Application of Pharmacokinetic-Pharmacodynamic Modeling and the Justification of a Novel Fusidic Acid Dosing Regimen: Raising Lazarus From the Dead

Brian T. Tsuji,1 Olanrewaju O. Okusanya,2 Jurgen B. Bulitta,1,3 Alan Forrest,1,2 Sujata M. Bhavnani,1,2 Prabhavathi Fernandes,4 and Paul G. Ambrose1,2,5

1School of Pharmacy and Pharmaceutical Sciences, University at Buffalo, 2Institute for Clinical Pharmacodynamics, Latham, 3Ordway Research Institute, Albany, New York, 4Cempra Pharmaceuticals, Chapel Hill, North Carolina, and 5University of Oxford, United Kingdom

Perhaps the most crucial step in the clinical development of an antimicrobial agent is the selection of a dosing regimen. Such decisions impact not only the success of a program but also the well being of individual patients, the emergence of resistance, and society as a whole. For fusidic acid, the selection of a dosing regimen for the treatment of patients with acute bacterial skin and skin-structure infection (ABSSSI) was based on the integration of knowledge gained from human population pharmacokinetic, in vitro infection, and mathematical models. The overarching goal of these studies was to identify a dosing regimen that would maximize the probabilities of positive clinical outcomes and limit the emergence of bacterial resistance during therapy. Novel dosing regimens identified included 1500 mg twice daily on day 1 followed by 600 mg twice daily for 10–14 days, a regimen that was subsequently found to be effective in a phase 2 clinical study of patients with ABSSSI. Herein, we review the data supporting the use of this novel fusidic acid dosing regimen, which will undergo further clinical evaluation in phase 3 clinical trials.

Fusidic acid, also known as sodium fusidate, is an oral antibiotic that was first introduced into clinical practice outside the United States in the 1960s and is currently in clinical development. Fusidic acid has activity against methicillin-susceptible (MSSA) and methicillin-resistant Staphylococcus aureus (MRSA) and has been used for the treatment of uncomplicated skin and skin-structure infections, predominantly in Europe and Australia [1, 2]. S. aureus develops resistance to fusidic acid when it is used as a single agent, most often in association with point mutations in the fusA gene [3]. For this reason, fusidic acid has not typically been used for the treatment of more serious complicated skin and skin-structure infections.

A fusidic acid dosing strategy of 500 mg administered 2 or 3 times daily has been used to treat skin and skin-structure infections outside the United States for decades [4, 5, 6]. The pharmacokinetics of fusidic acid differ notably among formulations [7], and major gaps exist regarding the mechanisms of saturable elimination and rate of accumulation of fusidic acid at steady state. Despite many years of clinical use, published pharmacokinetic-pharmacodynamic data and the rationale supporting the use of the aforementioned dosing regimens are lacking.

In an effort to select a dosing regimen for the development of fusidic acid as a single agent in the treatment of patients with acute bacterial skin and skin structure infection (ABSSSI) in the United States, knowledge gained from 2 mathematical models was combined through the use of Monte Carlo simulation. The first model, a population pharmacokinetic model...
describing the disposition of fusidic acid, was constructed using phase 1 pharmacokinetic data collected from healthy subjects [8]. The second model, a mechanism-based model that described the effect of the concentration-time course of fusidic acid on the time-course of bacterial growth and killing for MRSA, was derived from data generated using a 1-compartment in vitro infection model. These data were subsequently validated using a hollow fiber in vitro infection model [9]. The goal of the analyses described herein was to identify dosing regimen strategies for fusidic acid that would optimize bacterial eradication and delay the emergence of resistance during therapy.

POPULATION PHARMACOKINETIC MODEL

Intensively sampled plasma fusidic acid concentration data from 3 phase 1 studies involving a total of 76 healthy subjects provided the basis for the development of a population pharmacokinetic model [8]. Across these studies, which had single- or multiple-dose designs, subjects who fasted or had been fed received a wide range of doses (500–2200 mg/day).

In brief, the population pharmacokinetic model for fusidic acid was a 2-compartment disposition model with saturable absorption [10] that accounted for the effect of food and morning versus evening administration. Fusidic acid inhibited its own clearance, and this auto-inhibition affected the extent and time-course of accumulation at steady state. As evidenced by the coefficient of determination (R²) of 0.96 for observed versus individual fitted plasma concentrations, the population pharmacokinetic model provided excellent fits to the data despite the inherent variability seen in the absorption process. Parameter estimates for this model are reported elsewhere [8].

