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Comparative Pharmacodynamics of Single-Dose Oritavancin and Daily High-Dose Daptomycin Regimens against Vancomycin-Resistant *Enterococcus faecium* Isolates in an *In Vitro* Pharmacokinetic/Pharmacodynamic Model of Infection

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ABSTRACT There are limited therapeutic options to treat infections caused by vancomycin-resistant *Enterococcus faecium* (VREfm). The lipoglycopeptide oritavancin exhibits *in vitro* activity against this pathogen, although its utility against infections caused by VREfm has not been clinically established. In this study, the pharmacodynamic activity of free-drug levels associated with 12 mg/kg/day of daptomycin and a single 1,200-mg dose of oritavancin were determined against three VanA VREfm isolates in an *in vitro* pharmacokinetic/pharmacodynamic model.

KEYWORDS daptomycin, *Enterococcus faecium*, oritavancin, lipoglycopeptide, vancomycin resistance

Vancomycin-resistant enterococci (VRE) are an important cause of nosocomial infections in the United States (1, 2). The oxazolidinone linezolid remains the only agent indicated for infections caused by vancomycin-resistant *Enterococcus faecium* (VREfm). Although the lipopeptide daptomycin is not indicated for the treatment of VRE infections, high-dose regimens (≥8 mg/kg/day, exceeding the approved dose of 6 mg/kg/day for *Staphylococcus aureus* bloodstream infections) are often considered for first-line therapy (3, 4). Higher doses of daptomycin may improve outcomes by maximizing exposure to compensate for the elevated daptomycin MICs of VRE relative to *S. aureus* and by limiting the emergence of daptomycin nonsusceptibility (5, 6).

The long-acting lipoglycopeptide oritavancin is approved as a single 1,200-mg dose treatment of acute bacterial skin and skin structure infections caused by Gram-positive pathogens (7). Oritavancin exerts *in vitro* activity against VRE isolates expressing both the VanA and VanB phenotypes (8–10). It has shown efficacy in a rat model of vancomycin-resistant *E. faecium* bloodstream infection (11) and enhanced activity in combination with gentamicin in a rabbit model of enterococcal endocarditis (12, 13). In this study, we describe the pharmacodynamic (PD) activity of free-drug levels associated with 12 mg/kg/day of daptomycin and a single 1,200-mg dose of oritavancin against clinical isolates of VREfm in an *in vitro* pharmacokinetic (PK)/PD model.

(Part of this work was presented at IDWeek 2016, New Orleans, LA, 26 to 30 October 2016 [14].)

Oritavancin (The Medicines Company, Parsippany, NJ) and daptomycin (APIChem Technology Company, Hangzhou, China) broth microdilution MICs of the three VanA VREfm clinical isolates ATCC 51559, B7181440, and B7231527 were determined follow-

