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Evaluation of Standard- and High-Dose Daptomycin versus Linezolid against Vancomycin-Resistant *Enterococcus* Isolates in an *In Vitro* Pharmacokinetic/Pharmacodynamic Model with Simulated Endocardial Vegetations

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Daptomycin MICs for enterococci are typically 1- to 2-fold higher than those for *Staphylococcus aureus*, and there is an imminent need to establish the optimal dose for appropriate treatment of enterococcal infections. We investigated the bactericidal activity of daptomycin at various dose exposures compared to that of linezolid against vancomycin-resistant enterococcus (VRE) in an *in vitro* pharmacokinetic/pharmacodynamic model utilizing simulated endocardial vegetations over 96 h. Daptomycin at doses of 6, 8, 10, and 12 mg/kg of body weight/day and linezolid at a dose of 600 mg every 12 h were evaluated against two clinical vancomycin-resistant *Enterococcus faecium* strains (EFm11499 and 09-184D1051), one of which was linezolid resistant (09-184D1051), and one clinical vancomycin-resistant *Enterococcus faecalis* strain (EFs11496). Daptomycin MICs were 4, 2, and 0.5 μg/ml for EFm11499, 09-184D1051, and EFs11496, respectively. Bactericidal activity, defined as a ≥3 $\log_{10}$ CFU/g reduction from the initial colony count, was demonstrated against all three isolates with all doses of daptomycin; however, bactericidal activity was not sustained with the daptomycin 6- and 8-mg/kg/day regimens. Linezolid was bacteriostatic against EFm11499 and displayed no appreciable activity against 09-184D1051 or EFs11496. Concentration-dependent killing was displayed with more sustained reduction in colony count (3.58 to 6.46 and 5.89 to 6.56 $\log_{10}$ CFU/g) at 96 h for the simulated regimen of daptomycin at doses of 10 and 12 mg/kg/day, respectively ($P \leq 0.012$). No *E. faecium* mutants with reduced susceptibility were recovered at any dosage regimen; however, the *E. faecalis* strain developed reduced daptomycin susceptibility with daptomycin at 6, 8, and 10 but not at 12 mg/kg/day. Daptomycin displayed a dose-dependent response against three VRE isolates, with high-dose daptomycin producing sustained bactericidal activity. Further research is warranted.

Daptomycin (DAP) is a lipopeptide antibiotic with concentration-dependent activity against Gram-positive bacteria that is currently approved for the treatment of staphylococcus bacteremia and right-sided endocarditis at a dose of 6 mg/kg of body weight/day (7). Daptomycin also displays *in vitro* activity against almost all *Enterococcus* spp., including those resistant to other antibiotics, such as vancomycin, linezolid (LZD), and quinupristin-dalfopristin (3, 22, 38). Daptomycin exhibits a lower potency against enterococci than that against staphylococci, demonstrating higher Clinical Laboratory and Standards Institute (CLSI) breakpoints (≤4 μg/ml versus ≤1 μg/ml), MIC_{50} values (1 to 2 μg/ml versus 0.25 μg/ml), and MIC_{90} values (1 to 2 μg/ml versus 0.5 μg/ml) (11, 38). Based on *in vivo* neutropenic mice infection models, maximum concentration (C_{max})/MIC and area under the concentration-time curve (AUC)/MIC ratios are the best predictors for efficacy of daptomycin against infections caused by both *Staphylococcus* spp. and *Enterococcus* spp. (39) Additionally, *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) models have demonstrated a clear dose-effect relationship of daptomycin with reduction of $\log_{10}$ CFU/ml (9). The simulated effective dose to achieve 80% maximal kill activity was 3 mg/kg for the two staphylococcal isolates (MICs of 0.125 and 0.25 μg/ml) and 6.8 mg/kg for the two *Enterococcus faecium* isolates (MICs of 2 and 4 μg/ml) (9). Based on the available data, the current Food and Drug Administration-approved dose of 6 mg/kg/day for *Staphylococcus aureus* bacteremia and right-sided infective endocarditis infections is likely suboptimal for infections caused by most enterococcus due to the higher MIC values and leads to the logical conclusion that higher daptomycin doses (i.e., >6 mg/kg/day) will be required to adequately treat these infections.

