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Oritavancin Activity against *Enterococcus faecalis* In Vitro and in a Murine Thigh Infection Model

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Updated Abstract

Background: Oritavancin (ORI) is a lipoglycopeptide that has in vitro activity against enterococci. We evaluated the pharmacodynamics of a single dose of ORI against *Enterococcus faecalis* (EF) isolates in a neutropenic murine thigh infection (TI) model and in vitro time-kill (TK) assays.

Methods: 3 vancomycin-susceptible EF isolates were tested: ATCC 29212, 23241 and 33186. MIC determinations followed CLSI M7-A10 guidelines while MBC and TK assays followed M26-A guidelines. TK assays were conducted at both standard and high inoculum density (~10^6 and ~10^8 colony forming units (CFU)/ml, respectively) using ORI at concentrations approximating its mean free peak (fC_{max}, 16 µg/mL) in plasma based on a single 1200 mg dose. TI was established in neutropenic mice (n = 2/group) with an inoculum of 10^6 CFU in each thigh. ORI and vancomycin (VAN) were dosed 40 mg/kg once (IV) and 120 mg/kg BID (SQ), respectively, which produce *f*AUCs equivalent to human exposures. Efficacy was evaluated by determining mean log CFU/thigh changes from baseline after 24 h treatment.

Results: ORI MICs ranged from 0.015 – 0.03 µg/mL against the 3 EF isolates and ORI MBC/MIC ratios ranged from 4 - >16. In TK assays, ORI was bactericidal (≥ 3 log kill by 24h) against all isolates at both inoculum densities. Results in the TI model are shown in the table below.

Strain	Compound	Total Daily Dose (mg/kg)	MIC (µg/mL)	Change in Log CFU/thigh at 24h
E. faecalis ATCC 29212	ORI	40	0.016	-1.24
	VAN	240	2	-0.51
E. faecalis ATCC 23241	ORI	40	0.016	-1.50
	VAN	240	4	-1.24

Conclusion: ORI was bactericidal at fC_{max} concentrations in TK assays. In the TI model, ORI activity was similar to VAN and both drugs produced bacterial killing at human equivalent exposures. Further studies are warranted.

Introduction

- Oritavancin is a lipoglycopeptide with potent in vitro activity against most gram-positive bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE).
- Oritavancin was approved in 2014 for the treatment of acute bacterial skin and skin structure infections (ABSSSI) due to *S. aureus* (including MRSA), certain streptococci, and *E. faecalis* (vancomycin-susceptible only).
- The MIC/MBC and time-kill kinetics along with the oritavancin exposures required for activity against *S. aureus*, including MRSA, have been previously described (1, 2).
- The objective of these studies was to describe the activity and oritavancin exposures required for activity against *E. faecalis*.

Methods

Bacterial Strains

- *E. faecalis* ATCC 29212, ATCC 23241 and ATCC 33816 were used in these studies.

MIC, MBC, and Time-Kill Kinetic Determinations

- MIC determinations followed CLSI M7-A10 guidelines while MBC and TK assays followed M26-A guidelines (3, 4).
- Time-Kill assays were conducted at ~10^6 and ~10^8 CFU/mL using oritavancin at 0.25, 2, and 16 µg/mL and vancomycin at 4 and 16 µg/mL.

Mouse Pharmacokinetics:

- Neutropenic female Swiss-Webster mice were administered a single 40 mg/kg IV dose via the lateral tail vein.
- Blood samples were collected at various timepoints over 24 hours.
- Plasma levels were determined using an LC-MS/MS method and the data were fit to a non-compartmental model (WinNonlin).

