Pharmacodynamic Profile of Tigecycline against Methicillin-Resistant Staphylococcus aureus in an Experimental Pneumonia Model

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Tigecycline (TGC) is an extended-spectrum antibiotic with activity against Staphylococcus aureus, including methicillin ( meticillin)-resistant S. aureus strains, which are well-recognized pathogens in nosocomial pneumonia. The objective of this study was to characterize the exposure-response relationship for TGC against S. aureus in an immunocompromised BALB/c murine pneumonia model. Six S. aureus isolates were studied, and the TGC MICs for those isolates ranged from 0.125 to 0.5 mg/liter. The pharmacokinetics (PK) of TGC in serum and bronchoalveolar lavage (BAL) fluid were evaluated, as was the level of protein binding of the compound in this murine species. Administration of TGC at 1.56 to 150 mg/kg of body weight/day in single or two to three divided doses was used in the efficacy studies. TGC displayed linear PK and had a mean half-life of 10.9 ± 2.5 h. Efficacy was highly correlated with the area under the free concentration-time curve (AUC)/MIC (r² = 0.93). The 80% and 50% effective exposure indexes and the stasis exposure index were similar between the isolates (means ± standard deviations, 3.04 ± 1.12, 1.84 ± 1.3, and 1.9 ± 1.5, respectively). Maximal efficacy was predicted at a 2.85-log10-CFU reduction. TGC appeared to accumulate in the interstitial space, as the ratios of the fAUC from 0 to 8 h of epithelial lining fluid to plasma were 7.02, 15.11, and 23.95 for doses of 12.5, 25, and 50 mg/kg, respectively. TGC was highly effective in this murine pneumonia model. In light of current MIC distributions, the fAUC/MIC targets that we defined against S. aureus are readily achievable in humans given conventional doses of TGC.

Staphylococcus aureus has long been recognized as an important cause of infection, and the emergence of S. aureus strains with the methicillin ( meticillin)-resistant phenotype (methicillin-resistant S. aureus [MRSA]) has further complicated management. Both community-acquired MRSA (CA-MRSA) and hospital-acquired MRSA (HA-MRSA) strains have been associated with severe and difficult-to-treat infections. While the most common site of staphylococcal infection is the skin and skin structures, the surveillance of 8,792 invasive MRSA cases in the United States showed that pneumonia is the second most common clinical manifestation of MRSA infection (13.3% overall; 14% of the strains were CA-MRSA and 28% were HA-MRSA) (9).

Tigecycline (TGC) is a broad-spectrum glycycline with efficacy against gram-positive and gram-negative bacteria, including drug-resistant bacteria such as MRSA. The MIC₉₀ of TGC against meticillin-susceptible S. aureus (MSSA) and MRSA strains is reported to be ≤0.25 mg/liter (6). TGC is approved by the FDA for use for the treatment of complicated skin and skin structure infections and complicated intra-abdominal infections. Pneumonia is an important clinical manifestation of infection with drug-resistant bacteria; therefore, many in vivo and in vitro studies of TGC for the treatment of lower respiratory infection are ongoing (data available at http://www.clinicaltrialsearch.org /tigecycline_versus_imipenem_cilastatin_for_the_treatment_of


Previously, we demonstrated the efficacy of TGC against Acinetobacter spp. in a murine pneumonia model (10) and against S. aureus in a murine thigh infection model (3). We also found that TGC penetrated well into lung tissue, as displayed by high concentrations in bronchoalveolar (BAL) fluid (4). In the present study, we aimed to explore the exposure-response relationship for TGC against S. aureus in an immunocompromised BALB/c murine pneumonia model.

MATERIALS AND METHODS

Test antimicrobial agents. Standard analytical-grade TGC (lot RB5603; expiration date, September 2009; Wyeth, Madison, NJ) was used for all in vitro and in vivo experiments. For all animal studies, the TGC powder was weighed and reconstituted with normal saline to achieve the desired concentrations immediately prior to each experiment. The solution was used within 30 min of reconstitution.

Microorganisms. Six isolates of S. aureus (ATCC 29213, two HA-MRSA isolates, and three CA-MRSA isolates) were utilized in the study. The MIC of TGC for all organisms was determined in triplicate by the microdilution method, according to the guidelines of the CLSI (1). The modal MIC was utilized in all pharmacodynamic assessments.

Lung infection (pneumonia) model. Specific-pathogen-free, female BALB/c mice were obtained from Harlan Sprague-Dawley, Inc. (Indianapolis, IN), and were utilized throughout these experiments. This study was reviewed and approved by the Hartford Hospital Institutional Animal Care and Use Committee. The animals were maintained and used in accordance with the recommendations of the National Research Council and were provided food and water ad libitum. The mice were rendered transiently neutropenic by intraperitoneal injections of cyclophosphamide at 250 and 100 mg/kg of body weight at 4 days and 1 day prior to inoculation, respectively.

