AAC Journals.ASM.org

Comparison of *In Vivo* and *In Vitro* Pharmacodynamics of a Humanized Regimen of 600 Milligrams of Ceftaroline Fosamil Every 12 Hours against *Staphylococcus aureus* at Initial Inocula of 10^6 and 10^8 CFU per Milliliter

Wonhee So,[a] Jared L. Crandon,[a] George G. Zhanel,[b] David P. Nicolau[a,c]

Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, Connecticut, USA[a]; University of Manitoba, Winnipeg, Manitoba, Canada[b]; Division of Infectious Diseases, Hartford Hospital, Hartford, Connecticut, USA[c]

In light of the *in vivo*/*in vitro* discordance among beta-lactams against Gram-negative pathogens, we compared the *in vivo* pharmacodynamics of humanized ceftaroline against 9 *Staphylococcus aureus* strains (MICs of 0.13 to 1 mg/liter) from published *in vitro* studies using standard and high inocula in the murine thigh infection model. Consistent with the *in vitro* findings, mean reductions of ≥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 (PD) 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 or outer membrane proteins) (4, 6).

As for Gram-positive pathogens, such as *Staphylococcus aureus*, while many have been explored in *in vivo* and *in vitro* pharmacodynamic models, usage of varied isolates, initial inoculum 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-positive pathogens with non-enzyme-mediated resistance. To mimic the 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 600 mg ceftaroline fosamil (a ceftaroline prodrug) every 12 h against nine phenotypically diverse *S. aureus* isolates in the setting of two different initial inocula in the neutropenic murine thigh infection model.

Commercially available ceftaroline fosamil (lot 0008D36; Forest Pharmaceuticals, Inc., St. Louis, MO) was used for the *in vivo* studies. Vials were reconstituted and diluted to the desired concentrations according to the manufacturer's specifications.

Nine *S. aureus* isolates (ceftaroline MICs of 0.13 to 1 mg/liter) that were previously studied in published *in vitro* pharmacodynamic models (13, 14) were used for the standard-inoculum *in vivo* studies. Among these, three MRSA isolates were tested for the high-inoculum *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 with the 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 intraperitoneal injections of cyclophosphamide (100 and 150 mg/kg of body weight; Baxter Healthcare Corporation, Deerfield, IL) given 1 and 4 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 the antimicrobial therapy, each thigh was inoculated intramuscularly with 0.1 ml solution containing approximately 1×10^7 CFU/ml of the test isolate for the standard-inoculum and 1×10^9 CFU/ml for the high-inoculum studies.

Two hours postinoculation, subcutaneous doses of ceftaroline fosamil were administered as 0.2-ml subcutaneous injections to groups of three mice as two 12-h regimens over a 24-h period as follows: 20 mg/kg at 0 h, 2 mg/kg at 2.5 h and 4 h, and 1 mg/kg at 6 h and 9 h. This regimen was previously described to simulate the human concentration-time profile of 600 mg intravenous ceftaroline fosamil every 12 h, which achieved a percentage of the dosing interval where a free-drug concentration exceeds the MIC ($fT_{>MIC}$) of ≥60.8% at an MIC of ≤1 mg/liter (Table 1) (10). The

Received 16 June 2014 Returned for modification 19 July 2014
Accepted 8 August 2014
Published ahead of print 18 August 2014
Address correspondence to David P. Nicolau, david.nicolau@hhchealth.org.

doi:10.1128/AAC.03652-14

TABLE 1 Phenotypic profiles and corresponding $fT_{>MIC}$ of tested *S. aureus* isolates in standard- and high-inoculum studies[a]

S. aureus isolate	Isolate no. from referenced *in vitro* data (reference)	Phenotype[b]	Ceftaroline MIC (mg/liter)	$fT_{>MIC}$ (%)
477	89608 (13)	MSSA	0.13	100
478	89637 (13)	MSSA	0.13	100
479	83771 (13)	CA-MRSA	0.5	81.7
480	84495 (13)	HA-MRSA	0.5	81.7
456	VRS3a (13)	VRSA	0.5	81.7
435	NRS4 (13)	VISA	1	60.8
412[c]	412 (14)	MRSA	1	60.8
449[c]	449 (14)	hVISA	1	60.8
454[c]	454 (14)	VISA	1	60.8

[a] $fT_{>MIC}$ data from reference 10.
[b] CA, community acquired; HA, health care acquired; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; VISA, vancomycin-intermediate *Staphylococcus aureus*; VRSA, vancomycin-resistant *Staphylococcus aureus*; hVISA, heteroresistant vancomycin-intermediate *Staphylococcus aureus*.
[c] Isolates that were used in both standard- and high-inoculum studies.

