In the *Staphylococcus aureus* neutropenic murine thigh-infection model, the ratio of the free area under the 24-hour concentration-time curve to the minimum inhibitory concentration (fAUC/MIC) was found to be the pharmacodynamic index most closely linked to bacterial effect, with a ratio of approximately 50 producing a static effect. Further work was undertaken in neutropenic versus non-neutropenic animals. The presence of granulocytes increased the activity of tedizolid considerably, 25-fold on average, and maximal effect was achieved at an exposure equivalent to approximately 200 mg tedizolid phosphate per day in humans (dosing regimen used in phase 2 and 3 clinical trials). The fAUC/MIC was also found to be the pharmacodynamically linked variable in the *S. aureus* neutropenic murine pneumonia model; the fAUC/MIC ratio required for a static effect was approximately 20. Pharmacokinetic (PK) data demonstrate that tedizolid penetrates well into the epithelial lining fluid (ELF) of the lung. Data from the pneumonia infection model and ELF penetration PK study support exploring its use in pneumonia.

Antibiotic resistance among gram-positive pathogens remains a critical public health concern [1–4]. The main clinical problems with gram-positive resistance are due to methillin-resistant *Streptococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) [5–9]. Vancomycin is still considered the gold standard for treating most resistant gram-positive organisms, but there is a growing sentiment that its utility as a first-line agent is diminishing [10]. Over the past 10 years, a number of new agents with high in vitro activity against antibiotic-resistant gram-positive organisms has been added to our armamentarium. These agents have been viewed as alternatives for vancomycin in certain situations [11]. While these new agents have largely maintained high in vitro activity, it is not surprising that resistance to these newer agents is beginning to emerge [5, 7, 9, 12–15]. Despite our best efforts, antibiotic resistance is an inevitable consequence of antibiotic use. Truly, the only way to stay in front of the resistance curve is to practice “state-of-the-art” infection control techniques, use antibiotics prudently, and bring new agents to market.

To ensure the longevity of new antimicrobials, it is critical to develop them for “the right patient, at the right dose, for the right duration.” Inadequate dose selection is one of the most common reasons that drug development programs fail to achieve New Drug Application (NDA) approval. In the realm of antimicrobials, this is most often due to selection of a drug dose that is too low [16, 17]. Use of pharmacokinetic/pharmacodynamic (PK/PD) systems analyses in the dose-selection process can ameliorate this risk and improve the likelihood of selecting an effective dose, thereby increasing the likelihood of a successful NDA. In particular, valuable information for dose selection for phase 2 and phase 3 clinical studies can be gained from preclinical PK/PD infection models such as the murine thigh- or pneumonia-infection models. PK/PD infection model
studies have been shown to be effective in identifying the drug exposure targets (ie, PK/PD indices) that are most closely associated with therapeutic outcomes of interest in clinical studies for a given antimicrobial agent [16, 17]. For acute bacterial skin and skin structure infections (ABSSSIs), the exposure target associated with a 0 (static effect) - 1-log reduction in colony counts from baseline in the murine thigh-infection model has been shown to be predictive of efficacy in subsequent ABSSSI phase 3 trials [16, 17]. With knowledge of the exposure targets necessary for optimal clinical response, Monte Carlo simulation can be used to integrate PK, PD, and microbiologic data and inform dose selection for phase 2 and phase 3 studies [16–23].

Tedizolid phosphate (TR-701, formerly torezolid phosphate) is a pro-drug of the active moiety tedizolid (TR-700, formerly torezolid) and is a novel oxazolidinone that is currently being investigated for the treatment of ABSSSIs. Relative to the only commercially available oxazolidinone, linezolid, tedizolid has demonstrated greater in vitro activity against a number of key gram-positive pathogens, including MRSA, VRE, and PRSP [24–30]. Prior to initiating phase 3 trials, a number of PK/PD systems analyses were conducted to guide the dose-selection process [31–38]. The purpose of this review is to provide an overview of the S. aureus murine thigh-infection model studies that were used to inform the dose-selection process for the phase 3 ABSSSI trials [31, 34]. As a secondary objective, this article briefly details the PK/PD studies performed to date that provide critical insights into the potential utility of tedizolid in the setting of pneumonia [32, 33].

