Comparison of In Vivo and In Vitro Pharmacodynamics of Humanized Regimen of Ceftaroline Fosamil 600 mg Every 12 hours against Staphylococcus aureus at Initial Inocula of 10⁶ and 10⁸ CFU/ml

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In light of in vivo/in vitro discordance among beta-lactams against Gram-negatives, we compared in vivo pharmacodynamics of humanized ceftaroline against S. aureus (MIC 0.13-1mg/L) from published in vitro studies using standard and high inocula in the murine thigh infection model. Consistent with in vitro findings, mean reductions of $\geq 1\log_{10}$CFU were observed for ceftaroline against all strains using both standard and high inocula. These results suggest in vivo/in vitro concordance with no observed inoculum effect.
Discordant *in vivo* and *in vitro* pharmacodynamics have been reported among beta-lactams against Gram-negative pathogens (1-4). The discrepancy has been speculated to be due to the unnatural accumulation of enzymes within *in vitro* pharmacodynamic models and the resultant, rapid hydrolysis of antimicrobials against *Enterobacteriaceae* with enzyme-mediated resistance (4,5). However, the *in vivo/ in vitro* paradox has also been observed in other Gram-negative pathogens, such as *Pseudomonas aeruginosa*, whose mechanisms of resistance are mainly non-enzyme mediated (e.g. efflux pump, outer membrane proteins) (4,6).

As for Gram-positives, such as *S. aureus*, while many have explored *in vivo* and *in vitro* pharmacodynamics, usage of varied isolates, initial inocula concentrations, and antimicrobial regimens makes comparisons between *in vivo* and *in vitro* models difficult (7-10). Ceftaroline, a cephalosporin with anti-methicillin-resistant *S. aureus* (MRSA) activity via its high affinity to penicillin-binding protein 2a, is considered a viable option for resistant *S. aureus* infections. In lieu of the knowledge that increased ceftaroline MICs against *S. aureus* are mostly associated with mutations in penicillin-binding protein 2a (11,12), this study aimed to compare the *in vivo* and *in vitro* pharmacodynamics of ceftaroline against Gram-positives with non-enzyme mediated resistance. To mimic published *in vitro* pharmacodynamic studies that tested ceftaroline (active drug) against *S. aureus*, we evaluated the *in vivo* pharmacodynamics of a previously determined humanized regimen (simulating ceftaroline pharmacokinetics in humans versus mice) of ceftaroline fosamil (ceftaroline prodrug) 600 mg every 12h against nine phenotypically
diverse *S. aureus* in the setting of two different initial inocula in the neutropenic murine thigh infection model. Commercially available ceftaroline fosamil (Forest Pharmaceuticals, Inc., Lot: 0008D36, St. Louis, MO) was used for *in vivo* studies. Vials were reconstituted and diluted to the desired concentrations according to manufacturer specifications. Nine *S. aureus* isolates (ceftaroline MIC 0.13-1 mg/L) that were previously studied in published *in vitro* pharmacodynamics models (13,14) were used for the standard inocula *in vivo* studies. Among these, three MRSA were tested for the high inocula *in vivo* studies (Table 1) (14). The study protocol was reviewed and approved by the Hartford Hospital Institutional Animal Care and Use Committee. Animals were maintained and used in accordance to National Research Council recommendations. The previously-established neutropenic murine thigh infection model was employed to evaluate efficacy (10). Briefly, pathogen-free, female ICR mice weighing approximately 25 g were acquired from Harlan Sprague Dawley, Inc. (Indianapolis, IN). Mice were rendered neutropenic with 100 and 150 mg/kg intraperitoneal injections of cyclophosphamide (Baxter Healthcare Corporation, Deerfield, IL) given one and four days prior to inoculation, respectively. Three days prior to inoculation, mice were also given a single 5 mg/kg intraperitoneal injection of uranyl nitrate to produce a predictable degree of renal impairment to aid in humanizing the drug regimens (10). Two hours prior to the initiation of antimicrobial therapy, each thigh was inoculated intramuscularly with a 0.1 mL solution containing approximately 1x10^7 colony forming units (CFU)/mL of the test isolate for standard inocula and 1x10^9 CFU/ml for high inocula study.
Two hours post inoculation, subcutaneous doses of ceftaroline fosamil were administered as 0.2 ml subcutaneous injections to groups of three mice as two 12h regimens over a 24h period as follows: 20 mg/kg at 0h, 2 mg/kg at 2.5h and 4h, and 1 mg/kg at 6h and 9h. This regimen was previously described to simulate the human concentration-time profile of intravenous ceftaroline fosamil 600 mg every 12h, which achieved $T>MIC \geq 60.8\%$ at $MIC \leq 1$ mg/L (Table 1) (10). 24h control groups received normal saline with the same volume, route and frequency as the treatment regimen. Groups of three untreated mice were assigned to 0h control groups; they were euthanized by CO2 exposure, followed by cervical dislocation and harvested at the initiation of therapy. All other animals were harvested 24h after the initiation of therapy. Mice that failed to survive to 24h were harvested at the time of expiration. Following sacrifice, each thigh was harvested and homogenized in 5 ml of normal saline. Serially diluted thigh homogenates were plated onto Trypticase soy agar with 5% sheep blood to determine bacterial density. Efficacy was calculated as the change in bacterial density in log$_{10}$CFU at 24h compared with 0h.

