Antibiotics with novel mechanisms of action are urgently needed. Processes of cellular division are attractive targets for new drug development. FtsZ, an integral protein involved in cell cytokinesis, is a representative example. In the present study, the pharmacodynamic (PD) activity of an FtsZ inhibitor, TXA-709, and its active metabolite, TXA-707, was evaluated in the neutropenic murine thigh infection model against 5 *Staphylococcus aureus* isolates, including both methicillin-susceptible and methicillin-resistant isolates. The pharmacokinetics (PK) of the TXA-707 active metabolite were examined after oral administration of the TXA-707 prodrug at 10, 40, and 160 mg/kg of body weight. The half-life ranged from 3.2 to 4.4 h, and the area under the concentration-time curve (AUC) and maximum concentration of drug in serum ([C\text{max}]) were relatively linear over the doses studied. All organisms exhibited an MIC of 1 mg/liter. Dose fractionation demonstrated the area under the concentration-time curve (AUC/MIC) associated with optimal drug efficacy and (ii) identify the magnitude of the PK/PD index value required for efficacy among multiple *S. aureus* isolates, including those with beta-lactam resistance.

**MATERIALS AND METHODS**

Organisms, media, and antibiotics. Five isolates of *Staphylococcus aureus* (4 methicillin-resistant isolates and 1 methicillin-susceptible isolate) were studied (Table 1). The methicillin-resistant isolates included both hospital-acquired isolates and community-acquired isolates and three U.S. genotypes. Organisms were grown, subcultured, and quantified using Mueller-Hinton broth (MHB) and agar (Difo Laboratories, Detroit, MI). Prodruk TXA-709 and the active metabolite TXA-707 were supplied by the sponsor of the study, Taxis Pharmaceuticals, Inc.

**In vitro susceptibility testing.** MICs were determined in MHB using standard CLSI microdilution techniques (15). All MIC tests were performed in duplicate and on two separate occasions.

**Murine thigh infection model.** The neutropenic-mouse thigh infection model was used for *in vivo* study of TXA-709 and TXA-707 (TXA-709/707) (16). Animals were maintained in accordance with the American
Committees weighing 23 to 27 g were used for all studies. Mice were rendered neutropenic ICR/Swiss mice (Harlan Sprague-Dawley, Indianapolis, IN) from the University of Wisconsin. Six-week-old, specific-pathogen-free, fettopenic (neutrophils $S. aureus$) were grown in inoculum ranged from $10^{6.5}$ to $10^{6.7}$ CFU/ml. Thigh infections with this model for 5 days (18). Broth cultures of freshly plated bacteria were determined by the sponsor of $S. aureus$ isolates to TXA-707.

### Table 1: In vitro antimicrobial susceptibility of selected $S. aureus$ isolates to TXA-707

<table>
<thead>
<tr>
<th>Isolate</th>
<th>TXA-707 MIC (mg/liter)</th>
<th>Phenotype</th>
<th>Pulsed-field gel electrophoresis genetic lineage</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 25923</td>
<td>1</td>
<td>MSSA</td>
<td></td>
</tr>
<tr>
<td>307109</td>
<td>1</td>
<td>MRSA</td>
<td>USA 300</td>
</tr>
<tr>
<td>R-2527</td>
<td>1</td>
<td>MRSA</td>
<td>USA 200</td>
</tr>
<tr>
<td>ATCC 33591</td>
<td>1</td>
<td>MRSA</td>
<td>USA 400</td>
</tr>
<tr>
<td>MW2</td>
<td>1</td>
<td>MRSA</td>
<td>USA 400</td>
</tr>
</tbody>
</table>

*In addition to the antimicrobial susceptibility data, the presence or absence of beta-lactam (methicillin) resistance is shown for each isolate. MSSA, methicillin-susceptible $S. aureus$; MRSA, methicillin-resistant $S. aureus$.

Association for Accreditation of Laboratory Animal Care (AAALAC) criteria (17). All animal studies were approved by the Animal Research Committees of the William S. Middleton Memorial VA Hospital and the University of Wisconsin. Six-week-old, specific-pathogen-free, female ICR/Swiss mice (Harlan Sprague-Dawley, Indianapolis, IN) weighing 23 to 27 g were used for all studies. Mice were rendered neutropenic (neutrophils $S. aureus$) by injecting cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, IN) intraperitoneally 4 days (150 mg/kg of body weight) and 1 day (100 mg/kg) before thigh infection. Previous studies have shown that this regimen produces neutropenia in this model for 5 days (18). Broth cultures of freshly plated bacteria were grown in the logarithmic phase overnight to an absorbance of 0.3 at 580 nm using a Spectronic 88 spectrophotometer (Bausch and Lomb, Rochester, NY). After a 1:10 dilution into fresh MBH, bacterial counts of the inoculum ranged from $10^6$ to $10^7$ CFU/ml. Thigh infections with each of the isolates were produced by injection of 0.1 ml of inoculum into the thighs of isoflurane-anesthetized mice 2 h before therapy with TXA-709.

