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Evaluation of Ceftaroline, Vancomycin, Daptomycin, or Ceftaroline plus Daptomycin against Daptomycin-Nonsusceptible Methicillin-Resistant *Staphylococcus aureus* in an *In Vitro* Pharmacokinetic/Pharmacodynamic Model of Simulated Endocardial Vegetations

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Infective endocarditis (IE) caused by methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to vancomycin and daptomycin has few adequate therapeutic options. Ceftaroline (CPT) is bactericidal against daptomycin (DAP)-nonsusceptible (DNS) and vancomycin-intermediate MRSA, but supporting data are limited for IE. This study evaluated the activities of ceftaroline, vancomycin, daptomycin, and the combination of ceftaroline plus daptomycin against DNS MRSA in a pharmacokinetic/pharmacodynamic (PK/PD) model of simulated endocardial vegetations (SEVs). Simulations of ceftaroline-fosamil (600 mg) every 8 h (q8h) (maximum concentration of drug in serum [C_{max}], 21.3 mg/liter; half-life [$t_{1/2}$], 2.66 h), daptomycin (10 mg/kg of body weight/day) (C_{max}, 129.7 mg/liter; $t_{1/2}$, 8 h), vancomycin (1 g) q8h (minimum concentration of drug in serum [C_{min}], 20 mg/liter; $t_{1/2}$, 5 h), and ceftaroline plus daptomycin were evaluated against 3 clinical DNS, vancomycin-intermediate MRSA in a two-compartment, *in vitro*, PK/PD SEV model over 96 h with a starting inoculum of ~8 $\log_{10}$ CFU/g. Bactericidal activity was defined as a ≥3-$\log_{10}$ CFU/g reduction from the starting inoculum. Therapeutic enhancement of combinations was defined as ≥2-$\log_{10}$ CFU/g reduction over the most active agent alone. MIC values for daptomycin, vancomycin, and ceftaroline were 4 mg/liter, 4 to 8 mg/liter, and 0.5 to 1 mg/liter, respectively, for all strains. At simulated exposures, vancomycin was bacteriostatic, but daptomycin and ceftaroline were bactericidal. By 96 h, ceftaroline monotherapy offered significantly improved killing compared to other agents against one strain. The combination of DAP plus CPT demonstrated therapeutic enhancement, resulting in significantly improved killing versus either agent alone against 2/3 (67%) strains. CPT demonstrated bactericidal activity against DNS, vancomycin-intermediate MRSA at high bacterial densities. Ceftaroline plus daptomycin may offer more rapid and sustained activity against some MRSA in the setting of high-inoculum infections like IE and should also be considered.

Daptomycin (DAP) has been the main alternative to vancomycin (VAN) for the management of serious infections, such as infective endocarditis (IE), caused by methicillin-resistant *Staphylococcus aureus* (MRSA) with reduced susceptibility to VAN, such as VAN-intermediate *S. aureus* (VISA) and heterogeneous VISA (hVISA) (1). Unfortunately, as the MIC for VAN increases, the DAP MIC may also increase (2–4). DAP-nonsusceptible (DNS) MRSA, defined as a DAP MIC of >1 mg/liter, have now been reported (5–9). While DNS MRSA infections remain relatively uncommon, the alternatives available are limited by bacteriostatic activity, inadequate pharmacokinetics, and/or toxicity (10).

Ceftaroline (CPT), the active metabolite of the prodrug CPT-fosamil (CPT-F), demonstrates *in vitro* bactericidal activity against MRSA, including hVISA, VISA, and DNS strains (11–14). Furthermore, some *in vitro* data suggest that CPT is more active against MRSA with reduced susceptibility to lipo- and glycopeptides than fully susceptible strains due to the phenomenon referred to as the "seesaw" effect (14–16). CPT-F represents a favorable alternative for the management of serious infections with VAN- and DAP-nonsusceptible MRSA not only because of its bactericidal activity against these strains but also because of its excellent safety profile (17). However, because the use of CPT-F for the management of infections such as infective endocarditis and osteomyelitis is not approved by the FDA, the data supporting the use of this drug in these settings comes from *in vivo* rabbit models and 2 recently published case series (18–21). While the observations of these studies support the notion that CPT-F is a viable treatment for patients with infective endocarditis, additional studies are still needed to further describe the role of CPT-F in this setting. Currently, the MRSA guidelines endorsed by the Infectious Disease Society of America recommend the addition of another agent to high-dose DAP (10 mg/kg of body weight/day), including a beta-lactam, for the treatment of persistent bacteremia (1). The addition of CPT-F to high-dose DAP has been used successfully for the treatment of infective endocarditis after the

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emergence of DAP nonsusceptibility and *in vitro* data, including pharmacokinetic/pharmacodynamic (PK/PD) models have demonstrated therapeutic enhancement of this combination (13, 22). The studies that have been conducted so far, however, have not evaluated CPT or the combination of CPT with DAP in the simulated endocardial vegetation (SEV) model which simulates barriers such as high bacterial inocula and tissue penetration into large vegetations that would be found in humans.