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ing CLSI M07-A10 guidelines (6) using the quality control isolate *Enterococcus faecalis* ATCC 29212 to assess appropriate drug and assay performance (7). MICs of the derived mutants that survived daptomycin challenge were determined before and after serial passage on nonselective medium (Mueller-Hinton agar) for 5 days to assess the stability of the susceptibility changes. Subcultures of the VREfm isolates in exponential phase were inoculated at 10^6 CFU/ml into a dilutional one-compartment *in vitro* PK/PD model (15) containing 250 ml of cation-adjusted Mueller-Hinton broth (CAMHB) supplemented with either 50 μg/ml $CaCl_2$ (for daptomycin) or 0.01% polysorbate 80 (for oritavancin). Daptomycin was added as bolus daily doses and a pump flow rate (0.34 ml/min for 72 h) was used to simulate free-drug exposures expected from 12 mg/kg/day in healthy volunteers (assuming protein binding of 91.5%, a free peak concentration [fC_{max}] of 15.6 μg/ml, a half-life [$t_{1/2}$] of 8 h, and an area under the concentration-time curve from 0 to 24 h [$fAUC_{0-24\ h}$] of 171 $\mu g \cdot h/ml$) (16). For oritavancin, a single dose was infused over 3 h and flow rates (1.25 ml/min for 5 h, 0.94 ml/min for 1 h, 0.31 ml/min for 23 h, and 0.04 ml/min for 43 h) were used to approximate the mean free-drug concentration-time profile (assuming protein binding of 85% [17]; an fC_{max} of 20.7 μg/ml; alpha, beta and gamma $t_{1/2}$ of 2.3 h, 13.4 h, and 245 h, respectively, and an $fAUC_{0-24}$ of 178 $\mu g \cdot h/ml$) observed in patients receiving a 1,200-mg dose (15, 18). After 5 h of drug exposure, cultures were transferred to new sterilized *in vitro* PK/PD model systems to ensure that only drug-exposed bacteria were present. For control cultures (no drug exposure), fresh media were supplied using the flow rates indicated for oritavancin over 24 h (until turbid cultures were apparent). Aliquots were sampled at the indicated times for bacterial viability as previously described (15) and then frozen at −20°C until drug concentrations were determined. Statistical differences ($P < 0.05$) in mean changes in bacterial viability (log CFU/ml) relative to inoculum were compared by *t* test. Daptomycin concentrations were quantified using a described bioassay (15). Oritavancin was quantified by fluorescence polarization using a fluorescein-labeled D-Ala-D-Ala peptide (Ac-L-Lys-Ala-D-Ala-OH; Pharmaron, Irvine, CA) (19). Oritavancin standards (0.06 to 32 μg/ml) were prepared in CAMHB containing 0.01% P80 (assay linear range of sensitivity 0.25 μg/ml to 16 μg/ml); samples from the *in vitro* PK/PD model were diluted 1 in 4 in CAMHB. Assays (100 μl) were performed in 96-well plates (reference number 3694; Corning Inc., Corning, NY) using a final concentration of 90 nM of the fluorescein-labeled peptide and excitation and emission wavelengths of 485 nm and 535 nm, respectively. PCR amplification of the *liasFSR* locus and *cls* gene from genomic DNA (GenElute Bacterial Genomic DNA kit; Sigma-Aldrich, Oakville, Ontario, Canada) was performed using published primer sequences (20). For amplification of *liaS* in ATCC 51559, the primers 5′-AAAGGGATAGGCAGAACACG-3′ (forward) and 5′-CAATACCAGCTACTCGTTCTTTGA-3′ (reverse) were used due to allelic sequence variation. Sanger sequencing of the amplicons was performed at McGill University and the Génome Québec Innovation Centre (Montreal, Québec, Canada).

The three VREfm isolates were susceptible to daptomycin (MIC $\leq$ 4 μg/ml), exhibiting daptomycin MICs of 2 to 4 μg/ml (Table 1); oritavancin MICs ranged from 0.06 to 0.5 μg/ml. Exposure of the VREfm isolates to daptomycin at free-drug concentrations expected from dosing with 12 mg/kg/day (obtained PK parameters shown in Table 2) resulted in rapid bactericidal activity ($\geq$3 log kill relative to the starting inoculum) within 5 h and sustained suppression of regrowth for 24 h (Fig. 1 and Table 3). However, each isolate exhibited instances of bacterial regrowth by 48 h following exposure to the second dose of daptomycin (in 1 of 4 replicates of ATCC 51559 [Fig. 1A, inset], 4 of 4 replicates of B7181440 [Fig. 1B], and 3 of 4 replicates of B7231527 [Fig. 1C]). Regrowth among the three VREfm isolates was coincident with stable increases in daptomycin MICs ranging from 4- to 8-fold (daptomycin MICs of 16 μg/ml) relative to their corresponding parental isolates (Table 1). In one derived mutant (1440-141-1), the oritavancin MIC also increased 4-fold above its parental MIC (Table 1) and therefore it will be of interest to elucidate the genetic changes that provoke cross-reduced susceptibility to oritavancin in VREfm. Other studies have also shown the development of reduced susceptibility to daptomycin in VREfm (including ATCC 51559) when modeling