**Drug pharmacokinetics.** Analysis of the single-dose serum pharmacokinetics of the active drug, TXA-707, was performed in the same animal model. Dose levels of the prodrug, TXA-709, included 10, 40, and 160 mg/kg administered by the oral route. Groups of three mice were sampled at each time point (6 total time points) for each dose level. Sampling times ranged from 1 to 24 h. Serum concentrations of the active metabolite (TXA-707) and prodrug (TXA-709) were determined by the sponsor of the study using liquid chromatography-tandem mass spectrometry (LC-MS/MS) techniques. The assay limit of quantitation was 2.5 ng/ml, and the coefficient of variation was less than 10%. The values corresponding to the pharmacokinetic parameters, including the elimination half-life ($t_{1/2}$), 24-h area under the drug concentration-time curve (AUC), and maximum concentration of drug in serum ($C_{max}$), were calculated using a noncompartmental model. The half-life was determined by linear least-squares regression. The AUC was calculated from the mean concentration curve from time zero to infinity ($AUC_0–\infty$). The elimination half-life ranged from 2.7 to 96.4 mg · h/liter. The elimination half-life ranged from 3.2 to 4.4 h. The pharmacokinetics were relatively linear over the dose range ($AUC R^2 = 0.99$, $C_{max} R^2 = 0.96$).

### RESULTS

**In vitro susceptibility testing.** The MIC results were congruent between the two susceptibility experiments performed in duplicate for all 5 isolates and are shown in Table 1. All isolates demonstrated an MIC of 1 mg/liter. Beta-lactam resistance did not impact TXA-707 potency. The results are in agreement with a previous study that utilized a large and heterogeneous population of more than 60 clinical $S. aureus$ isolates, including those with beta-lactam resistance, where the MIC range was 0.5 to 2 mg/liter (unpublished data).

**Drug pharmacokinetics.** The single-dose serum pharmacokinetics of TXA-707 after oral administration of the prodrug TXA-709 at 10, 40, and 160 mg/kg are shown in Fig. 1. TXA-709 concentrations were below the limit of detection in all serum samples. Maximum TXA-707 concentrations ($C_{max}$) ranged from 0.5 to 13.7 mg/liter. Values corresponding to the area under the drug concentration curve from time zero to infinity ($AUC_0–\infty$) ranged from 2.7 to 96.4 mg · h/liter. The elimination half-life ranged from 3.2 to 4.4 h. The pharmacokinetics were relatively linear over the dose range ($AUC R^2 = 0.99$, $C_{max} R^2 = 0.96$).

**PK/PD of FtsZ Inhibitor TXA-709 in a Murine Model**

The relationship between the dose of TXA-709, the dosing interval, and the effect against $S. aureus$ ATCC 25923 is shown in Fig. 2. The dose-response curves for each fractionated dosing regimen were very similar. The similarity of dose-response curves among the dosing intervals suggests that AUC/MIC would be the predictive pharmacodynamic index. The relationships between the $log_{10}$ CFU/thigh and the PD indices AUC/MIC, $C_{max}$/MIC, and the percentage of time during which serum concentrations exceeded the MIC are illustrated in Fig. 3A, B, and C, respectively, for $S. aureus$ ATCC 25923. Analysis of these data suggests the impor-
tance of AUC/MIC as the predictive PK/PD index based on data fit and \(R^2\) values.

**Pharmacokinetic/pharmacodynamic index target for efficacy.** A total of 5 \(S. aureus\) isolates were studied to determine if the AUC/MIC targets required for an effect were similar in multiple pathogens. The initial burden at the start of therapy was 7.18 ± 0.41 \(\log_{10}\) CFU/thigh. The \(in vivo\) levels of fitness of the isolates were relatively similar in untreated control mice as determined on the basis of a burden increase of 1.88 ± 0.69 \(\log_{10}\) CFU/thigh over a 24-h period. The dose-response data for each of the five \(S. aureus\) isolates are shown in Fig. 4. The dose-response relationships were quite similar, which would be expected given that all isolates had the same drug MIC. Treatment of infection with all 5 isolates produced results showing net stasis. For 4 of 5 isolates, treatment regimens achieved at least a 1-log\(_{10}\) kill endpoint. The doses necessary to produce a bacteriostatic effect and a 1-log\(_{10}\) reduction in organism burden as well as the corresponding total and free drug 24-h AUC/MIC values are shown in Table 2. The static doses ranged from 186 mg/kg/24 h to 247 mg/kg/24 h. The doses associated with a 1-log\(_{10}\) kill were 326 mg/kg/24 h to 640 mg/kg/24 h. The presence of beta-lactam resistance did not alter the pharmacodynamic target required to produce efficacy. The relationships between TXA-707 exposure (expressed as the AUC/MIC) and efficacy against all \(S. aureus\) isolates are shown in Fig. 5. The relationship among the data for each of the five isolates studied was strong based on both visual inspection and calculation of an \(R^2\) value of 0.74. The mean total drug 24-h AUC/MIC value associated with stasis was 122 (free drug value, 17.1), and that needed for a 1-log\(_{10}\) reduction was approximately 2-fold higher at 243 (free drug value, 34).