The objective of the present study was to evaluate the activity of CPT alone or in combination with DAP against 3 strains of DNS MRSA with reduced susceptibility to VAN in an *in vitro* model of simulated endocardial vegetations.

MATERIALS AND METHODS

Bacterial strains. Three clinical strains of DNS MRSA with VAN MICs of ≥4 mg/liter were evaluated (R6386, R6913, and R5995).

Antimicrobial agents. DAP and VAN were purchased commercially from Cubist Pharmaceuticals (Lexington, MA) and Sigma Chemical Co. (St. Louis, MO), respectively. Analytical-grade CPT powder was provided by Forest Laboratories, Inc. (New York, NY).

Media. Due to the calcium-dependent nature of DAP, Mueller-Hinton broth (MHB) (Difco, Detroit, MI) supplemented with 50 mg/liter of calcium and 12.5 mg/liter magnesium was used for susceptibility testing, and MHB containing 75 mg/liter of calcium was used for *in vitro* simulated endocardial vegetation (SEV) model experiments (due to binding of calcium by albumin in SEVs). Colony counts were determined using tryptic soy agar (TSA) (Difco, Detroit, MI) plates. Brain heart infusion agar (BHIA) (Difco, Detroit, MI) plates were used for VAN and CPT resistance screening, and Mueller-Hinton agar (MHA) (Difco, Detroit, MI) supplemented with 50 mg/liter of calcium was used for DAP resistance screening.

Susceptibility testing. MICs were determined in duplicate by broth microdilution at $\sim 1 \times 10^6$ CFU/ml in MHB according to the Clinical Laboratory and Standards Institute guidelines (CLSI) for each study antimicrobial (23).

SEVs. Simulated endocardial vegetations were prepared by mixing 0.05 ml of organism suspension (final inoculum, 1×10^8 CFU/g), 0.5 ml of human cryoprecipitate antihemolytic factor (AHF) from volunteer donors (American Red Cross, Detroit, MI), and 0.025 ml of platelet suspension (platelets mixed with normal saline with 250,000 to 500,000 platelets per clot) in 1.5-ml siliconized Eppendorf tubes. Bovine thrombin (5,000 units/ml) (0.05 ml) was added to each tube after insertion of a sterile monofilament line into the mixture. The resultant simulated vegetations were then removed from the Eppendorf tubes with a sterile plastic needle and introduced into the infection model. This methodology results in SEVs consisting of approximately 3 to 3.5 g/dl of albumin and 6.8 to 7.4 g/dl of total protein.

***In vitro* pharmacodynamic infection model.** An *in vitro* infection model consisting of a 250-ml one-compartment glass apparatus with ports, where the SEVs were suspended, was utilized for all simulations. The apparatus was prefilled with medium, and antibiotics were administered as boluses over a 96-h period into the central compartment via an injection port. The model apparatus was placed in a 37°C incubator throughout the procedure, and a magnetic stir bar was placed in the medium for thorough mixing of the drug in the model. Fresh medium was continuously supplied and removed from the compartment along with the drug via a peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, IL) set to simulate the half-lives ($t_{1/2}$s) of the antibiotics. Supplemental DAP was added at an appropriate rate to CPT combination models to compensate for the higher flow rate required to simulate CPT clearance (24). A total of 4 simulated regimens were evaluated on each isolate. Total drug concentrations were utilized due to the protein content in the SEVs. These regimens were CPT-F simulations of 600 mg every 8 h (q8h) (peak concentration, 21.3 mg/liter; average $t_{1/2}$, 2.66 h) for 4 days, DAP simulations of 10 mg/kg every 24 h (q24h) (peak concentration, 129.7 mg/liter; average $t_{1/2}$, 8 h) (25) for 4 days, VAN simulations of 1 g q8h (trough concentration, 20 mg/liter; average $t_{1/2}$, 5 h) for 4 days, CPT-F simulations of 600 mg q8h plus DAP (10 mg/kg) q24h for 4 days, and a drug-free growth control for each isolate for 4 days.