Clinical experience with daptomycin for the treatment of enterococcal infections is limited to several retrospective, observational studies of patients with enterococcal bacteremia (12, 14, 17, 18, 20, 21, 28, 29, 31, 32). Success rates in these series vary from 58.1% to 90% depending on the severity of illness of the included patients and the inclusion of clinical or microbiological results in the definition of success. Although these retrospective studies have several limitations, they provide some clinical support that standard doses of daptomycin (6 mg/kg) may be suboptimal for serious enterococcal infections, such as bacteremia and endocarditis. Additional data to support the use of high-dose daptomycin

Received 23 December 2011 Returned for modification 1 February 2012
Accepted 23 March 2012
Published ahead of print 2 April 2012
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doi:10.1128/AAC.06439-11

for the treatment of enterococcal infections are vital to ensuring its appropriate use and efficacy in treating enterococcal infections and establishing daptomycin as the preferred bactericidal regimen for the treatment of serious enterococcal infections. A retrospective analysis of high-dose daptomycin (≥8 mg/kg/day) in 250 patients with both *S. aureus* and enterococcal infections reported that daptomycin was safe and well-tolerated with no dose-response relationship to changes in creatine phosphokinase (CPK) levels (24). That and other studies suggest that the routine use of high-dose daptomycin, ranging from 8 to 14 mg/kg/day, to treat enterococcal infections is safe and clinically feasible (6, 13, 15, 16, 23, 24, 33). The purpose of the current study was to examine the effects of standard and various high-dose daptomycin regimens on both bactericidal killing and the emergence of nonsusceptibility in an *in vitro* model of enterococcal infection compared to the effects of linezolid.

(This study was presented in part at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2011, and at the 22nd European Congress of Clinical Microbiology and Infectious Diseases, London, United Kingdom, 2012.)

MATERIALS AND METHODS

Bacterial strains. A total of three clinical vancomycin-resistant enterococcus (VRE) strains were evaluated. Two clinical *E. faecium* strains were evaluated (SF11499, daptomycin MIC of 4 μg/ml), one of which was linezolid resistant (09-184D1051, DAP MIC of 2 μg/ml), and one clinical *Enterococcus faecalis* strain (SF11496, DAP MIC of 0.5 μg/ml) was utilized. Isolates SF11499 (EFm11499) and SF11496 (EFs11496) were obtained from blood and urine sources, respectively, from a patient at Henry Ford Hospital in Detroit, MI. *E. faecium* isolate 09-184D1051 was recovered from a patient in Houston, TX.

Antimicrobials. Daptomycin (DAP) analytical powder (Cubist Pharmaceuticals, Inc., Lexington, MA) was provided by the manufacturer. Linezolid (LZD) 2-μg/ml solution was commercially purchased (Detroit Receiving Hospital, Detroit, MI).

Media. Mueller-Hinton broth II (MHB II; Difco, Detroit, MI) with 25 mg/liter of calcium and 12.5 μg/ml magnesium was used for susceptibility testing and *in vitro* pharmacodynamic simulated endocardial vegetation (SEV) models. Due to the dependency of daptomycin on calcium for antimicrobial activity, supplemented MHB II (SMHB II) containing 50 μg/ml of calcium was used for susceptibility testing, and that containing 75 μg/ml of calcium (50 and 75 SMHB, respectively) was used for *in vitro* SEV model experiments (due to binding of calcium by albumin) (25). Colony counts were determined using brain heart infusion agar (BHA; Difco, Detroit, MI). Nonsusceptibility was assessed with antibiotic-containing MHB II plus agar (Becton, Dickinson, Sparks, MD) supplemented to 50 mg/liter of calcium and BHA for daptomycin and linezolid, respectively.

Susceptibility testing. MICs and minimum bactericidal concentrations (MBCs) of daptomycin and linezolid were determined in duplicate by broth microdilution at ~5×10^5 CFU/ml in 50 SMHB II as specified above, according to the CLSI guidelines (11). Etest methodology, according to manufacturer recommendations, was used for any isolate observed to grow on DAP- or LZD-containing agar plates (Mueller-Hinton agar [MHA] for daptomycin, BHA for linezolid) used for screening changes in susceptibility during model experiments.