IN VITRO INFECTION MODELS

One-compartment and hollow fiber in vitro infection models over 48 and 240 h, respectively, were used to evaluate candidate fusidic acid dosing regimens that simulated human pharmacokinetics on the basis of phase 1 data. Data from these experiments were evaluated to determine the following: (1) to identify pharmacokinetic-pharmacodynamic targets associated with efficacy, and (2) to understand the impact of front-loaded dosing regimens on bacterial killing and suppression of the emergence of resistance during therapy. A brief summary of the results of these experiments is provided below, with further details reported elsewhere [9].

A MRSA USA300 clinical isolate (fusidic acid minimum inhibitory concentration [MIC], 0.25 mg/L) was obtained from JMI Laboratories (North Liberty, IA) and used in both in vitro infection model experiments. The determination of MIC values was performed in accordance with CLSI guidelines [11, 12]. Mueller Hinton Broth was supplemented with calcium, magnesium, and human albumin (to a final concentration of 4 g/dL), simulating human physiologic concentrations. Serial viable counts of antibiotic-resistant bacteria were quantified during the hollow fiber in vitro infection model experiments using drug-containing agar plates.

Figure 1 shows the comparative bacterial reduction of MRSA over time for various fusidic acid dosing regimens based on data from the 1-compartment in vitro infection model. There are a number of noteworthy observations including the bacterial regrowth associated with the dosing regimens of 550 mg every 12 h and of 1100 mg every 24 h and the suppression of bacterial regrowth associated with the front-loaded dosing regimens. It is also important to note that the extent of bacterial killing reached a plateau with the most intensive dosing regimens.

On the basis of the data from the 1-compartment in vitro infection model experiments, a subset of dosing regimens were prospectively designed and studied using a hollow fiber in vitro infection model. Figure 2 shows the change in the total and resistant bacterial populations over 240 h associated with 3 fusidic acid dosing regimens based on data from the hollow fiber in vitro infection model. Note that the front-loaded dosing regimens (Figures 2B and 2C) were associated with a delay in the emergence of resistant subpopulations over the study period. The magnitude of the change in the fusidic acid MIC value in the resistant subpopulations is also noteworthy, because it is consistent with that of a mutation in the fusA or fusE gene [3].

![Figure 1](cid:2011:52 (Suppl 7) Tsuji et al)
Subsequent hollow fiber in vitro infection model experiments were conducted over 240 h to evaluate the following fusidic acid dosing regimens against a *Streptococcus pyogenes* clinical isolate (fusidic acid MIC, 4.0 mg/L): 600 mg every 12 h; 1200 mg every 12 h on day 1, followed by 600 mg every 12 h; and 1500 mg every 12 h on day 1, followed by 600 mg every 12 h [9]. As shown in Figure 3, data from these experiments demonstrated complete eradication of *S. pyogenes* after 96–120 h, with suppression of resistant subpopulations over 240 h for all 3 dosing regimens evaluated.

**MECHANISM-BASED PHARMACOKINETIC-PHARMACODYNAMIC MODEL AND MONTE CARLO SIMULATION**

A mechanism-based pharmacokinetic-pharmacodynamic model describing the effect of the concentration-time course of fusidic acid on the time-course of bacterial growth and killing for a MRSA USA300 clinical isolate was developed on the basis of a previously-developed, mechanism-based pharmacokinetic-pharmacodynamic model [13] and data generated from the aforementioned 1-compartment in vitro infection model [9]. In brief, the mechanism-based pharmacokinetic-pharmacodynamic model described the bacterial population as consisting of 2 homogenous subpopulations, each differing in the baseline susceptibility to fusidic acid. The initial bacterial burden for each subpopulation was also assumed to differ at time zero. Within each subpopulation, bacteria were also modeled as existing in 2 states: a vegetative state preparing for replication (state 1), and a replicative state (bacteria immediately before replication; state 2). Bacteria were assumed to transition from state 2 to state 1 and could replicate (resulting in 2 daughter cells) when entering state 1 (100% probability of successful doubling or 0% probability of death during replication). Bacterial replication (doubling) was assumed to occur rapidly. As the total burden of bacteria in the system approaches the upper limit of what the system can sustain, the doubling efficiency decreased and the probability of cell death gradually increased.

![Figure 2](image-url) Change in the bacterial burden of methicillin-resistant *Staphylococcus aureus* for the total population and resistant subpopulations over 240 h associated with 3 fusidic acid dosing regimens based on data from a hollow fiber in vitro infection model: 600 mg every 12 h (A); 1200 mg every 12 h on day 1, followed by 600 mg every 12 h (B); and 1500 mg every 12 h on day 1, followed by 600 mg every 12 h (C). A log10 CFU/mL of 0 represented no observed colonies on the agar plate [9]. MIC, minimum inhibitory concentration.