TABLE 1 Characterization of VREfm parental isolates and derived mutants that survived daptomycin challenge in the *in vitro* PK/PD model

Parental isolate or derived mutant	MIC (μg/ml) of[a]:		Mutation[b]
	Daptomycin	Oritavancin	
ATCC 51559	2	0.25	NA[c]
51559-146-4[d]	16	0.25	Unknown
B7181440	2	0.06	NA
1440-140-1[d]	16	0.12	Unknown
1440-140-2[d]	16	0.12	LiaF truncation
1440-141-1[d]	16	0.25	Unknown
1440-141-2[d]	16	0.12	LiaF L181S
B7231527	4	0.5	NA
1527-140-4[d]	16	1	LiaS V129E
1527-141-3[d]	16	0.5	Unknown
1527-141-4[d]	16	0.25	Unknown

[a]Modal MICs are presented from ≥3 independent determinations.
[b]Mutations in the *liaFSR* system and cardiolipin synthase gene *cls* were determined by DNA sequencing.
[c]NA, not applicable.
[d]Daptomycin-nonsusceptible derived mutant. One of four replicates of ATCC 51559, four of four replicates of B7181440, and three of four replicates of B7231527 showed regrowth of derived mutants with reduced susceptibility to daptomycin following daptomycin exposure in the *in vitro* PK/PD model. MICs of the derived mutants were unchanged following 5 days of passage on nonselective medium.

free-drug exposures associated with ≤10 mg/kg/day (16, 21). In contrast, modeling of total drug exposures (in the presence of 3.5 g/dl of human albumin) associated with ≥10 mg/kg/day prevented the development of reduced susceptibility (5, 6) and established a total AUC_{0-24}/MIC (area under the concentration-time curve from 0 to 24 h divided by the MIC) value of 781 as a cutoff to prevent the emergence of reduced susceptibility for the tested VREfm isolate. The projected total AUC_{0-24}/MIC ratio for VREfm ATCC 51559 and B7181440 is 953 ($fAUC_{0-24}$ of 162, 91.5% protein binding, and daptomycin MICs of 2 μg/ml), an exposure that exceeded the cutoff value but did not prevent emergence of reduced susceptibility. In immunocompetent hosts, it is plausible that the small population of daptomycin-nonsusceptible bacteria that emerge (as shown in Fig. 1) could be eliminated by the immune system. Nevertheless, case reports of emergence of reduced susceptibility in patients receiving 10 mg/kg/day have been published (22, 23) and hence the appropriateness of the current daptomycin susceptibility breakpoint and high-dose daptomycin regimens for enterococci are under scrutiny (24).

TABLE 2 Pharmacokinetic parameters obtained for the indicated dosing regimens in the *in vitro* PK/PD model

Parameter[a]	Daptomycin, 12 mg/kg/day		Oritavancin, 1,200-mg single dose	
	Targeted[b]	Obtained ± SD	Targeted[c]	Obtained ± SD
fC_{max} (μg/ml)	15.6	15.1 ± 0.2	20.7	20.1 ± 3.1
$fAUC_{0-24}$ (μg · h/ml)	171	162 ± 7.7	178	164 ± 30.5
$fAUC_{0-72}$ (μg · h/ml)	ND	ND	246	223 ± 46.2
$t_{1/2}$ (h)	8	8.1 ± 0.5	ND	ND

[a]fC_{max}, free peak concentration; $fAUC_{0-24}$, area under the concentration-time curve from 0 to 24 h; $fAUC_{0-72}$, area under the concentration-time curve from 0 to 72 h; $t_{1/2}$, half-life; SD, standard deviation; ND, not determined.
[b]The targeted PK values for daptomycin were derived from Benvenuto et al. (28) and the prescribing information (29) with the assumption of 91.5% protein binding. The daptomycin $t_{1/2}$ in the *in vitro* PK/PD model was determined by nonlinear regression analysis using GraphPad Prism 6 software. The targeted fAUC (area under the concentration-time curve for the free, unbound fraction of a drug) values were calculated using a simulated daptomycin concentration-time profile (Prism 6) that respects the targeted PK parameters (fC_{max} of 15.6 μg/ml and $t_{1/2}$ = 8 h).
[c]The targeted PK values for oritavancin were derived from reference 18 and assuming 85% protein binding. The targeted fAUC values were calculated (Prism 6) from the mean oritavancin concentration-time profile obtained from population PK modeling (18).