Simulated endocardial vegetations. SEVs were prepared by mixing 0.05 ml of organism suspension (final inoculum, $10^{8.5}$ CFU/g), 0.5 ml of human cryoprecipitate antihemophilic factor from volunteer donors (American Red Cross, Detroit, MI), and 0.025 ml of platelet suspension (platelets mixed with normal saline, 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 (Becton, Dickinson, Sparks, MD) and introduced into the model. This methodology resulted in SEVs consisting of approximately 3 to 3.5 g/dl of albumin and 6.8 to 7.4 g/dl of total protein (1).

***In vitro* PK/PD model.** An *in vitro* PK/PD infection model consisting of a 250-ml glass apparatus with ports, where the SEVs were suspended, was utilized for all simulations. 75 SMHB II supplemented with 3.5 g/dl of human albumin (Baxter, Deerfield, IL) was used as the medium. The apparatus was prefilled with 250 ml of medium, and antibiotics were administered as boluses over a 96-h period into the central compartment via an injection port. The model apparatus was then placed into a 37°C incubator for the duration of 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 of the antibiotics. Simulated regimens included daptomycin at doses of 6 (DAP6), 8 (DAP8), 10 (DAP10), and 12 (DAP12) mg/kg daily (target peaks, 93.9, 123.3, 141.1, and 183.7 μg/ml, respectively; area under the concentration-time curve from 0 to 24 h [$AUC_{0\text{-}24}$], 631.8 to 1,277.4 μg · h/ml; average half-life, 8 h) (6) and linezolid (LZD) at a dose of 600 mg every 12 h (target peak, 15.1 μg/ml; average half-life, 5 h) (19, 36). All models were run in duplicate to ensure reproducibility.

Pharmacodynamic analysis. Two SEVs were removed from each model at 0, 4, 8, 24, 32, 48, 56, 72, and 96 h. The SEVs were homogenized and diluted in cold saline to be plated on BHI plates. For all samples, antimicrobial carryover was accounted for by serial dilution of the plated samples. If the anticipated dilution was near the MIC, samples were processed via vacuum filtration and washed through a 0.45-μm filter (Pall Corporation, Ann Arbor, MI) with normal saline to remove the antimicrobial agent. The limit of detection for determination of colony counts was 1 $\log_{10}$ CFU/g. Plates were then incubated at 37°C for 24 h, and the colony count was performed at the 24-h time point. 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 activity (99.9% kill) and bacteriostatic activity were defined as a ≥3 $\log_{10}$ CFU/g or a <3 $\log_{10}$ CFU/g reduction in colony count from that of the initial inocula, respectively. Inactivity was defined as no observed reduction compared to results for the initial inocula. The time to achieve a 99.9% bacterial load reduction was determined by linear regression (if $r^2 \geq 0.95$) or visual inspection.

Pharmacokinetic analysis. Pharmacokinetic samples were obtained, through the injection port of each model (duplicate samples) at 0, 0.5, 1, 2, 4, 8, 24, 32, 48, 56, 72, and 96 h for verification of target antibiotic concentrations. All samples were stored at −70°C until ready for analysis. Concentrations of DAP were determined by microbioassay utilizing *Micrococcus luteus* ATCC 9341. Briefly, blank quarter-inch disks were spotted with 10 μl of the standards or samples. Each standard was tested in duplicate by placing the disk on antibiotic medium 5 plates (Becton Dickinson, Sparks, MD) that were preswabbed with a 0.5 McFarland suspension of the test organism. Plates were incubated for 18 to 24 h at 37°C, and at 24 h, the zone sizes were measured using a protocol reader (Protocol; Microbiology International, Frederick, MD). Concentrations of 200, 100, 150, and 50 μg/ml were used as standards. This assay has a lower limit of detection of 5 μg/ml and demonstrates an interday coefficient of variation percentage (CV%) of ≤10.9% for daptomycin (2). Concentrations of LZD were determined using a validated high-performance liquid chromatography (HPLC) assay (30). Samples were measured using a system consisting of a ThermoFinnegan P4000 HPLC pump (San Jose, CA) with a model AS1000 fixed-volume autosampler, a model UV2000 UV detector, a Gateway Series e computer (Poway, CA), and the Chromquest HPLC data management system. The plasma standard curve for LZD ranged from 0.5 to 30 μg/ml and demonstrated a CV% of 1.04% to 4.39% for LZD (30). The half-life, area under the curve ($AUC_{0\text{-}24\ h}$), and peak concentration were determined by the trapezoidal method utilizing PK Ana-