The S. aureus isolates were frozen at −80°C in skim milk and were subcultured twice onto blood agar medium. For inoculation, a suspension of the test organ-
The in vitro potencies of TGC and the other compounds tested against the *S. aureus* isolates used in the pharmacodynamic studies are displayed in Table 1. The TGC MICs for the *S. aureus* isolates ranged from 0.125 to 0.5 mg/liter.

Figure 1 displays the total serum drug concentration-time profiles of TGC after various single subcutaneous doses; the values of the pharmacokinetic parameters are summarized in Table 2. The range of the total AUC from 0 to 24 h (AUC_{0-24}) was 10.4 to 103.5 mg·h/liter for the dosing regimens used.

**TABLE 1. MICs of TGC and other clinically utilized compounds for the *Staphylococcus aureus* isolates studied**

<table>
<thead>
<tr>
<th>Compound</th>
<th>MSSA</th>
<th>CA-MRSA</th>
<th>CA-MRSA</th>
<th>CA-MRSA</th>
<th>HA-MRSA</th>
<th>HA-MRSA</th>
<th>HA-MRSA</th>
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</thead>
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<tr>
<td>ATCC 29213</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>VAN</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ERY</td>
<td>0.125 &gt;32 &gt;32 &gt;32 &gt;32 &gt;32 &gt;32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CLI</td>
<td>0.125 &gt;16 =0.5 0.125 0.25 0.125 0.25 0.125</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVX</td>
<td>0.25 0.5 0.5 0.5 0.25 0.25 0.25 0.25</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>TMP-SXT</td>
<td>0.125 0.25 &lt;0.5 0.125 0.06 0.06 0.06 0.06</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>DOX</td>
<td>0.5 0.5 0.5 0.5 0.5 0.5 0.5 0.5</td>
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<td></td>
<td></td>
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<tr>
<td>TGCa</td>
<td>0.25 0.25 0.125 0.25 0.25 0.25 0.25 0.25</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

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*a* L2D, linezolid; VAN, vancomycin; ERY, erythromycin; CLI, clindamycin; LVX, levofloxacin; TMP-SXT, trimethoprim-sulfamethoxazole; DOX, doxycycline.

The FDA-approved breakpoint of TGC for susceptibility is an MIC of ≤0.5 mg/liter.

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**FIG. 1. Total concentrations of TGC after various single subcutaneous doses in *S. aureus*-infected mice.**

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**RESULTS**

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Given the concentration-dependent protein binding noted previously (4) and herein, a sigmoidal maximum-effect (EC_{50}) model explaining the relationship between the level of protein binding and the TGC concentration was constructed. Using this model, we calculated the free-drug exposures for each of the doses used in the bacterial density studies by uniformly correcting the concentration-time profile with the percentage of free drug noted at the peak serum drug concentration for each given dose (3).

**Pharmacodynamic analysis.** For each *S. aureus* isolate, a dose-response curve was constructed by plotting the change in the log_{10} CFU versus the ratio of the area under the free AUC to the MIC (fAUC/MIC) by using a sigmoidal maximum-effect model (WinNonlin, version 5.0.1; Pharsight, Mountain View, CA). This allowed determination of the effective exposure indices (EI) i.e., the exposure value required to produce 80% of the maximal effect [EI_{80}], the exposure value required to produce 50% of the maximal effect [EI_{50}], and the exposure value required to produce stasis. Only fAUC/MIC was measured in this study, as this pharmacodynamic parameter has previously been determined to be the most closely correlated to efficacy in other in vivo studies with this compound (3, 14).

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The percent protein binding with each concentration prepared was calculated by using the following equation: 

$$ [S - S_{\text{CB}}] \times 100, \text{ where } S \text{ is the TGC concentration in the initial serum solutions and } S_{\text{CB}} \text{ is the concentration in the ultrafiltrate.}$$

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The pharmacodynamic parameters for determination of the numbers of CFU. For the purposes of these studies, efficacy (the change in bacterial density) was calculated as the change in the log_{10} CFU versus the ratio of the area under the free AUC to the MIC (fAUC/MIC) by using a sigmoidal maximum-effect model (WinNonlin, version 5.0.1; Pharsight, Mountain View, CA). This allowed determination of the effective exposure indices (EI); i.e., the exposure value required to produce 80% of the maximal effect [EI_{80}], the exposure value required to produce 50% of the maximal effect [EI_{50}], and the exposure value required to produce stasis. Only fAUC/MIC was measured in this study, as this pharmacodynamic parameter has previously been determined to be the most closely correlated to efficacy in other in vivo studies with this compound (3, 14).

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**RESULTS**

The in vitro potencies of TGC and the other compounds tested against the *S. aureus* isolates used in the pharmacodynamic studies are displayed in Table 1. The TGC MICs for the *S. aureus* isolates ranged from 0.125 to 0.5 mg/liter.

