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Antibacterial Activity of Oritavancin and Daptomycin against Clinical Isolates of Vancomycin-Resistant *Enterococcus faecium* in In Vitro Pharmacokinetic/Pharmacodynamic Models

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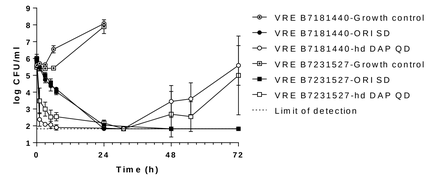
Abstract

Background: The lack of new therapies to treat infections caused by vancomycin-resistant Enterococcus (VRE) has led to clinical use of daily high-dose daptomycin (hd DAP QD) regimens (≥ 10 mg/kg) to optimize outcomes. The long-acting lipoglycopeptide oritavancin (ORI) exhibits in vitro activity against VRE, although its safety and efficacy in treating clinical VRE infections have not been established. This study tested simulated human dosing regimens of a single 1200 mg dose of ORI (ORI SD) and hd DAP QD against clinical isolates of vancomycin-resistant *Enterococcus faecium* (VREfm) in an in vitro pharmacokinetic/pharmacodynamic model (IVPM) over 72h.

Methods: Inocula (10^6 CFU/ml) of VREfm (VanA) clinical isolates B7181440 (ORI MIC= 0.06 µg/ml; DAP MIC= 2 µg/ml) and B7231527 (ORI MIC= 0.5 µg/ml; DAP MIC= 4 µg/ml) were exposed to simulated free drug (*f*d) plasma concentrations associated with ORI SD (3h infusion targeting a free peak [*f*Cmax] of 20.7 µg/ml based on 85% plasma protein binding [PPB]; α, β and γ T½ of 2.3h, 13.4h, and 245h, respectively) or hd DAP QD (12 mg/kg; bolus dose targeting a *f*Cmax of 15.6 µg/ml based on 91.5% PPB and T½ of 8h) over 72h in one-compartment IVPMs. ORI and DAP concentrations in the IVPM were determined by fluorescence polarization and bioassay, respectively. Results presented are from two independent experiments performed in duplicate. MICs were determined by broth microdilution following CLSI M7-A10 guidelines.

Results: *f*d levels associated with hd DAP QD exhibited rapid bactericidal activity (≥3-log reduction in CFU/ml) within 3h against both VREfm isolates whereas ORI SD exerted bactericidal activity over 24h (Figure). Sustained bactericidal activity was observed with hd DAP QD over 24h; however, both VRE isolates exhibited subsequent regrowth at 48h coincident with 2- to 16-fold increases in DAP MICs. In contrast, no regrowth of either VREfm isolate occurred with the ORI SD as bacterial counts remained below the limit of detection (<67 CFU/ml) between 24 to 72h.

Conclusion: The sustained bactericidal activity of ORI SD against VanA VREfm at simulated human exposures in the IVPM warrants further study of ORI as a treatment option for clinical VRE infections.



*Note that results for VREfm ATCC 51559 are not shown in the abstract but included in the poster.

Background

Nosocomial infections caused by vancomycin-resistant enterococci (VRE) pose serious healthcare challenges due to limited treatment options. The lipopeptide daptomycin exhibits in vitro activity against VRE and despite lacking regulatory approval for this organism, is now considered first-line therapy for severe VRE infections (1,2). High-dose regimens (≥ 10 mg/kg) are typically used and are thought to optimize outcomes by compensating for the elevated daptomycin MICs of enterococci relative to staphylococci and to prevent clinical development of reduced susceptibility (3).

The long-acting lipoglycopeptide oritavancin exhibits in vitro activity against VRE isolates expressing both the VanA and VanB phenotypes and has demonstrated activity in an animal model of VRE infection (4, 5). In this study, the pharmacodynamic (PD) activities of free-drug concentrations from a single 1200 mg dose of oritavancin and daily 12 mg/kg daptomycin were compared against clinical isolates of vancomycin-resistant *E. faecium* (VREfm) in an in vitro pharmacokinetic (PK)/PD model over 72h.

