In vitro activity of tigecycline against patient isolates collected during phase 3 clinical trials for hospital acquired pneumonia

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Abstract

The in vitro activity of tigecycline was evaluated against 819 baseline pathogens isolated from 383 patients enrolled in the phase 3 clinical trial investigating the efficacy of tigecycline in hospital acquired pneumonia (HAP). The trials were global, enrolling patients in 27 countries. Tigecycline was active against the most prevalent pathogens in HAP, including gram-positive and gram-negative strains (90% of MICs ≤2 μg/mL for the entire collection). The spectrum of activity of tigecycline included important pathogens such as Staphylococcus aureus (including methicillin-resistant S. aureus), Enterococcus faecalis, Streptococcus pneumoniae, Acinetobacter baumannii/calcoaceticus complex, Escherichia coli, Klebsiella pneumoniae, and Enterobacter cloacae. As reported previously, a few genera, such as Pseudomonas aeruginosa and the Proteaeae, were generally less susceptible to tigecycline by comparison to other gram-negative pathogens. The excellent in vitro, expanded broad-spectrum activity of tigecycline in the clinical isolates confirmed the potential utility of tigecycline for patient isolates associated with hospital acquired pneumonia infections.

Introduction

The glycylcycline class of antibiotics was developed by Wyeth in response to the threat of emerging antibiotic resistance throughout the world. Tigecycline, the first in the class glycylcycline, received market approvals for treatment of complicated skin and skin structure infections (cSSSI) and complicated intra-abdominal infections (cIAI) in 2005 and community acquired bacterial pneumonia (CABP) in 2008 (see Tygacil Label at http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021821s013s017s018lbl.pdf). Tigecycline binds to the 30S ribosomal subunit blocking access of amino-acyl tRNA molecules to the A site, and is not affected by tetracycline resistance mechanisms: efflux pumps and ribosomal protection. The expanded broad spectrum of activity of tigecycline includes a broad range of antibiotic-susceptible and -resistant gram-positive and gram-negative aerobes, anaerobes, and “atypical” bacteria.

Hospital acquired pneumonia (HAP) is second only to urinary tract infections as the most common nosocomial infection contracted, especially among patients admitted to the ICU. In critical care settings and following surgical treatment, nosocomial pneumonia is reported in approximately 20% of patients and mortality rates range from 20-70%. Methicillin-resistant Staphylococcus aureus (MRSA) as well as gram-negative pathogens – Acinetobacter spp., Escherichia coli, Pseudomonas aeruginosa – are predominant pathogens in HAP; in addition, antibiotic resistance rates are elevated in these organisms complicating therapeutic decision-making. To evaluate the safety and efficacy of tigecycline in treatment of HAP infections a randomized, double-blind trial was conducted with imipenem/cilastatin as the active comparator. This analysis was conducted in order to evaluate the susceptibility of the clinical isolates to tigecycline and selected comparator agents.

Materials and Methods

Clinical isolates

Baseline pathogens from all patients enrolled in the clinical trial were included in the analysis of susceptibility data. Site laboratories processed patient specimens and cultured bacterial pathogens according to local practices. Acute HAP was defined as pneumonia with onset of symptoms ≥48 hours after admission to an acute care hospital or chronic care facility (such as a skilled nursing home facility or rehabilitation unit), or <7 days after the subject was discharged from the hospital. The initial hospitalization must have been of ≥3 days duration. Subjects must have had the presence of a new or evolving infiltrate on chest X-ray and the chest X-ray must have been obtained ≥48 hours after the subject was admitted to the hospital or chronic care facility. Diagnosis required that the subjects have the presence of fever within 24 hours prior to randomization into the trial and leukocytosis or increased bands or leucopenia. In addition, subjects must have had at least two of the following: cough, dyspnea or tachypnea, pleuritic/inspiratory chest pain, auscultatory findings on pulmonary examination or rales and/or evidence of pulmonary consolidation, hypoxemia, purulent sputum or respiratory secretion or a change in sputum character occurring ≥48 hours after hospitalization, or respiratory failure requiring mechanical ventilation (in lieu of having two of the clinical signs and symptoms listed above). Respiratory tract specimens were obtained for Gram stain and culture at randomization. The majority of specimens submitted for culture were from: bronchoscopy, deep expectoration, or endotracheal aspiration; although it must be acknowledged that not all isolates described in the study were clinically relevant. Bacterial pathogens were sent to a central laboratory for identification and susceptibility testing. MICs were determined in Mueller-Hinton II broth (MHB); for streptococci MHB containing 5% lysed horse blood was used. MICs were determined using custom-prepared dehydrated microdilution panels (Trek Diagnostics, Westlake, OH, USA) and followed reference methodology as described by the CLSI. Cultures were grown in a 5% CO2-humidified incubator. The results of susceptibility testing were interpreted according to CLSI interpretive criteria.