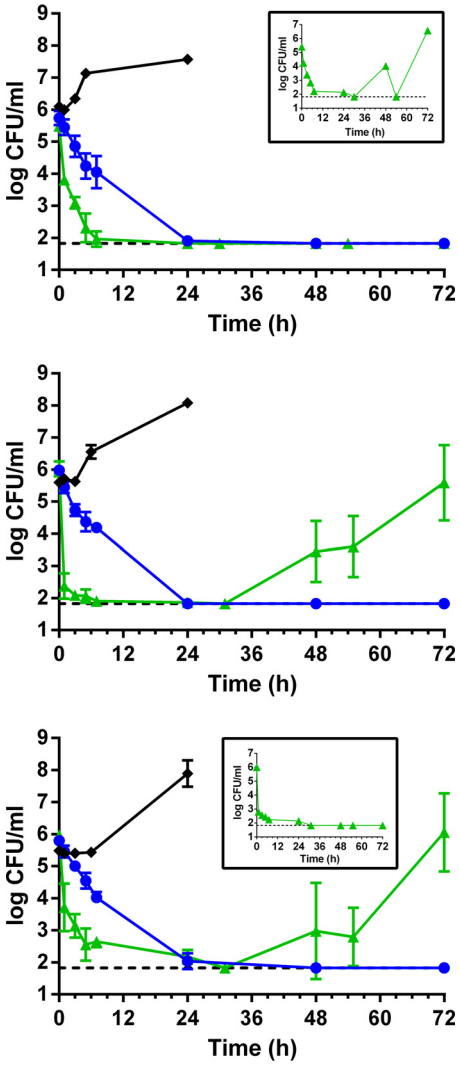

FIG 1 Pharmacodynamic activity of daptomycin and oritavancin at free-drug exposures associated with 12 mg/kg/day daptomycin (green triangles) and a single 1,200-mg dose of oritavancin (blue circles) against the clinical isolates of VanA VREfm ATCC 51559 (A), B7181440 (B), and B7231527 (C) in an *in vitro* PK/PD model over 72 h. Mean values ± standard deviation (SD) are from two independent experiments done in duplicate. Control cultures are shown for each isolate (black diamonds), using the flow rates for oritavancin to supply fresh drug-free medium over 24 h. The inset in panel A depicts the single occurrence of regrowth of ATCC 51559 following exposure to daptomycin. The inset in panel C depicts the single occurrence of eradication of B7231527 following exposure to daptomycin. The dashed line indicates the limit of detection (<66.7 CFU/ml).

Infusion of oritavancin over 3 h into the *in vitro* PK/PD model (obtained PK parameters shown in Table 2) resulted in bactericidal activity against the three VREfm isolates that was significantly less rapid than that of daptomycin over the first 7 h of exposure ($P < 0.05$) (Table 3 and Fig. 1), as bacterial counts were reduced by approximately 1.7 log. Whereas bacterial killing by oritavancin was not significantly different to that of daptomycin at 24 h ($P > 0.05$) (Table 3), oritavancin reduced counts of all three VREfm isolates to below the limit of detection (≤66.7 CFU/ml) between 48 and 72 h (Fig. 1) with suppression of regrowth of B7181440 and B7231527 that differed significantly from the regrowth of those isolates at 72 h following daptomycin exposure ($P < 0.05$) (Table 3). A limitation of this study is that the duration of oritavancin exposure was limited to 72 h and therefore it is unknown whether longer exposures that more completely represent the terminal half-life of oritavancin (245 h) would confirm the suppression of regrowth.