![Figure 3](image-url) Change in the bacterial burden of *Streptococcus pyogenes* for the total population and resistant subpopulations over 240 h associated with 3 fusidic acid dosing regimens based on data from a hollow fiber in vitro infection model: 600 mg every 12 h (A); 1200 mg every 12 h on day 1, followed by 600 mg every 12 h (B); and 1500 mg every 12 h on day 1, followed by 600 mg every 12 h (C). A log10 CFU/mL of 0 represented no observed colonies on the agar plate [9]. MIC, minimum inhibitory concentration.
Fusidic acid was modeled as both prolonging the rate of bacterial replication and increasing the probability of death during replication. The structure of the model described above for a given subpopulation is shown in Figure 4. Since the effect of fusidic acid on the bacterial growth and killing of each subpopulation was assumed to be the same, the schematic is identical for each subpopulation [9].

to 50%. Fusidic acid was modeled as both prolonging the rate of bacterial replication and increasing the probability of death during replication. The structure of the model described above for a given subpopulation is shown in Figure 4. Since the effect of fusidic acid on the bacterial growth and killing of each subpopulation was assumed to be the same, the schematic is identical for susceptible and resistant subpopulations. As evidenced by an R² of 0.946 for the observed versus population fitted log10 colony-forming units (CFUs) (Figure 1), the model provided unbiased and precise fits to the data.

Using the above-described population pharmacokinetic and mechanism-based pharmacokinetic-pharmacodynamic models for fusidic acid, Monte Carlo simulations were conducted to predict the time-course of CFU over 48 h for the following seven fusidic acid dosing regimens [9]:

- 500 mg every 12 h;
- 600 mg every 12 h;
- 500 mg every 8 h;
- 900 mg every 12 h on day 1, followed by 600 mg every 12 h;
- 1200 mg every 12 h on day 1, followed by 600 mg every 12 h;
- 1500 mg every 12 h on day 1, followed by 600 mg every 12 h;
- and 1500 mg every 12 h on day 1, followed by 900 mg every 12 h.

Fusidic acid plasma concentration versus time and associated CFU time-course profiles over 48 hours for 1,000 simulated subjects were predicted for each of the seven dosing regimens.

Log ratio analysis of the area under the CFU curve (AUC$_{CFU}$) [14] and log$_{10}$ CFU profile analyses were conducted to evaluate differences among simulated subjects who received 7 fusidic acid dosing regimens. Log ratio analysis was performed by comparing the AUC$_{CFU}$ for 0 to 24 h and 0 to 48 h for each dosing regimen relative to the AUC$_{CFU}$ of the growth control. The AUC$_{CFU}$ values and AUC$_{CFU}$ log ratio were computed as described elsewhere [14]. The median AUC$_{CFU}$ log ratios for simulated subjects who received the aforementioned 7 fusidic acid dosing regimens are shown in Table 1. Endpoints for log$_{10}$ CFU profile analyses included the lowest log$_{10}$ CFU value to which the bacteria were driven (nadir), the time to nadir, and the time to net bacterial stasis (time to return to baseline). Median values for these measures for simulated subjects administered each of the 7 different fusidic acid dosing regimens are shown in Table 2.

Table 1. Median Area Under the Colony-Forming Unit Curve (AUC$_{CFU}$) Log Ratio (With Interquartile Range [IQR]) for Simulated Subjects Administered 7 Different Fusidic Acid Dosing Regimens

<table>
<thead>
<tr>
<th>Dosing regimen</th>
<th>Day 1</th>
<th>Day 2</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mg every 12 h</td>
<td>500 mg every 12 h</td>
<td>-2.25</td>
<td>-0.860</td>
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</tr>
<tr>
<td>600 mg every 12 h</td>
<td>600 mg every 12 h</td>
<td>-2.28</td>
<td>-1.06</td>
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</tr>
<tr>
<td>500 mg every 8 h</td>
<td>500 mg every 8 h</td>
<td>-2.29</td>
<td>-1.31</td>
<td></td>
</tr>
<tr>
<td>900 mg every 12 h</td>
<td>600 mg every 12 h</td>
<td>-2.30</td>
<td>-1.59</td>
<td></td>
</tr>
<tr>
<td>1,200 mg every 12 h</td>
<td>600 mg every 12 h</td>
<td>-2.29</td>
<td>-1.79</td>
<td></td>
</tr>
<tr>
<td>1,500 mg every 12 h</td>
<td>600 mg every 12 h</td>
<td>-2.29</td>
<td>-2.29</td>
<td></td>
</tr>
<tr>
<td>1,500 mg every 12 h</td>
<td>900 mg every 12 h</td>
<td>-2.29</td>
<td>-2.34</td>
<td></td>
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</table>
DISCUSSION