TABLE 1 *In vitro* activity of daptomycin or linezolid in the pharmacokinetic/pharmacodynamic model

Strain	DAP MIC (μg/ml)	Regimen	T_{99}[b]	$T_{99}S$[c]	Reduction in $\log_{10}$ CFU/g from baseline (T_0)[d]		T_{96} mutant MIC (μg/ml)[e]
					24 h	96 h	
09-184D1051[a]	2	DAP6	32 h	NA	1.72 ± 0.10†	2.32 ± 0.05†	
		DAP8	4 h	NA	6 ± 0.32*†	2.87 ± 0.37†	
		DAP10	4 h	4 h	5.3 ± 0.39*†	4.71 ± 0.71*†	
		DAP12	4 h	4 h	6.4 ± 0.21*†	6.04 ± 1.46*†	
		LZD	NA	NA	−1.81 ± 0.09	−2.33 ± 0.03	
EFm11499[a]	4	DAP6	4 h	NA	2.31 ± 0.85†	−0.01 ± 0.19	
		DAP8	8 h	NA	3.34 ± 0.95†	1.17 ± 0.16	
		DAP10	4 h	4 h	4.46 ± 0.26*†	3.58 ± 1.45*†	
		DAP12	8 h	8 h	5.01 ± 0.18*†	6.56 ± 0.43*†	
		LZD	NA	NA	0.83 ± 0.27	1.08 ± 0.89	
EFs11496	0.5	DAP6	24 h	NA	4.18 ± 1.27†	0.65 ± 0.37	8
		DAP8	24 h	NA	4.5 ± 0.7†	2.27 ± 1.07†	16
		DAP10	4 h	4 h	5.61 ± 0.18†	6.46 ± 1.19*†	8
		DAP12	4 h	4 h	6.87 ± 0.12*†	5.89 ± 0.11*†	
		LZD	NA	NA	0.24 ± 0.07†	0.28 ± 0.25	

[a] No resistant mutants recovered from DAP or LZD simulated regimens.
[b] NA, not achieved; T_{99}, time to achieve a 99.9% colony reduction.
[c] NA, not achieved; $T_{99}S$, time to achieve a 99.9% colony reduction that was sustained to 96 h.
[d] T_0, time zero; *, P value of <0.05 for improved killing compared with that for the DAP 6 regimen; †, P value of <0.05 for improved killing compared with that for the LZD regimen.
[e] Recovered nonsusceptible mutants (performed via BMD).

lyst software (version 1.10; MicroMath Scientific Software, Salt Lake City, UT).

Nonsusceptibility. Development of nonsusceptibility was evaluated at multiple time points throughout the simulation, at 24, 48, 72, and 96 h for days 1 to 4. Samples (100 μl) from each time point were plated on agar plates (BHI for linezolid, MHA for daptomycin) containing three times the MIC of the respective antibiotic to assess the development of resistance. Plates were then examined for growth after 24 to 48 h of incubation at 37°C. Any observed growth was tested for changes in susceptibility by both Etest and broth microdilution.

Statistical analysis. Changes in CFU/g at 24, 48, 72, and 96 h were compared by one-way analysis of variance with Tukey's *post hoc* test. A P value of ≤0.05 was considered significant. All statistical analyses were performed using SPSS statistical software (release 20.0; SPSS, Inc., Chicago, IL).