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for each dose and at all time points. The AUC0–8s in ELF after than their corresponding concentrations in the serum samples regimens used. The mean correlation coefficient (aureus fitted curves was 0.93 (range, 0.802 to 0.991) (Table 3).

The TGC concentrations in the ELF samples were greater than their corresponding concentrations in the serum samples for each dose and at all time points. The AUC0–8 in ELF after doses of 12.5, 25, and 50 mg/kg were 11.0, 20.4, and 41.9 mg · h/liter, respectively; and the penetration ratios, which consisted of the AUC0–8 for ELF to the AUC0–8 for plasma, were 7.02, 15.11, and 23.95, respectively.

The mean starting (0-h) bacterial density in the lungs of the control mice was 7.69 × 10^5 CFU. The bacterial density increased 1.76 log CFU, on average (range, 1.03 to 2.3 log CFU), in the untreated control group at 24 h. The observed mean maximal reduction in the numbers of CFU in the TGC-treated animals after 24 h of exposure was 2.11 log10 CFU (range, 1.78 to 2.39 log10 CFU). This value was very similar to the mean maximal reduction in the numbers of CFU in the TGC-treated mice. When these results are compared with those of our previous study (3), in which we used the same staphylococcal isolates in a murine thigh infection model, the maximal reductions noted in the pneumonia model (1.9 log CFU), while the starting inocula and the growth after 24 h in the untreated mice were very similar. Moreover, the effective EIs required in the pneumonia model (mean EI_80, EI_50, and stasis values being 3.04, 1.84, and 1.88, respectively).

### DISCUSSION

Pneumonia is the second most common clinical manifestation of S. aureus and invasive MRSA infections (9). The appropriate antimicrobial agent for the treatment of such infections should possess not only activity against this organism but also the ability to penetrate into the site of infection. In vivo data describing the efficacy of TGC for the treatment of various staphylococcal infections exist (3, 8, 12, 18). In addition, data describing the efficacy of TGC against nonstaphylococcal pneumonia are available (10, 17). Taken collectively, it seems reasonable to suggest that TGC could be an option for the treatment of staphylococcal pneumonia. We explored the pharmacodynamic profile of TGC against S. aureus in a murine model of pneumonia as a bridge to the treatment of pneumonia in humans.

We found TGC to be highly active against the six S. aureus isolates tested in this pneumonia model. This appears to be consistent with the findings presented in a lone case report of the clinical efficacy of TGC against MRSA pneumonia complicated by sepsis in a 57-year-old man who had recently undergone liver transplantation and who was receiving immunosuppressive agents. The patient was successfully treated with TGC after he failed both vancomycin and linezolid treatments (15).

On the basis of our E_max model, we described an average maximal reduction in the numbers of CFU of 2.85 log units at 24 h after inoculation in TGC-treated mice. When these results are compared with those of our previous study (3), in which we used the same staphylococcal isolates in a murine thigh infection model, the maximal reductions noted in the pneumonia model were greater than those noted in the thigh infection model (1.9 log CFU), while the starting inocula and the growth after 24 h in the untreated mice were very similar. Moreover, the effective EIs required in the pneumonia model (mean EI_80, EI_50, and stasis values, 3.0, 1.8, and 1.9, respectively) were
lower than those required in the thigh infected mice (5.1, 2.2, and 2.4, respectively) and likely reflect enhanced penetration into the lung.

We demonstrated TGC concentrations in ELF to be greater than those in serum at each sampling time point. Moreover, we noted that the AUC for ELF ranged from 7 to 24 times the fAUC for serum, depending on the dose. This observation was also made in a previously conducted bronchopulmonary pharmacokinetic analysis by our group, in which AUC-based TGC penetration ratios in mice with lung infections ranged from 12.9 to 23.3 (5). While the previously noted thigh infection study did not describe the level of TGC penetration into thigh tissue, a blister fluid penetration study with healthy volunteers found decreased levels of exposure for tissue relative to those for blood (16). This comparison seems to be reasonable, given the similarities in ELF penetration noted between our murine data with the lowest dose (12.5 mg/kg) and the ELF penetration reported by Conte et al. in the study with human volunteers (2). In that study, volunteers given standard doses of TGC were found to have an ELF penetration ratio of 1.31 when the total drug AUC in serum was used. After correction for 79% protein binding (13), the ratio of the AUC for ELF to the fAUC for serum was 6.28. While the correlation between ELF TGC concentrations and clinical efficacy is unclear, in our murine model we demonstrated a high degree of ELF penetration to be consistent with enhanced bacterial killing.

In light of the current distributions of TGC MICs against S. aureus (MIC90, 0.12 mg/ml for MSSA strains and 0.12 to 0.25 mg/ml for MRSA strains) (6), the fAUC/MIC targets that we defined are readily achievable in humans given conventional (MIC90, 0.12 mg/ml for MSSA strains and 0.12 to 0.25 mg/ml for MRSA strains) (6).

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REFERENCES