PHARMACODYNAMICS OF TEDIZOLID PHOSPHATE IN THE S. AUREUS MURINE THIGH-INFECTION MODEL

The pharmacodynamics of tedizolid phosphate in the S. aureus neutropenic murine thigh-infection model, a good surrogate for efficacy in ABSSSIs, were first delineated by Louie et al [34]. Results of the dose fractionation study are shown in Figure 1. As mentioned above, results from these preclinical PK/PD infection models provide critical information for the logical development of an antimicrobial. In particular, the pharmacodynamically linked index can be delineated from these models, and this information can be used to identify the doses and dosing intervals for consideration in subsequent clinical trials [16, 17]. A MRSA strain (ATCC 33591) was used primarily for 24- and 48-hour dose-ranging and dose-fractionation studies, and comparative dose ranging was also conducted with a community-associated MRSA strain (strain 6-8548A) and a methicillin-susceptible S. aureus strain (MSSA; ATCC 29213). As seen in Figure 1, doses of tedizolid phosphate were administered once daily, half the daily dose every 12 hours, and one-fourth the daily dose every 6 hours. The pharmacokinetic profile for the drug in infected animals was ascertained, allowing transformation of dosing profiles into 3 pharmacodynamic indices: free maximal concentration to minimum inhibitory concentration (MIC) ratio ($f_{Cmax/MIC}$), free area under (concentration-time) curve to MIC ratio ($f_{AUC/MIC}$), and free drug time above the MIC ratio ($f_{T > MIC}$)

![Figure 1](https://example.com/figure1.png)

Figure 1. Dose fractionation study evaluating the relationship between the free maximal concentration to minimum inhibitory concentration (MIC) ratio ($f_{Cmax/MIC}$ ratio) (A), free area under (concentration-time) curve to MIC ratio ($f_{AUC/MIC}$ ratio) (B), and free drug time above the MIC ratio ($f_{T > MIC}$ ratio) (C) for tedizolid phosphate for methicillin-resistant Staphylococcus aureus strain ATCC 33591 with bacterial density (log CFU/g) in mouse thigh muscle. Error bars represent 1 standard deviation. Q 24 hours, every 24 hours. Reproduced from: Louie A, Liu W, Kulawy R, Drusano GL. In vivo pharmacodynamics of torezolid phosphate (TR-701), a new oxazolidinone antibiotic, against methicillin-susceptible and methicillin-resistant Staphylococcus aureus strains in a mouse thigh infection model. Antimicrob Agents Chemother 2011; 55: 3453–3460, with permission from the American Society of Microbiology.
free drug time above the MIC ratio ($fT > MIC$), and free area under (concentration-time) curve to MIC ratio ($fAUC/MIC$). Of these 3 indices, the $fAUC/MIC$ ratio explained more of the variance in the relationship than did the other candidate pharmacodynamic indices. The $fAUC/MIC$ ratio required to produce a static effect or 1-log CFU/g decrease in these studies was approximately 50 and 106, respectively, at 24 hours for ATCC 33591. In the 2 other cell lines, similar results were observed for stasis, with slight reductions in doses required to produce a decrease of 1 log CFU/g. Cell kills were also compared by dosing schedule in an analysis of variance (ANOVA), with no significant differences seen, again indicating that $fAUC/MIC$ is the most appropriate pharmacodynamically linked index to guide dose selection (ANOVA not shown) [34]. The animals in the study by Louie and colleagues were rendered granulocytopenic by cyclophosphamide [34]. While this is standard procedure, the impact of granulocytes on the ability of an antimicrobial agent to kill target microorganisms may alter the effectiveness of the drug. As demonstrated by Andes and Craig, the absence of granulocytes approximately doubles the exposure to the quinolone gatifloxacin required to achieve an equivalent microbiological effect in the mouse thigh model [39]. Because the target population was nonneutropenic patients with ABSSSI, further work was undertaken to study tedizolid in neutropenic vs nonneutropenic animals [31].