The efficacy of ceftaroline against the nine isolates in standard inocula study is displayed in Figure 1a. Respective log$_{10}$CFU at 0h and in 24h controls were 5.5-5.8 and 8.3-9.6. Ceftaroline treatment resulted in mean reductions of 1.1-2.4 log$_{10}$CFU, with no relationship to ceftaroline MIC. These observations were consistent with findings by Zhanel GG, et al who evaluated in vitro pharmacodynamics of humanized regimen of ceftaroline against isolates 477, 478, 479, 480, 456 and 435 at initial inocula of $1 \times 10^6$ CFU/ml (Table 1) (13). In their study, human simulated ceftaroline against all six strains achieved $>3$ log$_{10}$CFU decreases over 24h.
For the high inocula *in vivo* study, corresponding bacterial density for controls at 0h and 24h were 7.4-7.6 and 8.0-8.1, respectively. The use of high inocula did not affect ceftaroline efficacy against the three strains tested (Figure 1b) with mean decreases of 2.0-3.0 log\(_{10}\)CFU. These findings recapitulated the results from an *in vitro* pharmacodynamic study by Bhalodi AA, *et al*, which tested the same three *S. aureus* strains (i.e. 412, 449, 454 [MICs 1 mg/L]) at high inocula (14) and showed decreases of >5 log\(_{10}\)CFU after 24h. While the Bhalodi AA, *et al* study did not evaluate efficacy using a standard inoculum against these three strains, our data suggest ceftaroline does not exhibit an inoculum effect. Although previous *in vivo* and *in vitro* studies of penicillins and cephalosporins noted an inoculum effect secondary to reduction in penicillin-binding proteins during stationary growth phase (15), the absence of inoculum effect is consistent with the findings by Lee DG, *et al* (16), where another anti-MRSA cephalosporin, ceftobiprole, exhibited the smallest inoculum effect against *S. aureus* compared with daptomycin, linezolid and vancomycin. Of note, although 24h treatment groups in high inocula studies showed greater reduction (2.0-3.0) in log\(_{10}\)CFU than in standard inocula studies (1.1-2.4), this was an artifact of the elevated initial bacterial load (i.e. 0h) against which efficacy was compared.

While previous data have suggested discordance between *in vivo/in vitro* efficacy for beta-lactams and Gram-negatives, findings herein suggest *in vivo/in vitro* concordance for ceftaroline against *S. aureus*. This concordance extends to both standard and high inocula, in which ceftaroline did not show an inoculum effect.
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References


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Table 1. Phenotypic profiles and corresponding $f/T>MIC$ (10) of tested *S. aureus* isolates in standard and high inocula studies.

<table>
<thead>
<tr>
<th><em>S. aureus</em> Isolate</th>
<th>Isolate numbers from referenced <em>in vitro</em> data (reference)</th>
<th>Phenotype</th>
<th>Ceftaroline MIC (mg/L)</th>
<th>$f/T&gt;MIC$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>477</td>
<td>89608 (13)</td>
<td>MSSA</td>
<td>0.13</td>
<td>100</td>
</tr>
<tr>
<td>478</td>
<td>89637 (13)</td>
<td>MSSA</td>
<td>0.13</td>
<td>100</td>
</tr>
<tr>
<td>479</td>
<td>83771 (13)</td>
<td>CA-MRSA</td>
<td>0.5</td>
<td>81.7</td>
</tr>
<tr>
<td>480</td>
<td>84495 (13)</td>
<td>HA-MRSA</td>
<td>0.5</td>
<td>81.7</td>
</tr>
<tr>
<td>456</td>
<td>VRS3a (13)</td>
<td>VRSA</td>
<td>0.5</td>
<td>81.7</td>
</tr>
<tr>
<td>435</td>
<td>NRS4 (13)</td>
<td>VISA</td>
<td>1</td>
<td>60.8</td>
</tr>
<tr>
<td>412*</td>
<td>412 (14)</td>
<td>MRSA</td>
<td>1</td>
<td>60.8</td>
</tr>
<tr>
<td>449*</td>
<td>449 (14)</td>
<td>hVISA</td>
<td>1</td>
<td>60.8</td>
</tr>
<tr>
<td>454*</td>
<td>454 (14)</td>
<td>VISA</td>
<td>1</td>
<td>60.8</td>
</tr>
</tbody>
</table>

*: isolates that were used in both standard and high inocula studies; CA: community-acquired; HA: healthcare-acquired; MSSA: methicillin-susceptible *Staphylococcus aureus*; MRSA: methicillin-resistant *Staphylococcus aureus*; VISA: vancomycin-intermediate *Staphylococcus aureus*; VRSA: vancomycin-resistant *Staphylococcus aureus*; hVISA: heteroresistant vancomycin–intermediate *Staphylococcus aureus*.
Figure 1. Change log\textsubscript{10} CFU for humanized regimen of ceftaroline at 24h compared with 0h controls for a collection of \textit{S. aureus} isolates tested against standard inocula (a) and high inocula (b). Bars represent mean ± standard deviation.

(a)

(b)