Pharmacodynamic analysis. Two SEVs were removed from each infection model (total of 36) at 0, 4, 8, 24, 32, 48, 56, 72, and 96 h. The SEVs were homogenized, diluted in cold saline, and plated onto TSA plates. The plates were incubated at 37°C for 24 h at which time colony counts were performed. The total reduction in $\log_{10}$ CFU/g over 96 h was determined by plotting time-kill curves based on the number of remaining organisms over the time period. Bactericidal (99.9% kill) and bacteriostatic activity were defined as reductions in colony count from the initial inocula of ≥3-$\log_{10}$ CFU/g and <3-$\log_{10}$CFU/g, respectively. Inactivity was defined as no observed reductions in initial inocula. Therapeutic enhancement of activity was defined as an increase in kill of ≥2-$\log_{10}$ CFU/g by a combination of antimicrobials versus the most active single agent of that combination. Combinations that resulted in a ≥1-$\log_{10}$ bacterial growth in comparison to the least-active single agent were considered antagonistic.

Pharmacokinetic analysis. Pharmacokinetic samples were obtained through the injection port of each infection model at 0, 1, 2, 4, 8, 24, 32, 48, 56, 72, and 96 h for verification of target antibiotic concentrations. All samples were stored at −80°C until ready for analysis. CPT concentrations were determined by bioassay using *Bacillus subtilis* ATCC 6633. Blank 1/4-in. disks were spotted with 10 μl of standard concentrations or samples. Each standard was tested in duplicate by placing the disk on agar plates (antibiotic medium 11) inoculated with a 0.5 McFarland suspension of the test organism. This assay demonstrated an intraday coefficient of variance of less than 4.7% for high, medium, and low broth standards. The plates were incubated for 24 h at 37°C at which time the zone sizes were measured using a ProtoCOL plate reader (Microbiology International, Frederick, MD). DAP concentrations were determined using a validated high-performance liquid chromatography (HPLC) assay that conforms to the guidelines set forth by the College of American Pathologists and demonstrated an intraday coefficient of variance of less than 2% for high, medium, and low standards. Concentrations of VAN were determined using a fluorescence polarization immunoassay (TDx assay; Abbott Diagnostics). The VAN assay has a lower limit of detection of 2.0 mg/liter, with an interday coefficient of variance of less than 12% for low, medium, and high standards. The half-lives, peak concentrations, and area under the curve (AUC) (by trapezoidal method), or time spent above the MIC ($T>$MIC), were determined as appropriate for all antimicrobials utilizing PK Analyst software (version 1.10; MicroMath Scientific Software, Salt Lake City, UT).

Changes in susceptibility. Development of resistance was evaluated at 96 h. Samples of 100 μl from each time point were plated on BHIA or calcium-supplemented MHA containing 3-fold the MIC of the respective antibiotic to assess for increases in MIC. The plates were examined for growth after 48 h of incubation at 37°C. Broth microdilution MICs following CLSI guidelines were performed on any isolate observed to grow on drug-containing agar plates used for resistance screening. If resistance was detected by 96 h, earlier time points were then screened to detect the first occurrence of MIC elevation.

Statistical analysis. Changes in CFU/g at 24, 48, 72, and 96 h (days 1 to 4) were compared by one-way analysis of variance with Tukey's posthoc test. A P value of ≤0.05 was considered significant. All statistical analyses were performed using SPSS statistical software (release 21.0; SPSS, Inc., Chicago, IL).

RESULTS

Susceptibility testing. MIC values for DNS VISA strains R6913, R6386, and R5995 were 4, 8, and 4 mg/liter to VAN, 4, 4, and 4 mg/liter to DAP, and 0.5, 1, and 0.5 mg/liter to CPT, respectively.

***In vitro* PK/PD model.** Pharmacodynamic responses to simulated antimicrobial regimens for each strain are summarized in

Fig. 1A to C. VAN was bacteriostatic against all strains, but DAP and CPT were bactericidal at the simulated exposures. By 96 h, DAP monotherapy resulted in significantly improved killing compared to VAN against strain R6913 but was similar to that of CPT ($P = 0.38$); however, the combination of CPT plus DAP resulted in significantly improved killing compared to the effect of DAP against this strain ($P = 0.037$), although it was statistically similar to that of CPT. The combination of DAP plus CPT demonstrated therapeutic enhancement, resulting in significantly improved killing versus either agent alone against strains R6386 and R5995 ($P < 0.001$). DAP-containing regimens achieved bactericidal activity significantly faster than CPT monotherapy for all strains (4.5 ± 2.1 h versus 35.4 ± 17.5 h; $P = 0.0025$), but the combination of DAP plus CPT resulted in significantly faster time to bactericidal activity than DAP monotherapy (4.2 ± 1.6 h versus 6.5 ± 0.5 h; $P = 0.0023$).