For the comparative neutropenic vs nonneutropenic murine thigh-infection model studies, the same strain of MRSA (ATCC 33591) used in the previous study was employed (Figure 2) [31]. All doses were administered once daily, based on the results of the prior dose-ranging study. There were 2 striking findings from this investigation. The first is the dramatic impact of granulocyte presence on the antimicrobial effect of tedizolid. Dose-response enhancement was approximately 16-, 25-, and 35-fold (on average, 25-fold) greater for nonneutropenic vs neutropenic at the 24-, 48-, and 72-hour time points, respectively. The second striking finding was the impact of therapy duration on microbiological kill. In the nonneutropenic group, longer durations of therapy were associated with greater cell kill. There was a clear separation in extent of bacterial killing among the different doses of tedizolid phosphate administered for 24 hours vs 48 hours vs 72 hours. Indeed, the separation for any specific dose among the times of therapy is on the order of 2- to 3-log10 CFU/g. It should also be noted that the near maximal effect in the nonneutropenic group, irrespective of duration of therapy, was achieved at the lowest dose tested (an exposure of approximately 200 mg tedizolid phosphate per day in humans) [31]. Although the $fAUC/MIC$ ratio was not sought in this study, it is reasonable to assume that the target would be lower in immunocompetent animals. Assuming no PK differences in neutropenic vs nonneutropenic mice and accounting for the minimum enhancement of 16-fold in the 24-hour results, a $fAUC/MIC$ ratio of approximately 3 can be calculated from the $fAUC/MIC$ ratio of 50 from neutropenic mice [34].

**INTRACELLULAR ACCUMULATION OF TEDIZOLID**

The definitive reason for this remarkable increase in effect by granulocyte presence is unclear. However, intracellular accumulation of tedizolid may have a role in explaining the impact of granulocytes. Lemaire et al demonstrated that tedizolid accumulates...
more rapidly than linezolid in cultured THP-1 macrophages [40]. Figure 3 demonstrates that the intracellular concentration of tedizolid is at least 10-fold higher than the extracellular concentration, while the intracellular concentration of linezolid approximates the extracellular concentration at the end of the observation period [40]. However, it should be noted that Lemaire and colleagues investigated antimicrobial effects in THP-1 macrophages, not in granulocytes. Granulocyte penetration and alteration of the ability of granulocytes to kill target pathogens, particularly S. aureus, warrant further study.

DOSE SELECTION IN PHASE 2 AND PHASE 3 ABSSSI CLINICAL STUDIES

The preclinical evaluations described above were tested in a phase 2 complicated SSSI (cSSSI) clinical trial [41]. Three doses of tedizolid phosphate—200 mg, 300 mg and 400 mg—were tested in a randomized, double-blind, parallel-group study. Some of the results are displayed in Table 1. For all study subjects, there were no statistically significant differences seen between any of the tested doses regardless of lesion type, lesion size, and severity of infection. This result was true in both the modified intent-to-treat and the clinically evaluable populations, and it was seen at both end-of-therapy (EOT) and test-of-cure (TOC) visit (7 to 14 days post treatment) endpoints [41]. Since all doses were administered on a daily basis, the clinical trial validates the preclinical findings that support once-daily dosing, as well as the dose choice based on the non-neutropenic infection model studies.

Based on findings from the phase 2 cSSSI clinical trial, oral tedizolid phosphate 200 mg daily was compared to linezolid 600 mg twice daily in a phase 3 randomized, double-blind, dose-ranging study evaluating the safety, tolerability, population pharmacokinetics, and efficacy of oral torezolid phosphate in patients with complicated skin and skin structure infections. Antimicrob Agents Chemother 2011; 55:883–92. Copyright© 2011, American Society for Microbiology. All rights reserved. Reproduced with permission. Abbreviations: CE, clinically evaluable; EOT, end of therapy; MITT, modified intent-to-treat; TOC, test-of-cure.

