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Oritavancin Kills *Staphylococcus aureus* in Slow-growing Planktonic and Biofilm States

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Abstract

Background: Infections caused by *Staphylococus aureus* bacteria that are either in a state of slow growth or biofilm pose a serious clinical challenge. Oritavancin (ORI) is a glycopeptide antibiotic with rapid bactericidal activity against a broad spectrum of Gram-positive organisms including vancomycin (VAN)-resistant isolates. We tested the activity of ORI *in vitro* against slow-growing and biofilm bacteria.

Methods: 1) Time-kill studies of ORI and comparators were performed on stationary phase *S. aureus* ATCC 29213 inoculated into nutrient-depleted, cation-adjusted Mueller-Hinton broth (depCAMHB) following CLSI guidelines. 2) Biofilms were established in 96-well plates following incubation of static cultures of *S. aureus* ATCC 29213 for 24 h. Biofilms were challenged with increasing concentrations of ORI and comparators for 24 h. The minimal biofilm inhibitory concentration (MBIC) was determined by measuring residual dehydrogenase activity using a formazan dye. Alternatively, 24-h challenge plates were washed and drug-free CAMHB was added to each well and incubated for a further 48 h to determine bacterial regrowth following antibiotic challenge.

Results: 1) ORI exhibited time- and concentration-dependent bactericidal activity (≥3 log reduction in viable cell counts) against stationary phase cells inoculated into depCAMHB. At 2X MIC (1 µg/ml), ORI reduced viable cell counts by >4 log at 24 h. In contrast, no change in viable cell counts was detected with VAN and teicoplanin at 8X MIC (8 and 4 µg/ml, respectively) at 24 h. 2) ORI displayed a MBIC of 8 µg/ml after 24 h whereas VAN lacked activity (MBIC > 128 µg/ml) against the *in vitro* biofilm. ORI sterilized the *in vitro* biofilm as no bacterial regrowth was detected at 48 h. In contrast, bacterial regrowth occurred within 24 h even at the highest concentrations of all other antibiotics tested.

Conclusion: Under the conditions examined, ORI exhibits bactericidal activity *in vitro* against *S. aureus* in both slow-growing and biofilm states.

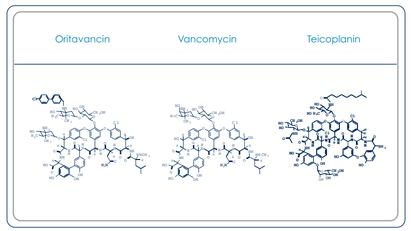

Figure 1.
Chemical structures of Oritavancin, Vancomycin, and Teicoplanin

Introduction

The antibacterial activity of most antibiotics depends largely on the growth state of the microorganism: maximal antibacterial activity is achieved against exponentially growing bacteria. However, in many infections, bacteria are not growing exponentially. This poses an important clinical challenge in treatment of infections in which bacteria are in a non-dividing state or growing in a biofilm (estimated by the NIH to be >60% of all human infections). Such recalcitrant infections are typified by nosocomially acquired staphylococcal infections of implants and catheters, and endocarditis.

Oritavancin is a semi-synthetic glycopeptide antibiotic with potent activity against a range of drug-resistant Gram-positive bacteria including vancomycin- and methicillin-resistant staphylococci and vancomycin-resistant enterococci. It differs from other glycopeptide antibiotics such as vancomycin and teicoplanin in that its antibacterial activity *in vitro* is rapid, bactericidal, and concentration-dependent.

In this study, we sought to determine whether oritavancin is active against *S. aureus* in a non-dividing state or growing in a biofilm *in vitro*.

Methods

Depleted cation-adjusted Mueller-Hinton broth: *S. aureus* ATCC 29213 was grown for 24 h in CAMHB at 37°C with rotation (225 rpm) then pelleted by centrifugation (8,000 X *g* for 30 minutes). The medium was re-inoculated with exponential-phase bacteria and incubated under the same conditions. Following centrifugation to remove the majority of bacteria, the depleted CAMHB was filter-sterilized (0.22 µM).

Time-kill studies: CAMHB containing antibacterial agents at varying concentrations was inoculated with exponential-phase *S. aureus* ATCC 29213 at approximately 5 X 10^5 CFU/ml following CLSI guidelines. Otherwise, depleted CAMHB containing antibacterial agents at varying concentrations was inoculated with stationary- or exponential-phase as indicated above. All time-kill studies were performed in 96-well deep-well plates at 37°C with rotation (225 rpm). Aliquots were removed at the indicated times and bacteria were enumerated by serial-dilution plating.

Biofilm studies: *S. aureus* ATCC 29213 biofilms were established by 2 methods: (1) In the first approach, bacteria (200 µl of 5 X 10^5 CFU/ml) were grown for 24 h in 96-well plates in tryptic soy broth containing 0.25% glucose. The following day, wells were washed 4 times with physiological saline. The adherent biofilms were then challenged with two-fold serial dilutions of antibacterial agents in CAMHB for 24 h. Wells were then washed twice with saline (200 µl) to remove non-adherent cells. The formazan dye MTT (250 µg/ml) was then added to each well and incubated at 37°C for 2 hours to assess the minimal biofilm inhibitory concentration (MBIC). Alternatively, 24-h challenge plates were washed 4 times with physiological saline, and drug-free CAMHB was added to each well and incubated for a further 48 h to determine bacterial regrowth following antibiotic challenge; (2) In the second approach, biofilms were established using the MBEC™ Physiology & Genetics Assay plate (Calgary biofilm device) following the manufacturer's protocol. To enumerate the biofilm CFUs on individual control pegs, pegs were broken off the lid using sterile forceps and placed in 1 ml saline, sonicated for 5 min and vortexed for 1 min at the highest setting. Bacteria were enumerated by serial-dilution plating.