Confirmation of extended spectrum β-lactamase

For those isolates of E. coli, Klebsiella pneumoniae or Proteus mirabilis resulting in a cephalosporin MIC of ≥2 μg/mL, confirmation of the presence of an extended spectrum β-lactamase (ESBL) was performed using Etest ESBL discs (Biomerieux, Durham, NC) in Mueller-Hinton II broth (MHB) at 35-37°C for 18-24 hours. Discs of 30μg cefotaxime, ceftazidime, aztreonam and clavulanate were used. An ESBL positive isolate was considered if all the following conditions were met: (1) an >2-fold difference in MICs between the presence and absence of clavulanate, (2) cefotaxime clavulanate MIC ≤4 μg/mL, and (3) cefotaxime MIC ≤32 μg/mL.

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Key words: tigecycline, clinical isolates, in vitro susceptibility, MIC, hospital acquired pneumonia.

Conflict of interest: all authors contributed significantly to the study. Study design was collaborative between HJ and PP, and all microbiology studies were conducted by PP. The molecular biology work was conducted by HJ and MT. The manuscript was written by HJ with editorial assistance from PP. All authors agree to the final draft of the manuscript as submitted.
PCR analysis of resistance determinants

Methicillin (S. aureus) and tetracycline resistance determinants (S. aureus, E. coli) were identified using diagnostic PCR assays as previously described. In addition, confirmed ESBL containing isolates were further examined by PCR to determine the class(es) of β-lactamase (e.g. TEM, SHV, CTX, OXA) encoded using protocols previously described.

Results

The most prevalent pathogens isolated from patients during the clinical trials (2004-2006) for HAP, including patients with ventilator associated pneumonia (VAP), are listed in Table 1. The distribution of pathogens was representative for the infection type and similar to reports from recent studies. A summary of the tigecycline susceptibility for the predominant baseline isolates obtained is presented in Table 2. The most prevalent pathogens isolated were Staphylococcus spp. (287 isolates) with S. aureus represented by 75 methicillin-resistant (MRSA) and 130 methicillin-sensitive (MSSA) isolates (Tables 1, 2). Acinetobacter baumannii/calcoaceticus complex was the most prevalent gram-negative pathogen isolated (82 baseline isolates), followed by E. coli (75 isolates), K. pneumonia (75 isolates), and P. aeruginosa (54 isolates) (Table 1, 2).

As shown in Table 2 and Supplementary Table, 92% of the MRSA isolates were susceptible to tigecycline (MIC<0.5 μg/mL). In the case of the MSSA isolates, 100% of the isolates were susceptible to tigecycline (MIC<0.25 μg/mL), and susceptibility rates for comparator agents were in excess of 91% with the exception of azithromycin (88%) and cefazidime (73%) in the VAP population. Twenty-two MRSA and five MSSA isolates were resistant to minocycline (MIC≥8 μg/mL); of these, 24 isolates encoded tet(M), two isolates encoded tet(K) and tet(M), and a single isolate encoded tet(K) alone as determined by PCR analysis as previously described (Supplementary Table and data not shown). In addition, 10 isolates were minocycline susceptible (MIC<4 μg/mL) and tetracycline resistant (MIC≥8 μg/mL); of these, four isolates encoded tet(M), five isolates encoded tet(K), and a single isolate encoded both determinants. All of the methicillin susceptible isolates of Staphylococcus epidermidis were fully susceptible to 0.5 μg/mL of tigecycline (MIC<0.5 μg/mL). Among the 38 isolates of MRSE there were three isolates with a tigecycline MIC of 1 μg/mL resulting in an overall susceptibility rate for these isolates of 92% (MIC<0.5 μg/mL). When considering all 287 strains of Staphylococcus spp., the tigecycline MIC<0.5 μg/mL.

Tigecycline had good activity against all 50 isolates of Enterococcus spp. collected (Table 2). The predominant species obtained was Enterococcus faecalis (44 isolates) and all of the isolates were susceptible to 0.25 μg/mL (FDA susceptible breakpoint). By contrast, the MIC<0.5 for both levofloxacin (>16 μg/mL) and minocycline (16 μg/mL) were at the resistant breakpoints for the E. faecalis isolates (Supplementary Table).

