000 system. DNA fingerprints were interpreted as recommended by Tenover et al.\textsuperscript{5} The available \textit{A. baumannii} isolates were exposed to phenyl-arginine-β-naphthylamide (PA\textsuperscript{B}N), an efflux pump inhibitor, and antibiotic susceptibility testing was repeated using agar dilution. PA\textsuperscript{B}N powder was re-suspended in pure water to a 25 mg/mL concentration and then added to 250 mL of Mueller–Hinton II agar cooled to 50°C.

Results

Clinical cases

Case 1 was a 76-year-old woman with a history of hypertension and diabetes mellitus who underwent lumbar laminectomy. Postoperatively, she developed multiple complications including atrial fibrillation, deep venous thrombosis and heparin-induced thrombocytopenia. During the course of her hospitalization she developed colonization with vancomycin-resistant \textit{Enterococcus faecium}. She developed fever and hypotension and was transferred to the intensive care unit. Intravenous tigecycline was commenced empirically as monotherapy (100 mg loading dose followed by 50 mg every 12 h). Nine days after initiation of tigecycline, the patient developed leucocytosis and hypothermia and two sets of blood cultures were drawn. \textit{A. baumannii} was grown from these cultures and from endotracheal aspirates. The MIC of tigecycline for this isolate was 4 mg/L. The isolate was susceptible to imipenem and piperacillin/tazobactam, but intermediate or resistant to ampicillin/sublactam, cefepime, ciprofloxacin and aminoglycosides. Therapy was switched initially to piperacillin/tazobactam but because of severe haemodynamic instability and respiratory failure, meropenem and gentamicin were substituted. Soon after, the patient died.

Case 2 was a 60-year-old man who had a ventricular-assist device inserted for ischaemic cardiomyopathy as a bridge to cardiac transplantation. Fourteen months later \textit{A. baumannii} and \textit{Enterobacter cloacae} were grown from wound cultures taken from around the drive line. \textit{E. cloacae}, of intermediate susceptibility to ertapenem, was thought to be the infecting organism and ertapenem was commenced as monotherapy. Despite being systemically well, repeat cultures were performed due to increased purulence from the wound. \textit{E. cloacae}, of intermediate susceptibility to ertapenem, was grown. Therapy was switched to intravenous tigecycline (100 mg loading dose followed by 50 mg every 12 h) and clinical response was achieved with a decrease in wound purulence. On the sixteenth day of tigecycline therapy the patient developed fevers and two sets of blood cultures were collected. These cultures grew \textit{A. baumannii}; the MIC of tigecycline being 16 mg/L. The isolate was susceptible to ampicillin/sublactam, imipenem and amikacin, but non-susceptible to piperacillin/tazobactam, cefepime, ciprofloxacin, gentamicin and tobramycin. Unfortunately, no tigecycline susceptibilities were available for the preceding \textit{A. baumannii} wound culture isolate. Therapy was switched to meropenem and amikacin, with subsequent clearance of his bacteriaemia within a week of their initiation. Repeated follow-up blood cultures over the following five months were negative despite the ventricular-assist device remaining in situ. The patient underwent heart transplantation with no recurrence of his \textit{A. baumannii} infection and without growth of the organism from the explanted device.

PFGE and exposure to PA\textsuperscript{B}N

Molecular typing identified that the two isolates were genotypically unrelated. After PA\textsuperscript{B}N exposure, the tigecycline MIC of the \textit{A. baumannii} isolate from Case 1 reduced from 4 to 1 mg/L. For Case 2, the tigecycline MIC reduced from 16 to 4 mg/L. The changes in other drug susceptibilities are shown in Table 1.

Discussion

This is the first report of bloodstream infection caused by tigecycline-non-susceptible \textit{A. baumannii} and raises important questions about the use of this drug in the setting of \textit{Acinetobacter} bacteraemia and the potential mechanism of resistance. Both patients reported herein developed \textit{Acinetobacter} bloodstream infection while receiving tigecycline. The bloodstream isolates from the two patients had elevated MICs of 4 and 16 mg/L, respectively. These MICs are above the susceptibility breakpoints for tigecycline defined by the US FDA or EUCAST for \textit{Enterobacteriaceae} (≤2 and ≤1 mg/L, respectively) and at this point, no interpretive criteria are available for non-fermentative bacteria such as \textit{Acinetobacter} spp from either organization. Neither CLSI nor BSAC have provided breakpoints for
Table 1. Susceptibility profiles of the two Acinetobacter baumannii clinical isolates before and after exposure to phenyl-arginine-β-naphthylamide (PAβN), an efflux pump inhibitor

