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Assessment of Oritavancin Activity in Combination with Beta-lactams against Vancomycin-resistant Enterococci

Francis F. Arhin, David Lalonde Seguin, Adam Belley, Greg Moeck The Medicines Company, Ville Saint Laurent, Québec, Canada

Francis Arhin
The Medicines Company
Ville Saint Laurent, Canada
francis.arhin@themedco.com

Abstract

Objective: Optimal treatments for infections caused by vancomycin-resistant enterococci (VRE) remain to be elucidated. Combinations of beta-lactams (BLs) and lipopeptides or glycopeptides exhibit synergy against VRE, as do combinations of gentamicin and BLs or glycopeptides. Because the lipoglycopeptide oritavancin (ORI) demonstrates *in vitro* activity against VRE, we assessed potentiation of its activity in combination with BLs against VRE.

Methods: Clinical isolates *of E. faecium* (Efm) VanA (n=5) and VanB (n=5) and *E. faecalis* (Efa) VanA (n=6) and VanB (n=5) were used in checkerboard experiments of ORI and the BLs ceftaroline (CPT), meropenem (MER) or penicillin (PEN). Potentiation of ORI activity was defined as a lowering of ORI MIC by at least two doubling dilutions in the combination, relative to the MIC of ORI alone, with the constraint that the MIC of the BLs in the combination had to be ≤32 µg/mL. Time-kill kinetics (TKK) were performed following CLSI guidelines. Potentiation of ORI activity in combination with BLs in TKK was defined as a ≥1-log decrease in CFU/mL from the most active single agent at 24h.

Results: ORI MIC range against the VREs was 0.004 - 1 µg/ml. MER MIC ranges were 512-1024 µg/ml (Efm VanA), 512 – 1024 µg/ml (Efm VanB), 16 -128 µg/ml (Efa VanA) and 64 -128 µg/ml (Efa VanB). CPT MIC ranges were 256 -1024 µg/ml (Efm VanA), 64– 1024 µg/ml (Efm VanB), 4 -128 µg/ml (Efa VanA) and 16 -128 µg/ml (Efa VanB). PEN MIC ranges were 1024-2048 µg/ml (Efm VanA), 256 - 512 µg/ml (Efm VanB), 4 -128 µg/ml (Efa VanA) and 8 -16 µg/ml (Efa VanB). Of the 21 VRE isolates tested, CPT, MER and PEN potentiated ORI MICs against 11, 11 and 8 isolates, respectively (Table), with the BL MICs being ≤32 µg/ml in each combination. ORI activity was potentiated by BL against the majority of Efa VanA, Efa VanB and Efm VanA isolates. BLs did not potentiate ORI activity against Efm VanB (Table). In TKK, potentiation by ORI-BL combinations compared to single agents was observed for VRE isolates.

Conclusions: MER, CPT and PEN potentiated ORI in vitro activity against VRE, with the exception of Efm VanB. Several of the VREs with high (≥256 µg/mL) BL MICs showed potentiation of ORI activity at BL MICs of ≤ 32 µg/mL.

Background

Clinical management of vancomycin-resistant enterococci (VRE) infections remains a challenge (Miller et al., 2016). Synergistic activity against VREs has been demonstrated for Beta-lactams (BLs) in combination with daptomycin, glycopeptides and gentamicin. Oritavancin, a semi-synthetic lipoglycopeptide, has in vitro activity against VRE (Mendes et al., 2016). Here, we assessed the potentiation of oritavancin activity against VRE by BLs.

Methods

Test strains: Clinical isolates *of E. faecium* (Efm) VanA (n=5) and VanB (n=5) and *E. faecalis* (Efa) VanA (n=6) and VanB (n=5) were tested.

Checkerboard experiments: Potentiation of ORI activity against VRE by the BLs ceftaroline (CPT), meropenem (MER) or penicillin (PEN) was assessed using the checkerboard approach (CLSI, 2015; AAC, instructions to authors). Potentiation of ORI activity was defined as a lowering of ORI MIC by at least two doubling dilutions in the combination, relative to the MIC of ORI alone, with the constraint that the MIC of the BLs in the combination had to be ≤32 µg/mL.