Tigecycline activity was determined against 27 isolates of Streptococcus pneumoniae that included two isolates that were penicillin intermediate and two isolates that were penicillin resistant according to the recently changed penicillin breakpoints for this organism. All of the isolates were susceptible to ≤0.12 μg/mL tigecycline.

Tigecycline showed good activity against gram-negative organisms of which A. baumannii/calcoaceticus complex was the predominant pathogen isolated (Supplementary Table). The MIC<0.5 for all of the comparator agents were above the respective resistance breakpoints except for imipenem for the non-VAP isolates (MIC<2 μg/mL). CLSI or FDA breakpoints for tigecycline have not been established for this organism.

For the 75 baseline patient isolates of E. coli, 100% were susceptible to 2 μg/mL tigecycline (MIC range 0.12-2 μg/mL) with an MIC<0.5 μg/mL (Supplementary Table). Thirteen (17%) of these isolates were multidrug resistant (MDR) strains showing resistance to cefazidime, levofloxacin, and tetracycline with MIC<64 μg/mL of >64 μg/mL, respectively. The E. coli collection included 48 tetracycline resistant (MIC≥8 μg/mL) strains, 26 of which were also resistant (MIC≥8 μg/mL) to minocycline. The tetracycline resistance determinants in these isolates were identified by PCR as previously described. Twenty-five of the minocycline resistant isolates were found to encode tet(B), with two isolates also encoding tet(A) and a single isolate also encoding tet(C). One minocycline resistant isolate encoded only tet(A). Twenty-two isolates were found to be susceptible to minocycline (MIC<4 μg/mL) and resistant to tetracycline (MIC≥8 μg/mL). All 22 isolates were found to encode tet(A), with four isolates also encoding tet(M) and two isolates also encoding tet(B). As previously shown, the presence of tetracycline-resistance determinants, specifically monospecific tetracycline efflux pumps, had no impact on tigecycline susceptibility of the isolates.

Twenty-one E. coli isolates were identified as encoding ESBLs owing to a cefazidime MIC ≥2 μg/mL and confirmed using E-test strips. As previously described, the class of β-lactamase responsible for the ESBL phenotype was deter-

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Table 1. Etiology of organisms cultured from patients with hospital acquired pneumonia.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Non-VAP</th>
<th>Non-VAP resistant isolates (%)</th>
<th>VAP</th>
<th>VAP resistant isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram-positive aerobes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>297 (30%)</td>
<td>101 (34%)</td>
<td>100 (12%)</td>
<td>42 (42%)</td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>147 (18%)</td>
<td>30 (34%)</td>
<td>38 (7%)</td>
<td>25 (43%)</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>37 (4.5%)</td>
<td>28 (76%)</td>
<td>12 (1.5%)</td>
<td>10 (3.9%)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>22 (2.7%)</td>
<td>19 (91%)</td>
<td>5 (0.6%)</td>
<td>3 (4.3%)</td>
</tr>
<tr>
<td>Staphylococcus haemolyticus</td>
<td>20 (2.4%)</td>
<td>16 (80%)</td>
<td>4 (0.5%)</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Enterococcus faecium</td>
<td>4 (0.5%)</td>
<td>2 (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram-negative aerobes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinetobacter baumannii/calcoaceticus complex</td>
<td>268 (33%)</td>
<td>27 (79%)</td>
<td>154 (18%)</td>
<td>48 (9%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>58 (7%)</td>
<td>12 (21%)</td>
<td>17 (2%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>61 (7%)</td>
<td>8 (13%)</td>
<td>14 (1.7%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>30 (3.6%)</td>
<td>4 (13%)</td>
<td>24 (3%)</td>
<td>7 (29%)</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>19 (2.3%)</td>
<td>3 (0.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>13 (1.5%)</td>
<td>9 (1.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella oxytoca</td>
<td>11 (1.3%)</td>
<td>2 (0.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>5 (0.6%)</td>
<td>7 (0.8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>6 (0.7%)</td>
<td>2 (33%)</td>
<td>6 (0.7%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>4 (0.5%)</td>
<td>1 (25%)</td>
<td>7 (0.8%)</td>
<td>2 (28%)</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>6 (0.7%)</td>
<td>1 (16%)</td>
<td>4 (0.5%)</td>
<td>1 (25%)</td>
</tr>
</tbody>
</table>
mined by PCR. Nineteen (90%) of the isolates were found to encode a βlactamase family enzyme with various combinations of βlactamases, βlactamase genes with fourteen isolates encoding the combination of blaCTX, blaTEM, and blaOXA genes. One isolate was found to carry both a blaTEM and blaOXA gene, whereas another isolate encoded an AmpC β-lactamase of the βlactam family. As previously described, E. coli encoding ESBLs are as susceptible as non-ESBL isolates to tigecycline.15