Thigh Infection Model:

- Female Swiss-Webster mice were used.
- Mice were rendered temporarily neutropenic by the administration of 150mg/kg of cyclophosphamide (Baxter, IL) on days −4 and −1 prior to infection.
- Strains were grown in Todd Hewitt Broth (THB) at 37˚C under constant aeration. After 20 hours, the inoculum was sub-cultured into fresh THB and allowed to regrow at 37˚C under constant aeration for ~ 4 hours. The bacterial suspensions were diluted in fresh THB to yield ~ 10^6 CFU/ml by correlation of absorbance at 600 nm with predetermined plate counts.
- Infection was initiated (under isoflurane anesthesia) *via* an intramuscular injection of 0.1 mL of inoculum (~ 10^6 CFU/thigh).
- Treatment was initiated 2 h post-infection; Oritavancin was administered as a single IV dose of 2.5 – 80 mg/kg; Vancomycin was administered subcutaneously at 120 mg/kg twice daily.
- Controls were euthanized at the start of treatment while treated animals were euthanized 24 hours post-treatment using CO_2; thighs were removed aseptically, homogenized in 5 mL of saline, and plated on Todd Hewitt Agar.

Pharmacodynamic Modeling:

- The relationship between AUC and reduction in log CFU/thigh 24 hours after the start of treatment was analyzed using a sigmoid E_{max} pharmacodynamic model (WinNonlin):

$$\text{Change in log CFU/Thigh} = (E_{max} \cdot X^{\gamma} / EC_{50}^{\gamma} + X^{\gamma}) - E_0;$$

Where E_{max} is the maximum reduction in log CFU/Thigh, X is the AUC, EC_{50} is the X value corresponding to 50% of the E_{max}, E_0 is the effect when X =0 and γ is a sigmoidicity factor.

Results

Table 1. Minimum Inhibitory and Bactericidal Concentrations

Strains	Oritavancin MIC (µg/mL)	Oritavancin MBC (µg/mL)	Vancomycin MIC (µg/mL)	Vancomycin MBC (µg/mL)
E. faecalis ATCC 29212	0.016	>0.25	2	>16
E. faecalis ATCC 23241	0.016	>0.25	4	>16
E. faecalis ATCC 33816	0.03	0.12	1	>16

Table 2. Pharmacokinetic Parameters of Oritavancin in Mice and Humans

Species	Dose	Protein Binding (%)	Total Cmax (mg/L)	Free Cmax (mg/L)	Total 24h AUC (mg*h/L)	Free 24h AUC (mg*h/L)
Mouse	40 mg/kg	85	238	35.7	1211	181.7
Human	1200 mg	85	138	20.7	1110	166.5

Table 3. Oritavancin Exposures Required for Stasis, 1-log, and 2-logs of Bacterial Killing over 24 hours in the Neutropenic Mouse Thigh Infection Model

Strain	24h AUC (mg*h/L)		
	Stasis	1-log Kill	2-log Kill
E. faecalis ATCC 29212	90.	265	1284
E. faecalis ATCC 23241	135	390	1336
***S. aureus* isolates (N = 14)[1]**	**143**	**242**	**ND**

Figure 1. Time-kill kinetics of ORI and VAN against *E. faecalis* ATCC 29212 at ~10^6 CFU/mL

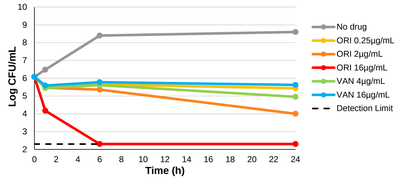


Figure 2. Time-kill kinetics of ORI and VAN against *E. faecalis* ATCC 23241 at ~10^6 CFU/mL

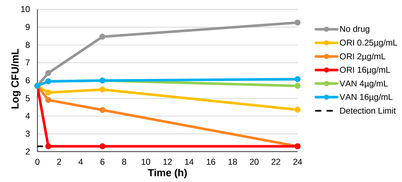


Figure 3. Time-kill kinetics of ORI and VAN against *E. faecalis* ATCC 33816 at ~10^6 CFU/mL

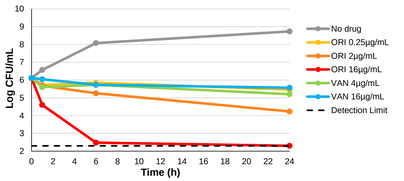


Figure 4. Time-kill kinetics of ORI and VAN against *E. faecalis* ATCC 29212 at ~10^8 CFU/mL