24-h control groups received normal saline with the same volume, route, and frequency as the treatment regimen. Groups of three untreated mice were assigned to 0-h control groups; they were euthanized by CO_2 exposure, followed by cervical dislocation, and harvested at the initiation of therapy. All other animals were harvested 24 h after the initiation of therapy. Mice that failed to survive to 24 h 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 the bacterial density. The efficacy was calculated as the change in bacterial density in $\log_{10}$ CFU at 24 h compared with that at 0 h.

The efficacy of ceftaroline against the nine isolates in the standard-inoculum study is displayed in Fig. 1A. The respective $\log_{10}$ CFU values at 0 h and in the 24-h controls were 5.5 to 5.8 and 8.3 to 9.6. The ceftaroline treatment resulted in mean reductions of 1.1 to 2.4 $\log_{10}$ CFU, with no relationship to the ceftaroline MIC. These observations were consistent with the findings of Zhanel et al., who evaluated the *in vitro* pharmacodynamics of the humanized regimen of ceftaroline against isolates 477, 478, 479, 480, 456, and 435 at initial inocula of 1×10^6 CFU/ml (Table 1) (13). In their study, human-simulated ceftaroline treatment against all six strains achieved >3 $\log_{10}$ CFU decreases over 24 h.

For the high-inoculum *in vivo* study, the corresponding bacterial densities for controls at 0 h and 24 h were 7.4 to 7.6 and 8.0 to 8.1, respectively. The use of high inocula did not affect the ceftaroline efficacy against the three strains tested (Fig. 1B), with mean decreases of 2.0 to 3.0 $\log_{10}$ CFU. These findings recapitulated the results from an *in vitro* pharmacodynamic study by Bhalodi et al., who tested the same three *S. aureus* strains (i.e., 412, 449, and 454 [MICs of 1 mg/liter]) at high inocula (14) and showed decreases of >5 $\log_{10}$ CFU after 24 h. While the study of Bhalodi et al. did not evaluate the efficacy using a standard inoculum against these three strains, our data suggest that 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 a reduction in penicillin-binding proteins during the stationary growth phase (15), the absence of an inoculum effect is consistent with the findings of Lee et al. (16), where another anti-MRSA cephalosporin, ceftobiprole, exhibited the smallest inoculum effect against *S. aureus* compared with those of daptomycin, linezolid, and vancomycin. Of note, although the 24-h treatment groups in the high-inoculum studies showed greater reductions (2.0 to 3.0) in $\log_{10}$ CFU values than those in standard-inoculum studies (1.1 to 2.4), this was an artifact of the elevated initial bacterial load (i.e., 0 h) against which the efficacy was compared.

While previous data have suggested discordance between *in vivo*/*in vitro* efficacy for beta-lactams and Gram-negative pathogens, the findings herein suggest *in vivo*/*in vitro* concordance for ceftaroline against *S. aureus*. This concordance extends to both