RESULTS

Organism susceptibilities to DAP are displayed in Table 1. The two *E. faecium* isolates (EFm11499, 09-184D1051) and the *E. faecalis* isolate (EFs11496) were susceptible to DAP, displaying MICs of 4, 2, and 0.5 μg/ml, respectively. One *E. faecium* isolate (09-184D1051) was LZD resistant, with an MIC of 16 μg/ml. EFm11499 and EFs11496 were susceptible to LZD, with MICs of 2 and 1 μg/ml, respectively. MBCs of DAP were 4, 8, and 4 μg/ml for 09-184D1051, EFm11499, and EFs11496, respectively. MBCs of LZD were >16 for all isolates. No change in DAP or LZD susceptibility was found for either of the *E. faecium* isolates during the study. *In vitro* changes in susceptibility at 96 h are displayed in Table 1. Decreased susceptibility to DAP developed in EFs11496 when exposed to DAP6 and DAP8, producing a 32-fold increase in MIC (which increased from 0.5 to 16 μg/ml). In DAP10, one SEV sample from a single model developed an increased MIC to DAP (MIC of 8 μg/ml). This organism was found stable to three overnight passes onto antibiotic-free medium. No resistance was seen with DAP12 or LZD.

PK parameters of simulated regimens are displayed in Table 2. Observed pharmacokinetic parameters for LZD were within 12% of the targeted range. The C_{max} and half-life for LZD were 14.4 ± 0.3 μg/ml and 4.4 ± 0.28 h (targeted values, 15.1 μg/ml and 5 h). Observed PK parameters for DAP were all within 11% of the targeted values. The C_{max} and half-life observed were 105.1 ± 10.5 μg/ml and 7.93 h, 123.1 ± 7.4 μg/ml and 8.54 h, 144.2 ± 4.0 μg/ml and 7.87 h, and 188.7 ± 4.9 μg/ml and 8.36 h (targeted C_{max}, 93.9, 123.3, 141.1, and 183.7 μg/ml; average half-life, 8 h) for DAP6, DAP8, DAP10, and DAP12, respectively. DAP AUC_{24}/MIC ratios ranged from 235 to 4,367 (Table 2) and varied depending on the organism MIC.

The *in vitro* activity of the simulated regimens is displayed in Table 1. LZD was bacteriostatic against EFm11499 and EFs11496 (Fig. 1) and displayed no appreciable activity against LZD-resistant 09-184D1051. All DAP regimens demonstrated bactericidal activity against LZD-resistant 09-184D1051. DAP6 and DAP8 displayed improved killing over that of LZD against 09-184D1051, EFm11499, and EFs11496, with times to a 99.9% kill (T_{99}) of 32, 4, and 24 h for DAP6 and 4, 8, and 24 h for DAP8, respectively. However, both DAP6 and DAP8 failed to maintain bactericidal activity at 96 h in all three strains. DAP6 exhibited the least effect with the most regrowth, patterned closely by DAP8. In contrast, the rapid bactericidal activity of DAP10 and DAP12 was sustained to 96 h. These two regimens were similar except against EFm11499, which had the highest MIC for daptomycin. Against 09-184D1051, EFm11499, and EFs11496, DAP10 and DAP12 displayed rapid and sustained bactericidal activity ($T_{99}S$) at 96 h, with a $T_{99}S$ of 4 h for all isolates for DAP10 and a $T_{99}S$ of 4, 8, and 4 h, respectively, for DAP12. These regimens were significantly more

TABLE 2 Pharmacokinetic parameters of daptomycin and linezolid achieved in the PK/PD model[a]

Drug, dosage, and strain	C_{max} (μg/ml) (target value)	Half-life (h)	AUC_{0-24} (μg · h/ml)	AUC_{24}/MIC[c]
Daptomycin, 6 mg/kg/day	105.1 ± 10.5 (93.9)	7.86 ± 0.8	941.5 ± 31.2	
09-184D1051				471
EFm11499				235
EFs11496				1,883
Daptomycin, 8 mg/kg/day	123.1 ± 7.4 (123.3)	8.54 ± 0.2	1,356.9 ± 121	
09-184D1051				678
EFm11499				339
EFs11496				2,714
Daptomycin, 10 mg/kg/day	144.2 ± 4.0 (141.1)	7.87 ± 2.6	1,540.3 ± 280.9	
09-184D1051				770
EFm11499				385
EFs11496				3,080
Daptomycin, 12 mg/kg/day	188.7 ± 4.9 (183.7)	8.36 ± 0.33	2,183.6 ± 95.7	
09-184D1051				1,092
EFm11499				546
EFs11496				4,367
Linezolid, 600 mg q12h[b]	14.4 ± 0.3 (15.1)	4.4 ± 0.28	158.3 ± 3.7	
09-184D1051				79
EFm11499				40
EFs11496				317