PHARMACODYNAMICS OF TEDIZOLID IN NEUTROPENIC S. AUREUS PNEUMONIA MODEL

Lepak and colleagues recently characterized the pharmacodynamics of tedizolid phosphate relative to linezolid against both

![Figure 3. Accumulation of linezolid and tedizolid in THP-1 macrophages (extracellular concentration, 250 mg/L). Uptake kinetics: the ordinate (Cc/Ce) shows the apparent cellular to extracellular concentration ratio (+ standard deviation; n = 3). Reproduced from: Lemaire S, Van Bambeke F, Appelbaum PC, Tulkens PM. Cellular pharmacokinetics and intracellular activity of torezolid (TR-700): studies with human macrophage (THP-1) and endothelial (HUVEC) cell lines. J Antimicrob Chemother 2009; 64: 1035–1043, with permission from Oxford University Press.](https://academic.oup.com/cid/article-abstract/58/suppl_1/S28/507739/16285973 by guest on 19 March 2019)

Table 1. Clinical Cure Rates With Tedizolid Phosphate at Test-of-Cure in Patients With Severe Complicated Skin and Skin Structure Infections

<table>
<thead>
<tr>
<th>Population and Visit</th>
<th>Tedizolid Phosphate Dose (No. of Patients Cured/Total No. of Patients in Group [%])</th>
</tr>
</thead>
<tbody>
<tr>
<td>MITT</td>
<td></td>
</tr>
<tr>
<td>EOT</td>
<td>24/26 (92.3)</td>
</tr>
<tr>
<td>TOC</td>
<td>23/26 (88.5)</td>
</tr>
<tr>
<td>CE</td>
<td></td>
</tr>
<tr>
<td>EOT</td>
<td>23/24 (95.8)</td>
</tr>
<tr>
<td>TOC</td>
<td>22/23 (95.7)</td>
</tr>
</tbody>
</table>

Severe complicated skin and skin structure infection is defined as the presence of systemic signs of infection or adjacent lymphadenopathy associated with lesions of ≥10 cm. Test-of-cure visit was 7 to 14 days post treatment. Reproduced from: Prokocimer P, Bien P, Surber J, et al. Phase 2, randomized, double-blind, dose-ranging study evaluating the safety, tolerability, population pharmacokinetics, and efficacy of oral torezolid phosphate in patients with complicated skin and skin structure infections. Antimicrob Agents Chemother 2011; 55:883–92. Copyright© 2011, American Society for Microbiology. All rights reserved. Reproduced with permission. Abbreviations: CE, clinically evaluable; EOT, end of therapy; MITT, modified intent-to-treat; TOC, test-of-cure.
Results illustrating the relationship between the 24-hour \( \text{fAUC/MIC} \) plasma value and antimicrobial effect as measured by change in \( \log_{10} \) CFU/lungs for linezolid and tedizolid are shown in Figure 4. Overall, the mean 24-hour plasma \( \text{fAUC/MIC} \) values needed for a static effect were similar for tedizolid and linezolid (20 vs 19, respectively). For a 1-log kill reduction in bacterial burden in lung tissue, an approximate doubling of exposure was needed for both tedizolid and linezolid (mean 24-hour plasma \( \text{fAUC/MIC} \) of 34.6 for tedizolid vs 46.1 for linezolid). Of note, the plasma 24-hour \( \text{fAUC/MIC} \) values associated with net stasis and 1-log kill were not significantly different between MSSA and MRSA isolates for tedizolid [33].