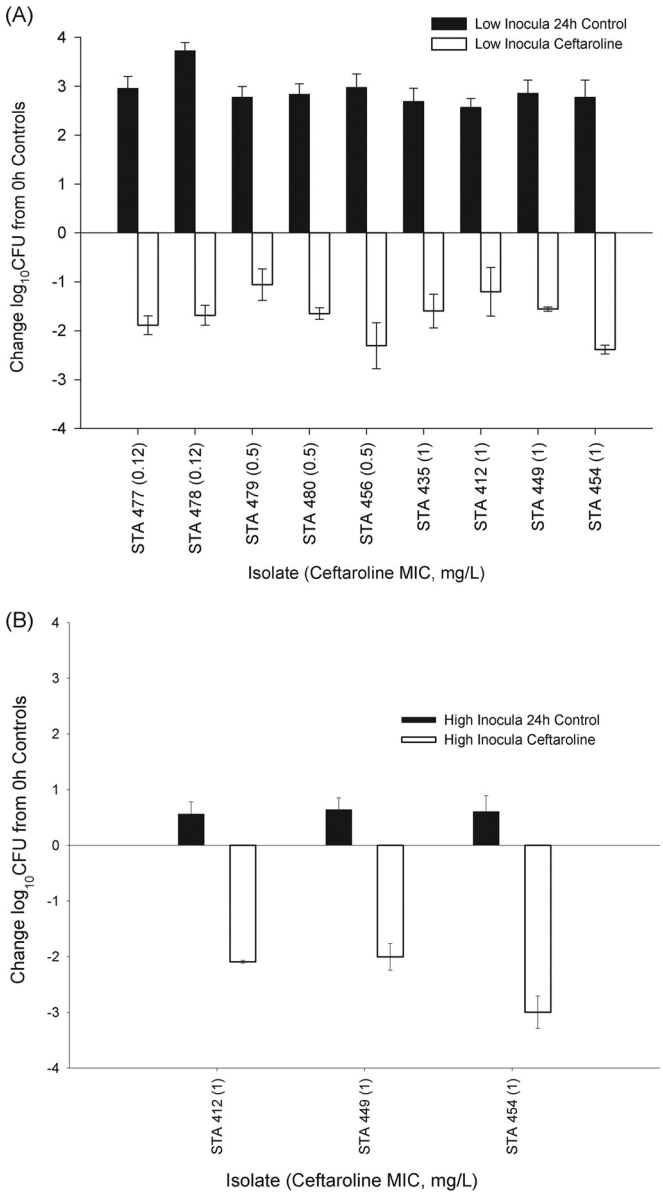

FIG 1 Changes in $\log_{10}$ CFU values for a humanized regimen of ceftaroline at 24 h compared with the 0-h controls for a collection of *S. aureus* isolates tested against standard inocula (A) and high inocula (B). Bars represent means ± standard deviations. STA, *Staphylococcus aureus*.

standard and high inocula, in which ceftaroline did not show an inoculum effect.

ACKNOWLEDGMENTS

We thank Mary Anne Banevicius, Henry Christensen, Jennifer Hull, Lucinda Lamb, Debora Santini, Sara Robinson, Shawn MacVane, Christina Sutherland, and Pamela Tessier for their assistance with the animal experimentation.

This study was internally funded by the Center for Anti-Infective Research and Development, Hartford Hospital (Hartford, CT).

D.P.N. has received grants and honoraria and participates in the advisory board for Forest Pharmaceuticals, Inc. G.G.Z. has received research grants from Forest. J.L.C. has received an honorarium from Forest. W.S. declares no conflicts of interest.