TABLE 3 Mean changes in bacterial viability relative to inoculum of the tested VREfm isolates exposed to daptomycin and oritavancin in the *in vitro* PK/PD model

	Mean decrease in bacterial viability relative to starting inoculum (log CFU/ml ± SD) for[a]:					
	VREfm ATCC 51559		VREfm B7181440		VREfm B7231527	
Time (h)	Daptomycin[b]	Oritavancin	Daptomycin	Oritavancin	Daptomycin	Oritavancin
1	−1.7 ± 0.2	−0.3 ± 0.2[c]	−3.6 ± 0.4	−0.5 ± 0.3[c]	−2.3 ± 0.8	−0.3 ± 0.1[c]
3	−2.4 ± 0.1	−0.9 ± 0.2[c]	−4.0 ± 0.2	−1.2 ± 0.3[c]	−2.8 ± 0.4	−0.8 ± 0.1[c]
5	−3.2 ± 0.4	−1.5 ± 0.2[c]	−4.0 ± 0.1	−1.6 ± 0.4[c]	−3.4 ± 0.5	−1.2 ± 0.2[c]
7	−3.5 ± 0.1	−1.7 ± 0.5[c]	−4.2 ± 0.3	−1.7 ± 0.1[c]	−3.3 ± 0.1	−1.7 ± 0.2[c]
24	−3.7 ± 0.1	−3.8 ± 0.2	−4.2 ± 0.1	−4.2 ± 0.2	−3.8 ± 0.1	−3.8 ± 0.2
48	−3.7 ± 0.1	−3.9 ± 0.2	−2.8 ± 1.2	−4.2 ± 0.2	−3.0 ± 1.5	−4.0 ± 0.1
72	−3.7 ± 0.1	−3.9 ± 0.2	−0.7 ± 1.5	−4.2 ± 0.2[c]	0.1 ± 1.2	−4.0 ± 0.1[c]

[a]Mean ± SD values shown are from two independent experiments done in duplicate ($n = 4$).
[b]The calculations of mean decrease in bacterial viability for the daptomycin exposures excluded both that of the single replicate of ATCC 51559 in which reduced susceptibility to daptomycin was observed and that of the single replicate of B7231527 in which no regrowth occurred.
[c]Decrease in log CFU/ml is significantly different ($P < 0.05$, *t* test) from the corresponding value obtained for daptomycin at the same exposure time.

Mutations in the cardiolipin synthase gene *cls* and the three-component regulatory system operon *liaFSR* have been implicated in clinical development of reduced susceptibility to daptomycin in VREfm isolates (20, 25). No mutations in *cls* were observed in the derived daptomycin-nonsusceptible mutants. A total of 171 singlenucleotide polymorphisms (SNPs) were observed within the *liaFSR* operon of ATCC 51559 relative to its counterpart shared by B7181440 and B7231527 (data not shown); the SNPs accounted for 7, 12, and 3 amino acid differences in LiaF, LiaS, and LiaR, respectively. Queries of the GenBank database revealed that both allelic variations are conserved in different *E. faecium* isolates (data not shown). Three of the eight derived mutants had incurred mutations within the *liaFSR* locus (Table 1). In mutant 1440-140-2, a deletion of the thymine residue at position 24 of *liaF* caused a frameshift mutation, presumably truncating the resultant protein. In mutant 1440-141-2, a nonsynonymous point mutation resulted in a change of leucine to serine at position 181 of LiaF. Impairment of LiaF function in an *E. faecalis* isolate was shown to cause a 3-fold increase in its daptomycin MIC and abolished the bactericidal activity of the drug (26). In mutant 1527-140-4, a nonsynonymous point mutation resulted in a change of valine to glutamic acid at position 129 of LiaS. For the other five derived mutants, no changes in *liaFSR* were observed and consequently analysis of other genes that have been implicated in reduced susceptibility to daptomycin (27) is warranted.

In conclusion, oritavancin demonstrated sustained bactericidal activity *in vitro* against VREfm isolates at free-drug exposures expected to occur in patients receiving a single 1,200 mg-dose. These results support further investigation of its safety and efficacy in clinical VREfm infections.

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