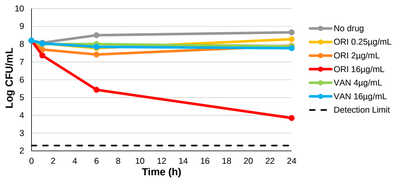


Figure 5. Time-kill kinetics of ORI and VAN against *E. faecalis* ATCC 23241 at ~10^8 CFU/mL

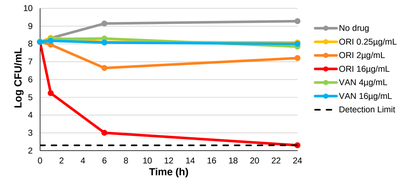


Figure 6. Time-kill kinetics of ORI and VAN against *E. faecalis* ATCC 33816 at ~10^8 CFU/mL

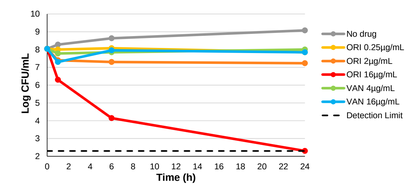


Figure 7. Activity of Oritavancin and Vancomycin against *E. faecalis* in a Neutropenic Mouse Thigh Infection Model

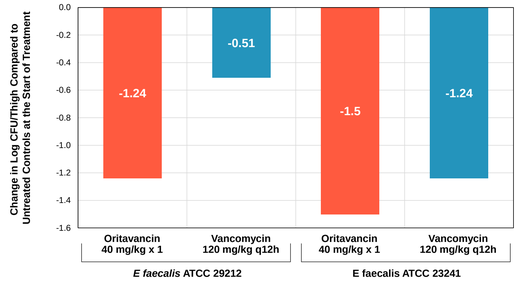
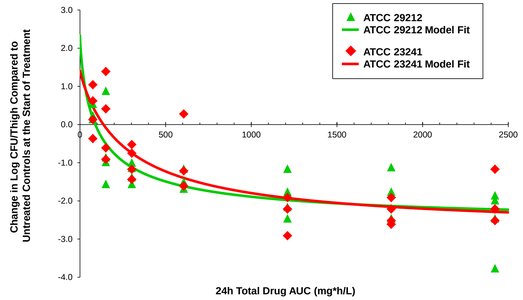


Figure 8. Relationship between Oritavancin Total Drug AUC and Change in Log CFU in a Neutropenic Mouse Thigh Infection Model Due to *E. faecalis*



Summary & Conclusions

- The MICs of oritavancin for these isolates were 0.016 – 0.03 µg/mL and the MBCs were 0.12- >0.25 µg/mL. Vancomycin MICs were 1-4 µg/mL and the MBC for all strains was >16 µg/mL (Table 1).
- A single 40 mg/kg dose of oritavancin produces a 24 h AUC that is similar to that achieved in with 1200 mg in humans (5) (Table 2).
- While there is an inoculum effect, oritavancin 16 µg/mL produced >3-log reduction of bacterial counts against all 3 isolates at both standard and high inoculum densities. Vancomycin at 16 µg/mL did not achieve a 3-log kill against any isolates at either of the inoculum densities tested. (Figures 1-6).
- Oritavancin and vancomycin produced similar levels of bacterial killing against both strains in the neutropenic mouse thigh infection model using 24h exposures equivalent to the human exposures of both drugs (Figure 7).
- In dose ranging pharmacodynamic experiments against *E. faecalis* strains ATCC 29212 and 23241 in the neutropenic thigh infection model, oritavancin produced stasis and 1-log of bacterial killing at exposures similar to those observed previously with *S. aureus* in the same model (Table 3; Figure 8).
- Overall, these data suggest that oritavancin is bactericidal against some *E. faecalis* both in vitro and in vivo at exposures that are similar to those observed following a single 1200 mg dose in humans.
- Further study of oritavancin in the treatment of infections due to Enterococci is warranted.

Disclaimers/Acknowledgments:

Authors are employees of The Medicines Company

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