The overarching goal of the analyses described above was to support dose selection for fusidic acid in a future phase 2 study of patients with ABSSSI. We successfully developed a population pharmacokinetic model describing the saturable disposition of fusidic acid in plasma and a mechanism-based pharmacokinetic-pharmacodynamic model. The latter model describes the effect of the concentration-time course of fusidic acid on the time-course of bacterial growth and killing for MRSA based on data from a 1-compartment in vitro infection model. These models were integrated using Monte Carlo simulation to prospectively identify a fusidic acid dosing regimen for study in the aforementioned phase 2 clinical study, which has since been completed [15].

The population pharmacokinetic analysis demonstrated that fusidic acid disposition was best described by a 2-compartment disposition model (ie, a model containing central and peripheral compartments) with time-dependent, mixed-order absorption and auto-inhibition of clearance [8]. As described below, results of this analysis provided important insights about the absorption under fasting and fed conditions, saturable elimination, and accumulation after multiple doses of fusidic acid.

The aforementioned population pharmacokinetic model explained the nonlinear pharmacokinetics and accumulation of fusidic acid after multiple doses using an indirect response model approach [16, 17]. The observed approximate linear increase in exposure with doses from 550 mg to 2200 mg and the dose-independent degree of accumulation after multiple doses was best explained by an auto-inhibition of fusidic acid clearance. Thus, multiple dosing (without front-loaded doses) would result in extensive and slow accumulation, and depending on the dose level, would potentially require over 2 weeks to achieve steady state. This slow accumulation to steady state supports the use of front-loaded dosing regimens for therapy, which may substantially reduce the time to steady state and to efficacious concentrations.

One-compartment and hollow fiber in vitro infection models were used to evaluate candidate fusidic acid dosing regimens [9]. Results from the 1-compartment in vitro infection model experiments revealed 4 important findings: (1) the ratio of the AUC to the MIC was well correlated with effect, 2) fusidic acid dosing regimens of 550 mg every 12 h and 1100 mg every 24 h without front-loading displayed regrowth; (3) front-loaded dosing regimens prevented the regrowth of bacteria back to baseline over the 48-h study period; and (4) little to no additional benefit was associated with a higher fusidic acid dosing regimen of 1100 mg every 12 h [9].

Dosing regimens evaluated in subsequent experiments that used the hollow fiber in vitro infection model over 240 h provided additional support for the benefit of the front-loaded fusidic acid dosing regimens in comparison with a dosing regimen without front-loading of fusidic acid. The 2 important insights gained based on data from these studies were as follows: (1) a fusidic acid dosing regimen of 600 mg every 12 h was associated with the rapid emergence of a resistant subpopulation that replaced the total population in 48 h, and (2) front-loading significantly increased the magnitude of bacterial eradication and delayed the emergence of resistance by up to 72 h for MRSA. Specifically, the front-loaded dosing regimen of 1500 mg every 12 h performed better than that of 1200 mg every 12 h by increasing the time to the emergence of resistance [9].

Subsequently, Monte Carlo simulations were conducted using parameter estimates from the aforementioned population

<table>
<thead>
<tr>
<th>Dosing regimen</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Nadir, log_{10} CFU</th>
<th>Time to nadir, h</th>
<th>Time to return to baseline, h</th>
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<tbody>
<tr>
<td>500 mg every 12 h</td>
<td>500 mg every 12 h</td>
<td>5.35</td>
<td>16.6</td>
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</tr>
<tr>
<td>600 mg every 12 h</td>
<td>600 mg every 12 h</td>
<td>5.33</td>
<td>17.9</td>
<td>30.3</td>
<td></td>
</tr>
<tr>
<td>500 mg every 8 h</td>
<td>500 mg every 8 h</td>
<td>5.32</td>
<td>18.6</td>
<td>32.3</td>
<td></td>
</tr>
<tr>
<td>900 mg every 12 h</td>
<td>600 mg every 12 h</td>
<td>5.27</td>
<td>21.1</td>
<td>36.3</td>
<td></td>
</tr>
<tr>
<td>1,200 mg every 12 h</td>
<td>600 mg every 12 h</td>
<td>5.22</td>
<td>24</td>
<td>39.1</td>
<td></td>
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<tr>
<td>1,500 mg every 12 h</td>
<td>600 mg every 12 h</td>
<td>5.16</td>
<td>26.9</td>
<td>46.7</td>
<td></td>
</tr>
<tr>
<td>1,500 mg every 12 h</td>
<td>900 mg every 12 h</td>
<td>5.12</td>
<td>25.2</td>
<td>=48.0</td>
<td></td>
</tr>
</tbody>
</table>