Time-kill kinetics: Time-kill assays followed CLSI guideline M26-A (NCCLS, 1999) and were performed in 24-well deep-well plates. Viable cell counts were determined by serial dilution plating, including the use of 25 g/L charcoal suspension to limit antibiotic carryover. Potentiation of ORI activity in combination with BLs in time-kill kinetics was defined as a ≥1-log decrease in CFU/mL from the most active single agent at 24h. Time-kill assays were repeated twice independently; results presented are from a representative experiment.

Results

- BL and ORI MICs against the tested VRE isolates are shown in Table 1
- Of the 21 VRE isolates tested in checkerboard experiments, CPT, MER and PEN potentiated ORI MICs against 11, 11 and 8 isolates, respectively (Table 2), with the BL MICs being ≤32 µg/ml in each combination that demonstrated potentiation
- Time-kill studies (Figures 1 – 6) demonstrated synergistic or potentiated activity of ORI/BL combinations against VRE

Table 1. MIC ranges (µg/mL) of ORI and beta-lactams for the tested strains

	MIC range (µg/mL)			
Agent	**Efm VanA (n=5)**	**Efm VanB (n=5)**	**Efa VanA (n=6)**	**Efa VanB (n=5)**
Oritavancin	0.016 -0.5	0.004 -0.03	0.25 - 1	0.016 -0.03
Ceftaroline	256 -1024	64 - 1024	4 -128	16 -128
Meropenem	512- 1024	512 - 1024	16 - 128	64 - 128
Penicillin	1024 -2048	256 -512	4 -128	8 -16

Efm, *E. faecium*; Efa, *E. faecalis*; VanA, VanA type VRE; VanB, VanB-type VRE

Table 2. Number of VRE isolates in which potentiation of ORI activity by BL was demonstrated

	# of isolates exhibiting potentiation of oritavancin activity/# of isolates tested (Oritavancin MIC range [µg/mL] alone/in combination with BL for strains showing potentiation)			
Agent	**Efm VanA**	**Efm VanB**	**Efa VanA**	**Efa VanB**
Ceftaroline	4 /5 (0.016 – 0.5/0.004 -0.12)	1/5 (0.008/0.00006)	3/6 (0.25 – 1/0.06 -0.25)	3/5 (0.016 -0.03/0.002 -0.008)
Meropenem	3/5 (0.016 – 0.5/0.004 -0.03)	0/5 (NA)	3/6 (0.25 -0.5/0.008 -0.12)	5/5 (0.016 -0.03/0.004 -0.008)
Penicillin	2/5 (0.03 - 0.5/0.004 -0.12)	0/5 (NA)	2/6 (0.25 -0.5/0.004 -0.03)	4/5 (0.03/0.004 -0.008)

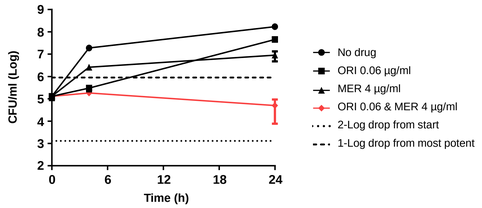
Efm, *E. faecium*; Efa, *E. faecalis*; VanA, VanA type VRE; VanB, VanB-type VRE; ORI, oritavancin; BL, beta-lactam; NA, not applicable.

MIC ranges of ORI alone are in black; ranges of ORI MIC in combination with BL are in red.

Beta lactam MICs in ORI MIC-potentiated strains were ≤32 µg/mL.

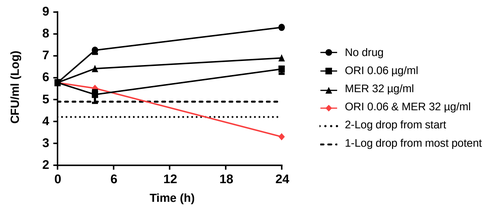
Results

Figure 1. Time-kill kinetics of ORI, MER, and ORI in combination with MER against *E. faecalis* B2140164 (VanB).