Antibiotic	MIC[1] (µg/ml)
Oritavancin	0.5
Vancomycin	1
Teicoplanin	0.5

Table 1.
Antibacterial activity of glycopeptides against *S. aureus* ATCC 29213

[1]MICs were determined by the broth microdilution method in CAMHB following the guidelines of the Clinical Laboratory Standards Institute (CLSI) protocol M7-A7 and fell within the acceptable QC limits established for each drug (table 3 of document M100-S16).

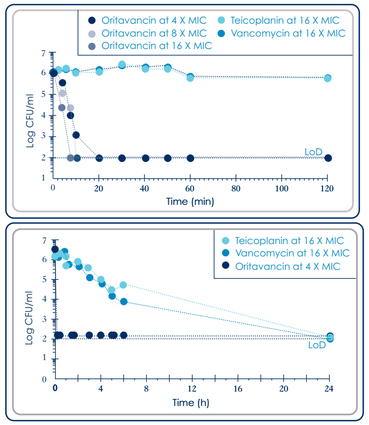

Figure 2.
Oritavancin exhibits rapid bactericidal activity against exponentially growing *S. aureus* ATCC 29213. Top panel, 0-120 min; bottom panel, 0-24 h

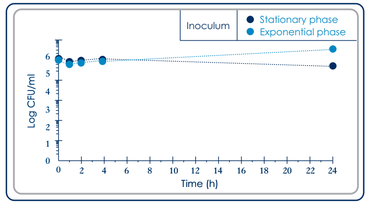

Figure 3.
Depleted CAMHB maintains stasis of *S. aureus* ATCC 29213 over 24 hours

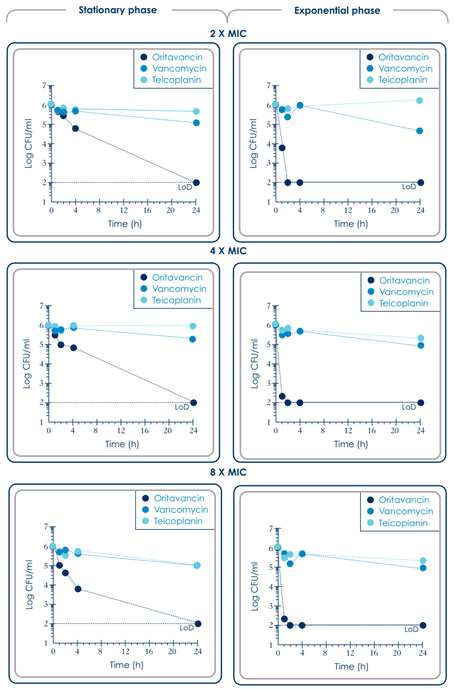

Figure 4.
Only oritavancin exhibits bactericidal activity against *S. aureus* ATCC 29213 in depleted CAMHB

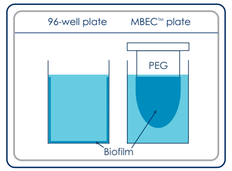

Figure 5.
Methods used to establish *S. aureus* ATCC 29213 biofilms

Antibiotic	96-well plate		MBEC™ plate	
	MIC (µg/ml)	MBIC[1] (µg/ml)	MIC[1] (µg/ml)	MBEC[2] (µg/ml)
Oritavancin	0.5	4-16[2]	2-4	4-8
Vancomycin	1	>128[2]	2-4	≥128
Teicoplanin	0.5	64-128[2]	1-2	16-32
Rifampicin	0.008	0.004[2]	<0.125	8

Table 2.
Oritavancin displays antibacterial activity against *S. aureus* ATCC 29213 biofilms established in 96-well and MBEC™ plates

[1]MBIC, minimal biofilm inhibitory concentration.
[2]As assessed 48 hours after addition of fresh CAMHB to the drug-exposed wells, no growth was observed in wells above the oritavancin MBIC breakpoint whereas bacterial regrowth occurred for all other antibiotics.
[3]MICs were determined in MBEC™ plates and represent the antibacterial activity against planktonic cells shed from the peg biofilms.
[4]The minimal biofilm eradication concentration (MBEC) was determined following the manufacturer's protocol. Biofilms on control pegs contained an average of 8.7 ± 5.5 X 10^7 CFU/peg.

Conclusions

- Depleted CAMHB maintains the viable counts of *S. aureus* ATCC 29213 at a static level over the 24-h incubation period.
- Although the activity of oritavancin against stationary-phase *S. aureus* ATCC 29213 was delayed compared to exponential-phase cells, oritavancin exhibited bactericidal activity against stationary-phase cells (4 log CFU kill at 24 h) whereas vancomycin and teicoplanin did not.
- Oritavancin exhibited antibacterial activity against *S. aureus* ATCC 29213 biofilms established *in vitro* by two different methods. Furthermore, oritavancin was capable of sterilizing the biofilm. In contrast, vancomycin and teicoplanin exhibited more significant reductions in their antibacterial activity (MICs increased 16- to >128-fold) and were incapable of sterilizing the biofilm.
- In light of our findings, the bactericidal activity of oritavancin against non-dividing cells and biofilms will be tested against other drug sensitive- and drug-resistant strains of *S. aureus*.

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