Methods

Antibacterial agents

Oritavancin diphosphate was from The Medicines Company (Parsippany, NJ). Daptomycin was obtained from APIChem Technology Company (Hangzhou, China).

Bacterial isolates and broth microdilution MIC testing

The reference VanA VREfm isolate ATCC 51559 and VanA VREfm clinical isolates B7181440 and B7231527 were used in this study. All isolates were susceptible to daptomycin. Broth microdilution MICs for the parental isolates and isolates that survived drug challenge in the in vitro PK/PD model were determined following CLSI M07-A10 guidelines (6) using the quality control isolate *E. faecalis* ATCC 29212 to assess whether appropriate assay performance was within the parameters described in CLSI M100-S26 (7). MICs of the surviving isolates were assessed after serial passage on nonselective agar for 5 consecutive days to determine whether the changes in susceptibility were stable.

In vitro PK/PD modeling

A dilutional, one-compartment in vitro PK/PD model that has previously been described was used to investigate the PD of simulated dosing regimens of oritavancin and daptomycin (8). The central flask containing 250 ml of cation-adjusted Mueller-Hinton broth (CAAMHB) supplemented with either 0.01% polysorbate-80 (oritavancin) or 50 µg/ml $CaCl_2$ (daptomycin) was inoculated with exponentially-growing VREfm isolates at 10^6 CFU/ml and incubated for 72h at 37ºC with stirring (200 rpm). After 5 h of drug exposure, volumes in the central flask were transferred to a new sterilized flask to ensure that only drug-exposed bacteria remaining in solution were present in the model (i.e. eliminating unexposed bacteria that may have attached to either the vessel walls above the air-liquid interface). At the indicated time points, aliquots were sampled for bacterial viability (serial dilution plating using activated charcoal to reduce the antibiotic carry-over effect) then frozen at -20ºC until drug concentrations determined. Values presented are log CFU/ml of the mean from two independent experiments done in duplicate (n=4).

Exposure to oritavancin consisted of simulating the free-drug concentration-time profile (assuming protein binding of 85%) from a single, 3 h infusion of 1200 mg into the in vitro PK/PD model as previously described to approximate the population PK parameters (*f*Cmax of 20.7 µg/ml, alpha, beta and gamma t½ of 2.3h, 13.4h, and 245h, respectively, *f*$AUC_{0\text{-}24}$ of 178.1 µg•h/ml) determined from the SOLO clinical trials (9). Daptomycin was added as a bolus dose to approximate PK parameters associated with free-drug levels from 12 mg/kg (assuming protein binding of 91.5%, a *f*Cmax of 15.6 µg/ml, a *f*$AUC_{0\text{-}24h}$ of 170.9 µg•h/ml and a t½ of 8 h; [10]).

Quantification of oritavancin and daptomycin

For quantification of oritavancin in the in vitro PK/PD model, a fluorescence polarization assay with a fluorescein labelled D-Ala-d-Ala peptide substrate was used (11). Standard curves for oritavancin were prepared in CAMHB containing 0.01% P80 and the fluorescein-labelled d-Ala-d-Ala peptide substrate. Excitation and emission wavelengths of 485 nm and 535 nm, respectively, were used. The assay linear range of sensitivity was 0.25 µg/ml to 16 µg/ml for oritavancin. For quantification of daptomycin in the in vitro PK/PD model, a previously described bioassay using the indicator isolate *Streptococcus pyogenes* ATCC 19692 was used (8).

Results

Figure 1. Pharmacodynamic activity of simulated free-drug exposures of a single dose (SD) of 1200 mg oritavancin (ORI) and daily dosing (QD) of 12 mg/kg daptomycin (DAP) against the VREfm isolates in the in vitro PK/PD model.