When tested against *K. pneumoniae*, tigecycline performed well with 96% of isolates susceptible and an MIC50 of 2 μg/mL for the 75 isolates tested (Table 2). Whereas in earlier studies *K. pneumoniae* had shown a tendency for elevated tigecycline MICs, only three isolates in the present study had an MIC of 4 μg/mL. Eight (11%) of the baseline isolates were MDR strains, resistant to a β-lactam and at least two other classes of agents, in this case levofloxacin and minocycline. The ESBL status of 31 isolates was confirmed (ceftazidime MIC ≥ 2 μg/mL and Etest positive) and the class of determinant responsible for the ESBL status identified by PCR.15 Twenty-four isolates encoded a blaTEM gene with 23 of the isolates encoding additional determinants in various combinations of the blaTEM, blaCTX, and blaOXA classes. In the case of blaCTX, 19 (79%) isolates encoded this determinant with 16 isolates encoding both the blaTEM and blaCTX determinants. Two isolates encoded AmpC β-lactamases of the βlactam family.

The 54 *P. aeruginosa* isolates collected during the clinical trial had MIC50s in the resistant range for all of the comparator agents for which a breakpoint has been established. The tigecycline MIC50 was 32 μg/mL, which is reflective of earlier studies demonstrating reduced susceptibility of this organism to tigecycline.16 *P. aeruginosa* expresses a family of multidrug efflux pumps (Mex pumps) that efficiently remove tigecycline from the cytoplasm, reducing its effectiveness. As would be expected, *P. aeruginosa* displayed low levels of susceptibility to ceftazidime (63-73%; non-VAP, VAP), levofloxacin (57-63%), and aminoglycosides (63-73%) (Supplementary Table).

The activity of tigecycline was evaluated against 22 *Enterobacter cloacae* isolates with the result that all isolates were susceptible to 1 μg/mL. The findings were similar for the small collection (11) of *Enterobacter aerogenes* isolates: 10 of the 11 isolates were susceptible to 2 μg/mL tigecycline with one isolate having an MIC = 8 μg/mL. Prior mechanistic studies revealed that a multidrug efflux system, AcrAB, is responsible for reduced tigecycline susceptibility in *Enterobacter spp.* against the small collection of 12 *P. mirabilis* isolates, tigecycline showed results in agreement with what has been seen in prior studies: MIC50 8 μg/mL.17 All of the *P. mirabilis* isolates were resistant to minocycline (MIC range 16 – >64 μg/mL). In addition, two baseline isolates were found to express the ESBL phenotype (ceftaxoxime MIC ≥ 2 μg/mL and Etest positive), and PCR analysis revealed that one of the isolates encoded blaTEM, blaOXA, and blaOXA family enzymes whereas the other isolate only encoded a blaTEM family enzyme.

### Discussion

Tigecycline was specifically designed to overcome the two classical tetracycline resistance mechanisms, ribosomal protection proteins and monospecific tetracycline efflux pumps, while maintaining the broad spectrum of activity of the tetracycline class.1 During preclinical development, tigecycline was shown to have activity against a broad range of clinically important pathogens, including MRSA, VRE, and antibiotic resistant gram-neg-
The treatment of patients with this disease.

Gram-negative pathogens isolated from non-VAP isolates: MIC 90 2 μg/mL. By comparison, for the VAP population, P. aeruginosa is only 5% (30 isolates) of isolates and, although the tigecycline MIC 90 for these isolates is 32 μg/mL, has less of an impact on the MIC 90 for the 566 non-VAP isolates: MIC 90 2 μg/mL. In the case of A. calcoaceticus/baumannii complex, the imipenem MIC 90 was 2 μg/mL for the non-VAP population and 32 μg/mL for the VAP population with 94% and 77% corresponding imipenem susceptibility. Tigecycline was the only agent tested with good activity (MIC 90 2 μg/mL) against the A. calcoaceticus/baumannii complex isolates from VAP patients. Tigecycline has been shown to be safe and effective in double-blind, multicenter, global clinical trials for cSSSI, cIAI, and, most recently, CAPB (see Tygacil label at http://www.accessdata.fda.gov/drugsatfda_docs/label/2009/021821s013s017s018lbl.pdf). In summary, the in vitro activity of tigecycline against a broad spectrum of gram-positive and gram-negative pathogens isolated from patients enrolled in phase 3 clinical trials conducted worldwide for HAP showed an excellent susceptibility profile and suggests utility in the treatment of patients with this disease.

References