<table>
<thead>
<tr>
<th>Strains</th>
<th>TGC</th>
<th>MIN</th>
<th>GEN</th>
<th>TOB</th>
<th>CIP</th>
<th>CHL</th>
<th>PIP</th>
<th>CAZ</th>
<th>FEP</th>
<th>IPM</th>
<th>MEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1 isolate</td>
<td>4.0</td>
<td>0.25</td>
<td>8.0</td>
<td>I</td>
<td>24 R</td>
<td>&gt;32 R</td>
<td>32 R</td>
<td>256 R</td>
<td>96 R</td>
<td>&gt;256 R</td>
<td>1.5</td>
</tr>
<tr>
<td>+PAβN</td>
<td>1.0</td>
<td>0.25</td>
<td>4.0</td>
<td>S</td>
<td>16 R</td>
<td>32 R</td>
<td>8.0</td>
<td>256 R</td>
<td>8.0</td>
<td>128 R</td>
<td>0.25</td>
</tr>
<tr>
<td>Case 2 isolate</td>
<td>16</td>
<td>2.0</td>
<td>128</td>
<td>R</td>
<td>16 R</td>
<td>&gt;32 R</td>
<td>32 R</td>
<td>48 I</td>
<td>192 R</td>
<td>&gt;256 R</td>
<td>1.5</td>
</tr>
<tr>
<td>+PAβN</td>
<td>4.0</td>
<td>1.0</td>
<td>16</td>
<td>R</td>
<td>6 I</td>
<td>32 R</td>
<td>8.0</td>
<td>8.0</td>
<td>8.0</td>
<td>16 I</td>
<td>0.125</td>
</tr>
</tbody>
</table>

S, susceptible; I, intermediate; R, resistant.
TGC, tigecycline; MIN, minocycline; GEN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; CHL, chloramphenicol; PIP, piperacillin; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem.

Tigecycline. Unfortunately, the initial A. baumannii isolate in Case 2 was not available for tigecycline susceptibility or clonality assessment to confirm the acquisition of resistance during tigecycline exposure. However, given the improvement in the patient’s wound infection, the prolonged time from initiation of tigecycline to the development of A. baumannii bacteraemia (16 days) and the presence of a foreign body, we feel that acquired resistance is most likely. Of interest, the time from initiation of tigecycline to A. baumannii bacteraemia for Case 1 was also prolonged (9 days).

Despite the ability of tigecycline to evade the highly specific, single-component tet-type efflux pumps, it is susceptible to the RND family of multidrug efflux pumps, including MexXY-OpRM found in Pseudomonas aeruginosa and AcrAB found in Proteus mirabilis, Escherichia coli and Klebsiella pneumoniae.67 RND-type efflux pumps have recently been described from Acinetobacter (AdeABC) and confer reduced susceptibility to aminoglycosides, fluoroquinolones, erythromycin, chloramphenicol and tetracyclines.7 Of interest, our A. baumannii isolates were multidrug-resistant; suggesting that such pumps may be responsible. To provide supporting evidence for this theory we assessed the tigecycline MICs of our isolates after exposure to the pump inhibitor, PAβN. For Case 1, the tigecycline MIC reduced from 4 to 1 mg/L and for Case 2, it reduced from 16 to 4 mg/L. Also, susceptibility results for other known substrates of the AdeABC pump reduced after PAβN exposure, including chloramphenicol and aminoglycosides. Such preliminary results suggest that multidrug efflux pumps may be important contributors to tigecycline non-susceptibility in Acinetobacter. Further work is in progress to characterize this mechanism.

Pharmacokinetic studies indicate that tigecycline, given in the regimen validated in clinical trials (100 mg loading dose followed by 50 mg every 12 h), achieves a mean (±SD) maximum serum steady-state concentration of 0.63 (±0.28) mg/L in hospitalized patients.8 The drug is distributed widely and undergoes rapid and extensive transfer from the bloodstream to the tissues, with levels far exceeding that of serum. The only pharmacodynamic study published thus far, using a murine model, indicates that tigecycline exhibits time-dependent antimicrobial activity and suggests that concentrations should be maintained above the MIC for 50–75% of the dosing interval.9 However, because of tigecycline’s relatively long half-life and post-antibiotic effect, the AUC/MIC ratio was also shown to be reasonably predictive of efficacy.9 Regardless, it would seem intuitive that if the drug is to be successfully employed for bloodstream infection, its concentration in the blood should exceed the MIC. Furthermore, tigecycline, as with other tetracycline derivatives, is bacteriostatic for most organisms, including A. baumannii.

In vitro studies reporting on tigecycline MICs in A. baumannii, have demonstrated that ~50% isolates had a tigecycline MIC of ≥1 mg/L, whereas up to 32% isolates had a tigecycline MIC of ≥2 mg/L.10 Such MICs are well above the mean peak serum concentration of tigecycline at recommended doses (0.63 mg/L) and therefore, raise serious concerns about the use of this agent for treating most bloodstream infections caused by A. baumannii. Of interest, pooled data from trials evaluating tigecycline in treating skin, soft tissue and intra-abdominal infections, showed equal clinical cure rates with the corresponding comparator in patients with concomitant bacteraemia.11,12 However, organism types were predominately those with predicted tigecycline MICs of <1 mg/L, such as staphylococci, streptococci and E. coli, with no cases of bacteraemia due to Acinetobacter spp. reported.

In conclusion, we present two cases of Acinetobacter bloodstream infection occurring in patients receiving tigecycline. The current pharmacokinetic and dynamic data of tigecycline do not support its use for bloodstream infection caused by organisms with MICs ≥1 mg/L. Given the propensity of Acinetobacter to acquire resistance to other antimicrobials, exposure to subtherapeutic levels of tigecycline for even short periods of time may promote the rapid emergence of tigecycline resistance. The observed reduction in tigecycline MICs with PAβN in our cases, suggests an efflux-based mechanism is at least partly responsible for tigecycline non-susceptibility and further work is ongoing to elucidate this mechanism.

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