Pharmacokinetics. The PK parameters for CPT achieved were a maximum concentration of drug in serum (C_{max}) of 21.39 ± 1.12 mg/liter (target C_{max} of 21.3 mg/liter) and half-life ($t_{1/2}$) of 2.64 ± 0.23 h (target $t_{1/2}$ of 2.66 h) for the central compartment. The average time above the MIC was 100% of the dosing interval for all 3 strains. The PK parameters for DAP achieved in the model were a C_{max} of 131.9 ± 4.24 mg/liter (target, 129.7 mg/liter) and $t_{1/2}$ of 8.41 ± 0.41 h (target, 8 h) with an average area under the curve from 0 to 24 h (AUC_{0-24}) of 1,375.1 ± 0.79 mg · h/liter. The PK parameters for VAN achieved in the model were a minimum concentration of drug in serum (C_{min}) of 20.3 ± 0.1 mg/liter (target, 20 mg/liter) and $t_{1/2}$ of 4.5 ± 0.01 h (target, 5 h) with an AUC_{0-24} of 1,360 ± 6.8 mg · h/liter correlating with a VAN AUC_{0-24}/MIC ratio of 340 for all strains.

Changes in susceptibility. Isolates with MICs that were higher than the baseline MICs to CPT, DAP, and VAN were not detected on resistance screening plates.

DISCUSSION

The prevalence of DNS- and VISA-related MRSA infections remains relatively low; however, these organisms tend to arise from difficult-to-treat, high-inoculum infections such as infective endocarditis and osteomyelitis (10). Only limited data exist to support alternatives to VAN and DAP in the setting of serious infections with DNS and VISA strains of MRSA, but cases of successful management of these infections with CPT-F continue to be reported (26). To address the lack of data in this area, we evaluated the pharmacodynamics of humanized CPT exposures in a PK/PD model of simulated endocardial vegetations. CPT demonstrated bactericidal activity against DNS VISA strains of MRSA even at the high bacterial densities present in the simulated vegetations. At the simulated exposure of VAN, with a target trough concentration of 20 mg/liter, VAN demonstrated modest, bacteriostatic activity against the DNS VISA strains tested. This is unsurprising, as the AUC/MIC ratios (areas under the curve over 24 h in the steady state divided by the MICs) achieved against these strains were not optimal, since optimization would be clinically unrealistic. DAP demonstrated bactericidal activity against these strains; however, this was at exposures typically achieved with a 10-mg/kg/day dose. Even at this high-dose simulation, bacterial regrowth was observed. The synergistic combination of DAP plus CPT appears to offer more rapid activity against DNS MRSA strains than CPT alone and provides more sustained bacterial suppression with less