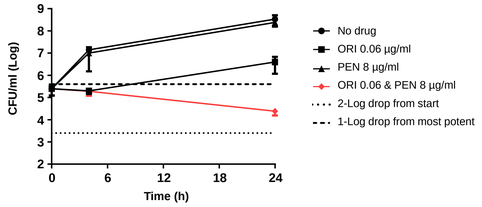
Oritavancin (ORI) MIC, 0.03 µg/mL; Meropenem (MER) MIC, 64 µg/mL

Figure 2. Time-kill kinetics of ORI, MER, and ORI in combination with MER against *E. faecium* B7231527 (VanA).



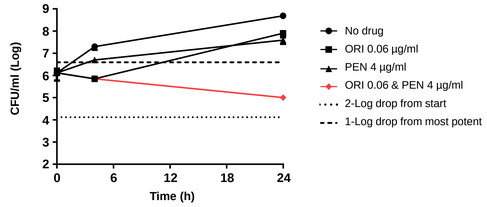
Oritavancin (ORI) MIC, 0.5 µg/mL; Meropenem (MER) MIC, 1024 µg/mL

Figure 3. Time-kill kinetics of ORI, PEN, and ORI in combination with PEN against *E. faecium* B7231527 (VanA).



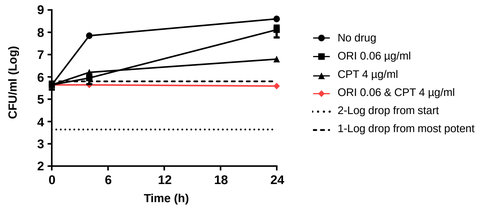
Oritavancin (ORI) MIC, 0.5 µg/mL; Penicillin (PEN) MIC, 1024 µg/mL

Figure 4. Time-kill kinetics of ORI, PEN, and ORI in combination with PEN against *E. faecalis* 1058964 (VanA).



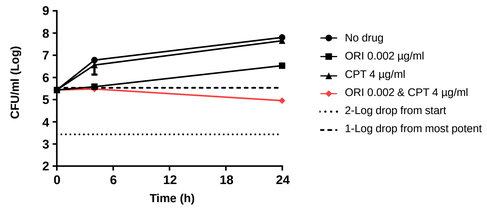
Oritavancin (ORI) MIC, 0.25 µg/mL; Penicillin (PEN) MIC, 64 µg/mL

Figure 5. Time-kill kinetics of ORI, CPT, and ORI in combination with CPT against *E. faecalis* 1058968 (VanA).



Oritavancin (ORI) MIC, 0.25 µg/mL; Ceftaroline (CPT) MIC, 64 µg/mL

Figure 6. Time-kill kinetics of ORI, CPT, and ORI in combination with CPT against *E. faecium* 7030254 (VanA).



Oritavancin (ORI) MIC, 0.03 µg/mL; Ceftaroline (CPT) MIC, 512 µg/mL

Conclusions

- CPT, MER and PEN potentiated ORI in vitro activity against VRE, with the exception of Efm VanB
- Among the 21 VRE isolates tested in this study, CPT, MER and PEN potentiated ORI MICs in 11, 11 and 8 isolates, respectively, with the BL MICs ≤32 µg/ml in each combination that demonstrated potentiation
- The 5 tested isolates of Efm VanA had high BL MICs (256 to 2048 µg/mL); potentiation of ORI activity at BL MICs of ≤ 32 µg/mL was demonstrated in 4, 3 and 2 of these isolates using CPT, MER and PEN combinations, respectively
- Time-kill kinetics studies confirmed the increased activity of ORI/BL combinations against VRE compared to the activity of each respective agent tested alone

Disclosures

Support and funding and for this study was provided by The Medicines Company.

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Presented at IDWeek 2016, October 26-30, 2016, New Orleans, LA