[a] C_{max}, maximum concentration; AUC_{0-24}; area under the concentration-time curve from 0 to 24 h. Results are expressed as means ± standard deviations.
[b] q12h, every 12 h.
[c] Varied based on organism MIC.

efficacious at decreasing the $\log_{10}$ CFU/g than DAP6, DAP8, and LZD at 72 and 96 h against 09-184D1051 ($P = 0.008$), at 96 h against EFm11499 ($P = 0.012$), and at 48 to 96 h against EFs11496 ($P = 0.011$). DAP10 had an overall kill count reduction of 4.46 to 5.61 $\log_{10}$ CFU/g at 24 h and 3.58 to 6.46 $\log_{10}$ CFU/g at 96 h. DAP12 had an overall kill count reduction of 5.01 to 6.87 $\log_{10}$ CFU/g at 24 h and 5.89 to 6.56 $\log_{10}$ CFU/g at 96 h. For EFm11499, DAP12 demonstrated significantly more killing than DAP10 at 72 and 96 h ($P < 0.001$), but activity was not significantly improved over that of DAP10 for 09-184D1051 or EFs11496 at 96 h.

DISCUSSION

Enterococcal infections are difficult to treat, especially in immunocompromised hosts and in those with deep-seated, high-inoculum infections, such as device-related infections and infective endocarditis (5, 26, 34, 37). Few therapeutic options are available, and bactericidal agents or combination therapy have been preferred for life-threatening infections (23). Linezolid demonstrates bacteriostatic activity, and prolonged therapy can result in thrombocytopenia. Quinupristin-dalfopristin is a last-line effort, being poorly tolerated with substantial toxicities. Daptomycin is a concentration-dependent cyclic lipopeptide with demonstrated *in vitro* bactericidal activity against enterococci. Daptomycin MIC values are higher for enterococci than for *S. aureus* (11, 38). Maximum effect (E_{max}) models suggest that increased doses (>7.9 mg/kg/day) may be needed to surmount this; however, a paucity of data exist evaluating escalating doses for activity against enterococci (9). Daptomycin resistance is still relatively rare; however, clinical cases are emerging for both *E. faecium* and *E. faecalis*, notably in patients with more complicated conditions (e.g., osteomyelitis, endocarditis, device-related infections, biofilm) (5, 26, 34). A recent review of daptomycin nonsusceptibility in enterococci from 23 studies from 2003 to 2010 reported an overall prevalence rate of 0.6% (23). The majority of the strains reported were vancomycin resistant (93.3%), with 88% being reported as *E. faecium*. Of interest, the most common dosage of daptomycin associated with resistance was 6 mg/kg/day. Although the optimal dosage of daptomycin for treatment of enterococcal infections is unknown, the authors suggested that dosages greater than what is currently recommended (4 to 6 mg/kg/day) may be required (23). The exact mechanism of enterococcal resistance to daptomycin is not fully elucidated. Similar to *S. aureus* resistance, enterococcal resistance is thought to result from several factors, including altered cell membrane composition and increased positive surface charge, altered ability of daptomycin to depolarize the cell, and cell wall thickening associated with genetic mutations; however, the affected genes appear to be different from those observed for *S. aureus* resistance (4, 23, 35, 40). Insights into the mechanism of daptomycin resistance in enterococci have recently been provided (4, 35). Whole-genome sequencing of a clinical strain pair of daptomycin-susceptible and -resistant *E. faecalis* obtained from the blood of a patient before and after daptomycin therapy, respectively, indicated that changes in two genes were necessary and sufficient for daptomycin resistance: (i) the *liaF* gene, which encodes a member of a three-component regulator (LiaFRS) that is likely to be involved in the stress-sensing response to cell envelope antibiotics and antimicrobial peptides, and (ii) the glycerophosphoryl diester phosphodiesterase gene, predicted to be involved in phospholipid metabolism. The genetic changes were associated with important ultrastructural alterations of the cell envelope and