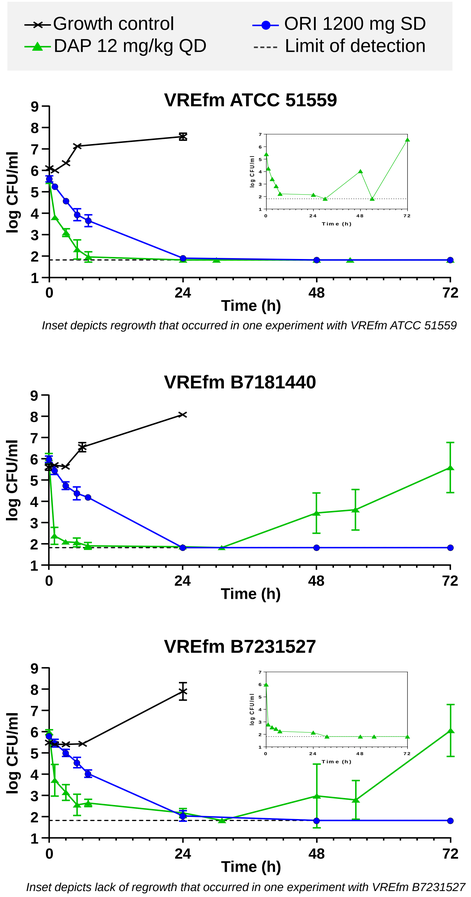


Table 1. Pharmacokinetic parameters obtained from simulated free-drug plasma levels associated with a single dose (SD) of 1200 mg oritavancin and daily dosing (QD) of 12 mg/kg daptomycin in the in vitro PK/PD model.

	Oritavancin 1200 mg SD		Daptomycin 12 mg/kg QD	
Parameter	**Targeted**	**Obtained**	**Targeted**	**Obtained**
*f*Cmax (µg/ml)	20.6	20.1 ± 3.1	15.6	15.1 ± 0.2
T½ (h)	nd	nd	8	8.1 ± 0.5
f$AUC_{0\text{-}24}$ (µg•h/ml)	178.1	164.2 ± 30.5	170.9	162.1 ± 7.7
f$AUC_{0\text{-}72}$ (µg•h/ml)	245.7	223.3 ± 46.2	nd	nd

nd = not determined

Table 2. MIC determinations of VREfm isolates that developed reduced susceptibility to daptomycin in the in vitro PK/PD model.

			Daptomycin MIC[b] (µg/ml)		Oritavancin MIC (µg/ml)	
VREfm isolate	**No. of occurrences[a]**	**Isolate designation**	**Pre**	**Post**	**Pre**	**Post**
ATCC 51559	1 of 4	150-4	2	16	0.25	0.25
B7181440	4 of 4	140-1	2	16	0.03	0.06
		140-2	2	16	0.03	0.06
		141-1	2	32	0.03	0.25
		141-2	2	16	0.03	0.12
B7231527	3 of 4	140-4	4	8	0.25	0.5
		141-3	4	16	0.25	0.5
		141-4	4	16	0.25	0.5

[a] Number of occurrences of development of reduced susceptibility to daptomycin in two independent experiments done in duplicate (n =4).

[b] Modal MICs are shown of the parental isolates prior to exposure (Pre) and for surviving isolates after 72h exposure to daptomycin that were then passaged for 5 days on nonselective agar (Post).

Conclusions

- Daptomycin (12 mg/kg QD) exerted rapid bactericidal activity against the VREfm isolates.
- Following exposure to daptomycin (12 mg/kg QD), all three tested VREfm isolates exhibited regrowth and concomitant reduced susceptibility (ranging from 2- to 16-fold increases in daptomycin MIC) at various frequencies.
- Oritavancin (1200 mg SD) exerted sustained bactericidal activity against the VREfm isolates with the absence of regrowth and concurrent changes in susceptibility.
- These results support further study of oritavancin as a treatment option for clinical VREfm infections.

Disclosures

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