REFERENCES

1. **Bulik CC, Christensen H, Li P, Sutherland CA, Nicolau DP, Kuti JL.** 2010. Comparison of the activity of a human simulated, high-dose, prolonged infusion of meropenem against *Klebsiella pneumoniae* producing the KPC carbapenemase versus that against *Pseudomonas aeruginosa* in an *in vitro* pharmacodynamics model. Antimicrob. Agents Chemother. **54:** 804–810. http://dx.doi.org/10.1128/AAC.01190-09.
2. **Bulik CC, Nicolau DP.** 2010. *In vivo* efficacy of simulated human dosing regimens of prolonged-infusion doripenem against carbapenemase-producing *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **54:** 4112–4115. http://dx.doi.org/10.1128/AAC.00026-10.
3. **Bulik CC, Nicolau DP.** 2011. Double-carbapenem therapy for carbapenemase-producing *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **55:**3002–3004. http://dx.doi.org/10.1128/AAC.01420-10.
4. **Crandon JL, Schuck VJ, Banevicius MA, Beaudoin ME, Nichols WW, Tanudra MA, Nicolau DP.** 2012. Comparative *in vitro* and *in vivo* efficacies of human simulated doses of ceftazidime and ceftazidime-avibactam against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **56:** 6137–6146. http://dx.doi.org/10.1128/AAC.00851-12.
5. **Coleman K, Levasseur P, Girard AM, Borgonovi M, Miossec C, Merdjan H, Drusano G, Shlaes D, Nichols WW.** 2014. Activities of ceftazidime and avibactam against β-lactamase-producing *Enterobacteriaceae* in a hollow-fiber pharmacodynamic model. Antimicrob. Agents Chemother. **58:**3366–3372. http://dx.doi.org/10.1128/AAC.00080-14.
6. **Fusté E, Lopez-Jimenez L, Segura C, Gainza E, Vinuesa T, Vinas M.** 2013. Carbapenem-resistance mechanisms of multidrug-resistant *Pseudomonas aeruginosa*. J. Med. Microbiol. **62**(Part 9)**:**1317–1325. http://dx.doi.org/10.1099/jmm.0.058354-0.
7. **LaPlante KL, Leonard SN, Andes DR, Craig WA, Rybak MJ.** 2008. Activities of clindamycin, daptomycin, doxycycline, linezolid, trimethoprim-sulfamethoxazole, and vancomycin against community-associated methicillin-resistant *Staphylococcus aureus* with inducible clindamycin resistance in murine thigh infection and *in vitro* pharmacodynamics models. Antimicrob. Agents Chemother. **52:**2156–2162. http://dx.doi.org/10.1128/AAC.01046-07.
8. **Steed M, Vidaillac C, Rybak MJ.** 2011. Evaluation of ceftaroline activity versus daptomycin (DAP) against DAP-nonsusceptible methicillin-resistant *Staphylococcus aureus* strains in an *in vitro* pharmacokinetic/pharmacodynamic model. Antimicrob. Agents Chemother. **55:**3522–3526. http://dx.doi.org/10.1128/AAC.00347-11.
9. **MacGowan AP, Noel AR, Tomaselli S, Bowker KE.** 2013. Pharmacodynamics of ceftaroline against *Staphylococcus aureus* studied in an *in vitro* pharmacokinetic model of infection. Antimicrob. Agents Chemother. **57:** 2451–2456. http://dx.doi.org/10.1128/AAC.01386-12.
10. **Keel RA, Crandon JL, Nicolau DP.** 2011. Efficacy of human simulated exposures of ceftaroline administered at 600 milligrams every 12 hours against phenotypically diverse *Staphylococcus aureus* isolates. Antimicrob. Agents Chemother. **55:**4028–4032. http://dx.doi.org/10.1128/AAC.00372-11.
11. **Mendes RE, Tsakris A, Sader HS, Jones RN, Biek D, McGhee P, Appelbaum PC, Kosowska-Shick K.** 2012. Characterization of methicillin-resistant *Staphylococcus aureus* displaying increased MICs of ceftaroline. J. Antimicrob. Chemother. **67:**1321–1324. http://dx.doi.org/10.1093/jac/dks069.
12. **Alm RA, McLaughlin RE, Kos VN, Sader H, Iaconis JP, Lahiri SD.** 2014. Analysis of *Staphylococcus aureus* clinical isolates with reduced susceptibility to ceftaroline: an epidemiological and structural perspective. J. Antimicrob. Chemother. **69:**2065–2075. http://dx.doi.org/10.1093/jac/dku114.
13. **Zhanel GG, Rossnagel E, Nichol K, Cox L, Karlowsky JA, Zelenitsky S, Noreddin AM, Hoban DJ.** 2011. Ceftaroline pharmacodynamics activity versus community-associated and healthcare-associated methicillin-resistant *Staphylococcus aureus*, heteroresistant vancomycin-intermediate *S. aureus*, vancomycin-intermediate *S. aureus* and vancomycin-resistant *S. aureus* using an *in vitro* model. J. Antimicrob. Chemother. **66:**1301–1305. http://dx.doi.org/10.1093/jac/dkr110.
14. **Bhalodi AA, Hagihara M, Nicolau DP, Kuti JL.** 2014. In vitro pharmacodynamics of human simulated exposures of ceftaroline and daptomycin against MRSA, hVISA, and VISA with and without prior vancomycin exposure. Antimicrob. Agents Chemother. **58:**672–677. http://dx.doi.org/10.1128/AAC.01516-13.
15. **Stevens DL, Yan S, Bryant AE.** 1993. Penicillin-binding protein expression at different growth stages determines penicillin efficacy *in vitro* and *in vivo:* an explanation for the inoculum effect. J. Infect. Dis. **167:**1401–1405. http://dx.doi.org/10.1093/infdis/167.6.1401.
16. **Lee DG, Murakami Y, Nades DR, Craig WA.** 2013. Inoculum effects of ceftobiprole, daptomycin, linezolid, and vancomycin with *Staphylococcus aureus* and *Streptococcus pneumoniae* at inocula of 10^5 and 10^7 CFU injected into opposite thighs of neutropenic mice. Antimicrob. Agents Chemother. **57:**1434–1441. http://dx.doi.org/10.1128/AAC.00362-12.