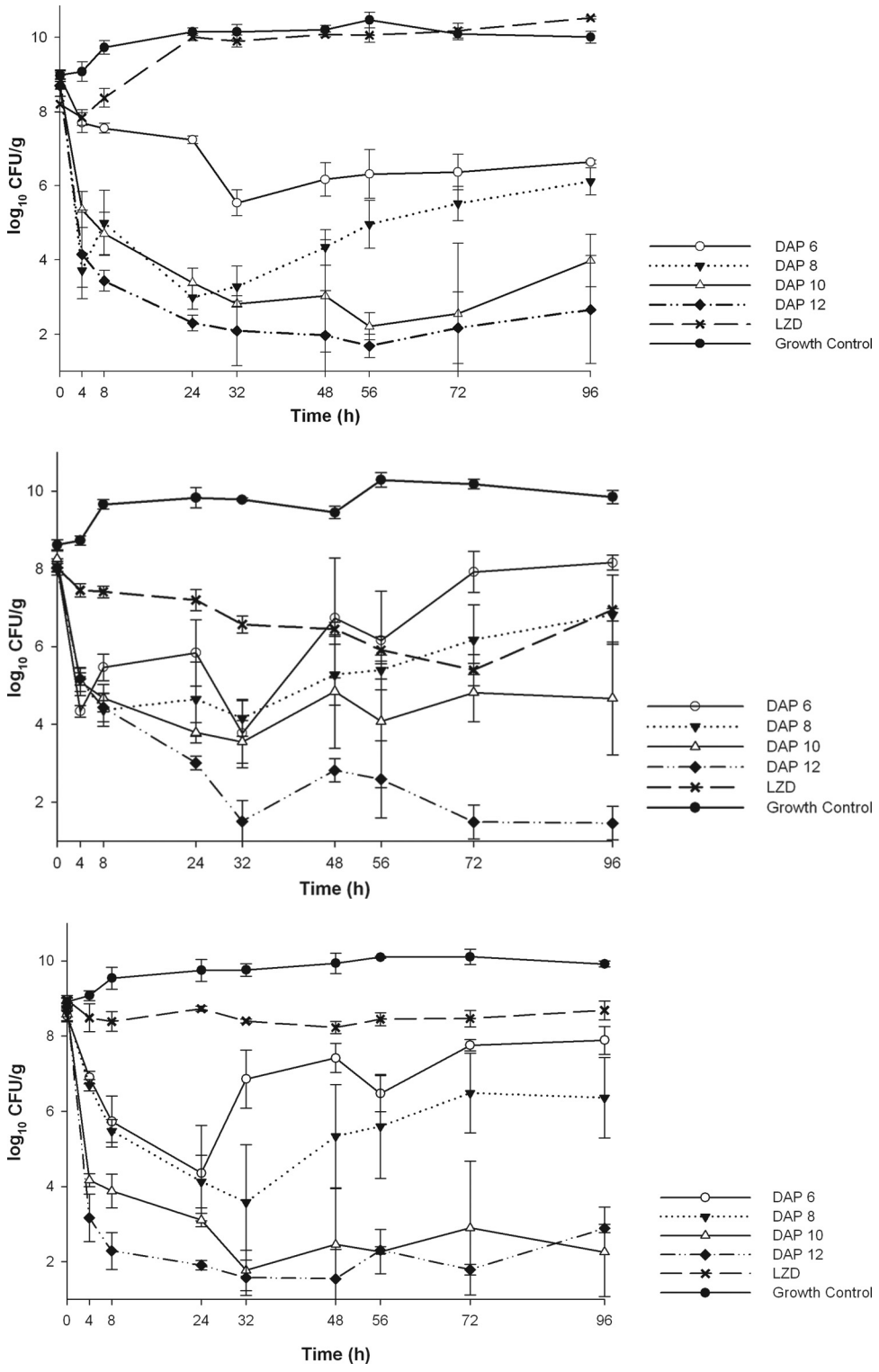

FIG 1 Activities of LZD, DAP6, DAP8, DAP10, and DAP12 against 09-184D1051 (A), EFm11499 (B), and EFs11496 (C).