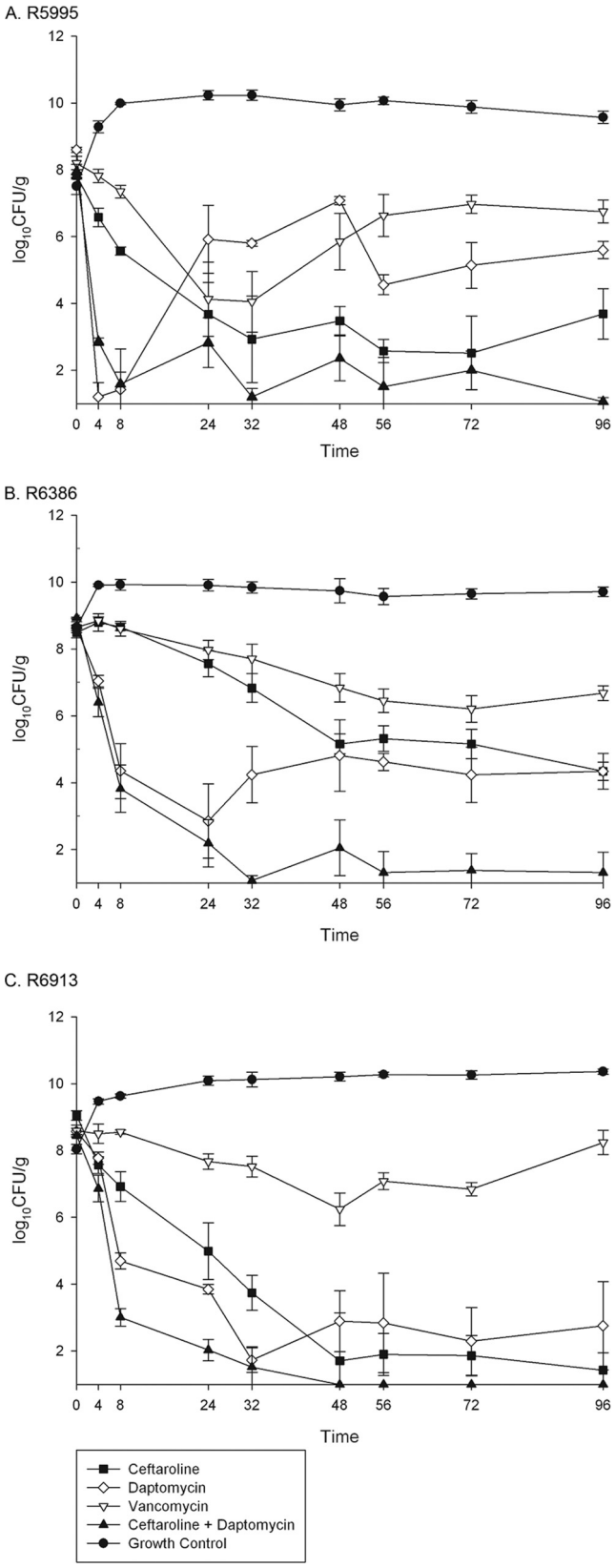

FIG 1 Pharmacodynamics of simulated antimicrobial regimens over 96 h against 3 strains of daptomycin-nonsusceptible, vancomycin-intermediate MRSA in a pharmacokinetic/pharmacodynamic model of simulated endocardial vegetations. Time is shown in hours on the *x* axes.

regrowth than DAP alone. Against strain R6913, CPT, DAP, and CPT plus DAP had similar amounts of activity, and final colony counts at 96 h were not significantly different. Although all three strains had a daptomycin MIC of 4 mg/liter, we observed a surprising degree of heterogeneity in the response to the simulated DAP exposures. R6913 was killed effectively by DAP monotherapy in the model, while R6386 and R5995 responded initially but ultimately regrew. Interestingly, DAP monotherapy against R5995 resulted in >7-$\log_{10}$ CFU/g reduction in colony counts by just 4 h but regrew to ~6 $\log_{10}$ CFU/g by 24 h. This response seems to suggest heterogeneous resistance to DAP, where initial exposure eradicates the more susceptible population and selects for the resistant subpopulations capable of growing in high concentrations of DAP. DAP nonsusceptibility is known to be a complex phenotype that may arise from a variety of possible genetic abnormalities (27). This variability in genetic pathways leading to the vancomycin-intermediate phenotype as well as the DNS phenotype may explain why responses to DAP were varied among strains with the same MICs.

The combination of CPT plus DAP is known to enhance DAP binding by ~7-fold and to increase the activity of human cationic antimicrobial peptides produced by the immune system (13). This enhancement of activity appears to be related to an alteration of cell surface charge that makes DAP or cationic peptide binding more favorable (13, 28). Given that there were no immune cells or antimicrobial peptides in our model, it is possible that CPT may be more active *in vivo* due to synergy with peptides such as cathelicidins, defensins, and platelet-derived microbicidal proteins (29). Furthermore, the synergy with DAP may be potent enough to allow for reduced doses of CPT and/or DAP to achieve similar pharmacodynamics, which may offer cost savings in the clinic and reduce risk of toxicity without sacrificing efficacy (30). Further research should focus on evaluating less frequent dosing of CPT and lower doses of DAP to determine the minimum exposures necessary to prevent resistance and achieve pharmacodynamics similar to the pharmacodynamics of higher doses.

Given the antibacterial efficacy of CPT observed in our PK/PD model of SEVs and the emerging clinical and animal data, CPT-F appears to be a suitable alternative to manage patients with IE secondary to DNS or VISA MRSA. Although clinical data are still lacking, the combination of DAP plus CPT-F may also be a suitable choice for these infections and may offer faster clearance of bacteremia and more sustained suppression than either agent alone. Further clinical evaluation of this combination is warranted.

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