PULMONARY DISPOSITION OF TEDIZOLID IN HEALTHY VOLUNTEERS

To put the findings of Lepak et al [33] into proper perspective, it is important to consider the concentrations achieved in the plasma and the epithelial lining fluid (ELF) of the lungs. The best data to date on the plasma and pulmonary dispositions of oral tedizolid 200 mg daily comes from a study of noninfected, healthy volunteers by Housman et al [32]. Results detailing ELF penetration and \( \text{AUC}_{0-24} \) values in plasma and ELF after 9999-subject Monte Carlo simulation are shown in Table 2. The median \( \text{fAUC}_{0-24} \) in plasma was 2.6 and the 5th to 95th percentile ranged from 1.7 to 3.9 [32]. Interestingly, higher

<table>
<thead>
<tr>
<th>Calculation</th>
<th>Penetration Ratio</th>
<th>Free Plasma(^a)</th>
<th>Epithelial Lining Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>39.7</td>
<td>2.67</td>
<td>106.0</td>
</tr>
<tr>
<td>Median</td>
<td>36.3</td>
<td>2.59</td>
<td>93.9</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>82.3</td>
<td>0.68</td>
<td>55.9</td>
</tr>
<tr>
<td>Percentile of the distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5(^{th})</td>
<td>23.9</td>
<td>1.71</td>
<td>40.9</td>
</tr>
<tr>
<td>10(^{th})</td>
<td>26.3</td>
<td>1.87</td>
<td>49.3</td>
</tr>
<tr>
<td>25(^{th})</td>
<td>30.6</td>
<td>2.19</td>
<td>66.9</td>
</tr>
<tr>
<td>50(^{th})</td>
<td>36.3</td>
<td>2.59</td>
<td>93.9</td>
</tr>
<tr>
<td>75(^{th})</td>
<td>42.8</td>
<td>3.07</td>
<td>131.4</td>
</tr>
<tr>
<td>90(^{th})</td>
<td>49.7</td>
<td>3.57</td>
<td>177.7</td>
</tr>
<tr>
<td>95(^{th})</td>
<td>54.6</td>
<td>3.90</td>
<td>213.0</td>
</tr>
</tbody>
</table>

\(^a\) \text{AUC}_{0-24}, \text{area under the 24-hour concentration-time curve.}

\(^b\) The plasma AUC is corrected for mean protein binding (89.4%).

AUC₀₋₂₄ₕₒᵤᵣ were observed in ELF relative to plasma (Table 2) [32]. On average, AUC₀₋₂₄ₕₒᵤᵣ were 40-fold higher in ELF relative to free plasma concentrations; the median fAUC₀₋₂₄ₕₒᵤᵣ in ELF was 93.9 and the 5th to 95th percentile ranged from 40.9 to 213.0. Of note, plasma concentrations were corrected for 89.4% protein binding when generating the penetration ratio; ELF concentrations were not corrected for protein binding since protein binding of tedizolid in ELF is unknown. Even without adjusting for protein binding, the AUC₀₋₂₄ₕₒᵤᵣ are still 4- to 5-fold higher in ELF relative to plasma [32]. The exact mechanism for higher concentrations of tedizolid in ELF is unclear, but it may be a function of passive diffusion across the alveolar capillary wall, active transport by macrophages or other cells, active transport by pumps, or additional undefined mechanisms [32, 44]. Further study is needed to elucidate the mechanism, but the robust penetration into ELF supports exploration of the use of tedizolid phosphate in the setting of pneumonia.

CONCLUSION

Tremendous strides have been made in our understanding of the relationship between antimicrobial exposure and effect over the past quarter century. The exposure profile associated with a high probability of a successful outcome has been identified for a number of antimicrobials, leading to improvements in drug dosing in clinical practice. For new agents seeking regulatory approval, PK/PD systems analyses are also being used in the dose-selection process to improve the likelihood of selecting an effective dose and, thereby, increasing the likelihood of a successful NDA [16–19]. For tedizolid phosphate, a series of preclinical PK/PD infection models were conducted to determine the exposures necessary for a high probability of success in ABSSSI [31–38]. Based on the available S. aureus murine thigh-infection model data, 200 mg of tedizolid phosphate daily was selected as the dosing scheme for the ABSSSI phase 3 trials. Overall, the results of the first phase 3 ABSSSI trial support the use of this regimen in clinical practice for this condition. Beyond its use in ABSSSI, data from a neutropenic S. aureus pneumonia model study and a healthy volunteer ELF penetration study also speak to its potential use in pneumonia [32, 33].

Notes

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Both authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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