affected the ability of daptomycin to depolarize and permeabilize the cell membrane (4). *In vitro* selection of *E. faecalis* V583 in high concentrations of daptomycin resulted in changes in seven different genes. The predominant alteration was found in a gene encoding a putative cardiolipin synthase found in all resistant mutants observed. Cloning of the mutated allele of the cardiolipin synthase gene in a multicopy plasmid resulted in reduced susceptibility to daptomycin of V583, supporting the role of phospholipid enzymes in the resistance mechanism (35). Of note, changes in both the putative LiaFRS system and cardiolipin synthase have been

observed with unrelated daptomycin-resistant clinical isolates of *E. faecium* and other *E. faecalis* isolates (4, 35).

The present study demonstrates a dose-response curve utilizing escalating doses of daptomycin compared with standard-dose linezolid against clinical vancomycin-resistant *E. faecium* and *E. faecalis* strains. In this study, we found that DAP6, as predicted, did not maintain bactericidal activity against *E. faecium* and *E. faecalis*, and regrowth was noted at 96 h. DAP10 and DAP12 displayed the most significant and sustained killing over the 96-h duration. We noted a concentration-dependent effect; as doses were escalated, bactericidal activity was prolonged. Overall, there was not a profound difference between results for the isolates. Although the overall doses and AUC requirements for effective and sustained activity were similar for *E. faecalis* and *E. faecium*, the AUC_{24}/MIC ratios varied considerably based on the MIC (range, 0.5 to 4 μg/ml).

The PD parameter for daptomycin that best predicts outcome for *S. aureus* is the AUC_{24}/MIC ratio (8, 27). Louie et al. reported an 80% maximal kill for *S. aureus*, and the AUC_{24}/MIC ratio that correlated with bactericidal activity for the daptomycin dose of 6 mg/kg in animals was 245 to 516, depending on the organism MIC (27). Cha et al. reported an AUC_{24}/MIC ratio of 502 and 705 for daptomycin doses of 6 and 8 mg/kg daily, respectively, for vancomycin-resistant *E. faecium* (10). In the current investigation, the $AUC_{0\text{-}24}$ was proportional to the dose administered (6 to 12 mg/kg/day), and the corresponding AUC_{24}/MIC ratio ranged from 235 to 4,367 and varied according to the organism MIC. The minimum $AUC_{0\text{-}24}$ needed for sustained bactericidal activity was 1,540, and the corresponding AUC_{24}/MIC ratio was 214 to 1,715, dependent upon the MIC (DAP MIC of 0.5 to 4 μg/ml).

Limitations of this study include the utilization of only three isolates for testing; therefore, the results may not be representative of those for all daptomycin and enterococcal interactions. A longer duration of exposure (e.g., >96 h) is needed to verify that killing is sustained and that there is suppression of resistance. In addition, a specific dose breakpoint should be pursued to determine the optimal AUC_{24}/MIC exposure for each *Enterococcus* sp. best correlating with sustained bactericidal killing and suppression of emergence of resistance.

In conclusion, daptomycin doses of ≥10 mg/kg per day may be necessary to treat high-inoculum vancomycin-resistant *E. faecium* and *E. faecalis* infections, such as those found in patients with infective endocarditis.

ACKNOWLEDGMENTS

This investigator-initiated study was funded by a research grant from Cubist Pharmaceuticals.

We are grateful to Audrey Wanger for providing isolate 09-184D1051 and to Marcus Zervos for providing isolates SF11496 and SF11499.

M.J.R. has received grant support, has served as a consultant, or has participated as a speaker for Astellas, Cubist, Forest Laboratories, Pfizer, Rib-X, and Novartis and is supported in part by grant R21AI092055 from the NIAID. C.A.A. has received lecture fees, research support, and consulting fees from Pfizer, Inc., and Cubist and has served as a speaker for Novartis. C.A.A. is supported in part by NIH grants R00 AI72961 and R01 AI093749 from the NIAID. B.E.M. has received grant support from Johnson & Johnson, Astellas, Palumed, Intercell, and Cubist, has served as a consultant for Astellas, Cubist, The Medicines Company, Pfizer, Rib-X, AstraZeneca, and Duranta Therapeutics, and was supported in part by NIH grant R01 AI72961.

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