**DISCUSSION**

Methicillin-resistant \(S. aureus\) infections have steadily increased in number since they were first recognized in the 1960s (1, 19, 20) and continue to represent a significant cause of morbidity and mortality (2). For example, more people die of MRSA infection in the United States health care setting than of HIV infection and tuberculosis combined (21, 22). Unfortunately, new antibiotic development specifically targeting resistant Gram-positive infections has been sparse. Since the introduction of vancomycin in 1972, very few new classes of antibiotics, including the oxazolidinones (linezolid and tedizolid), lipopeptides (daptomycin), and beta-lactams (ceftaroline), have been developed for treatment of MRSA infections. A recent addition has been the result of approval of lipoglycopeptides (telavancin, dalbavancin, and oritavancin). Importantly, only the oxazolidinones are orally bioavailable, representing a significant limitation of treatment of MRSA infections, especially skin and soft tissue infection. Additionally, drug resistance to these newer therapies, save lipoglycopeptides,
has been repeatedly exhibited very soon after clinical introduction (23–39).

There is an urgent need for the development of antibiotic compounds that exhibit novel mechanisms of action against drug-resistant pathogens (7, 9, 10). An encouraging area of drug development research over the previous decade has been the preclinical investigation of compounds that inhibit cell division. One of the more promising targets identified is the divisome, a macromolecular complex of cell division proteins (40,41). FtsZ has been identified as a key component of the divisome and therefore is an attractive drug target (11, 12, 40–44). Its appeal is further supported for several reasons: (i) it is an essential bacterial protein for survival; (ii) it is highly conserved across many bacterial species, making it a potential broad target; (iii) it is not present in eukaryotes, and toxicity therefore would be expected to be low; and (iv) it is a novel target currently unexploited by other therapeutic options and therefore would be expected to present a low probability of cross-resistance with other therapies. Previous in vitro studies have demonstrated that a number of FtsZ inhibitor compounds contain antibacterial potency against Gram-positive pathogens, including isolates with phenotypic resistance to other antibiotics (14, 45–51).

These data represent the first preclinical animal model pharmacodynamic characterization of a novel, orally bioavailable methylbenzamide antibiotic compound, TXA-709, and its active metabolite, TXA-707, which targets bacterial cell division via FtsZ inhibition. Prior in vivo study of methoxybenzamide compounds which inhibit FtsZ was limited to a few proof-of-concept studies (14,52, 53). In two of these studies, a single dose of the study compound led to improved survival in a murine methicillin-susceptible S. aureus (MSSA) septicemia model, whereas a third study demonstrated a decrease in bacterial burden for a single dose in a murine MSSA thigh model.

Note that methoxybenzamide derivatives have been shown to be equipotent against susceptible as well as beta-lactam-resistant pathogens. 
S. aureus isolates (53). Indeed, we found a very narrow MIC range with similar potencies for MSSA and MRSA isolates, similarly to previous reports. We demonstrated in vivo efficacy against a diverse group of 5 S. aureus isolates, including 4 MRSA isolates and three different U.S. genotypes, with the achievement of net stasis and 1-log$_{10}$ kill endpoints. Pharmacodynamic evaluation of the dose fractionation experiments demonstrated the importance of AUC/MIC as the PD index that best predicts optimal efficacy. Modeling the AUC/MIC drug-response data for the entire organism data set also demonstrated a strong fit, with an $R^2$ of 0.74. A 24-h AUC/MIC target of approximately 120 was needed for net stasis, with a 2-fold increase in the target yielding a 1-log$_{10}$ kill level. Importantly, as suggested by the in vitro potency, the pharmacodynamic target was not influenced by beta-lactam resistance or genotype.

In conclusion, these studies demonstrated that TXA-709/707 exhibits dose-dependent in vivo activity against S. aureus isolates, including those with beta-lactam resistance. The AUC/MIC was the PK/PD index that best predicted efficacy, and we were able to demonstrate both net stasis and cidal endpoints in a clinically relevant animal infection model. The PD index and targets identified in this study, along with human PK data, will be useful in guiding appropriate dosing regimen design for future clinical studies. These findings suggest that TXA-709 and TXA-707 are a promising novel antibiotic class and compound against S. aureus. Further development and study are warranted, especially in light of the urgent need for novel drugs to address the rise of drug-resistant infections.

ACKNOWLEDGMENT

This study was funded by Taxis Pharmaceuticals, Inc.

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