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pharmacokinetic and mechanism-based pharmacokinetic-pharmacodynamic models. The results of these Monte Carlo simulations were used to discriminate among potential phase 2 fusidic acid dosing regimens.

The 3 front-loaded fusidic acid dosing regimens employing 1200 or 1500 mg every 12 h on day 1, followed by 600 or 900 mg every 12 h, were associated with median AUC_{CFU} log ratio values of $2.34$ at 48 h, whereas that for the 3 dosing regimens without a front-loaded dose ranged between $-0.86$ to $-1.31$ (1 unit of difference is a 10-fold difference in AUC_{CFU}). However, the front-loaded dosing regimen of 1500 mg every 12 h on day 1 followed by 600 mg every 12 h performed moderately better than the front-loaded dosing regimen of 1200 mg every 12 h on day 1 followed by 600 mg every 12 h. These 2 front-loaded dosing regimens achieved similar nadirs of 5.16 and 5.22 log_{10} CFU, respectively, with a time to nadir of 26.9 and 24 h, respectively. The time for CFU to return to baseline (ie, time to net bacterial stasis) was 46.7 and 39.1 h, respectively. The implication of the nadirs reached and the suppression of regrowth of bacteria back to baseline is that optimizing such end points could give the immune system more time to eliminate the remaining bacteria.

With the results of the aforementioned analyses serving as the basis for the selection of a fusidic acid dose regimen, a phase 2, randomized, double-blind, multiple-center study evaluating efficacy and safety of an oral front-loaded fusidic acid dosing regimen (1500 mg twice daily on day 1 followed by 600 mg twice daily, compared with oral linezolid 600 mg twice daily, both administered for 10–14 days) was initiated. Clinical success rates for the front-loaded fusidic acid regimen and linezolid were comparable in the intent-to-treat and clinically and microbiologically evaluable populations at the test-of-cure [15].

In conclusion, we successfully developed a population pharmacokinetic model describing the disposition of fusidic acid and a mechanism-based model that described the effect of the concentration-time course of fusidic acid on the time-course of bacterial growth and killing for MRSA based on data from a 1-compartment in vitro infection models. Both mathematical models were integrated using Monte Carlo simulation to prospectively identify a fusidic acid dosing regimen for evaluation in a phase 2 randomized, double-blind, multiple-center study. Results from the phase 2 study demonstrated that the fusidic acid dosing regimen chosen using the approach described herein had comparable efficacy relative to the active control, linezolid. Additionally, results from the hollow fiber in vitro infection model for this dosing regimen demonstrated a delay in the emergence of resistance as compared to that for other dosing regimens evaluated. The approach undertaken, which allows for dosing regimens to be selected that are associated with a high probability of a successful outcome, has the potential to reduce uncertainty and mitigate the risk of poor patient outcomes in late-stage clinical drug development due to selection of an inadequate dosing regimen. In the case of fusidic acid, this approach provided a rational strategy to prospectively design a novel dosing regimen with the potential for increased efficacy and delayed emergence of resistance during therapy as compared with the previously used standard dosing regimens. Implementation of a pharmacokinetic-pharmacodynamic–driven approach early in clinical drug development will increase the likelihood of the successful development of an antimicrobial agent.

Acknowledgments

Supplement sponsorship. This article was published as part of a supplement entitled “Fusidic Acid Enters the United States” sponsored by Cempra Pharmaceuticals.

Potential conflicts of interest. O. O. O., A. F., S. M. B., and P. G. A. have received consulting fees and/or research grants from Cempra Pharmaceuticals, Cerexa, Cubist Pharmaceuticals, Durata Therapeutics, the Medicines Company, Nabriva Therapeutics AG, Polymedix, and Rib-X Pharmaceuticals. B. T. T. has received research grants from Cempra Pharmaceuticals, Cubist Pharmaceuticals, and Pfizer Inc. J. B. B. has received research grants from Pfizer, Inc. and Trius Therapeutics. P. F. is an employee of Cempra Pharmaceuticals.

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