Intracellular Activity of Oritavancin against MSSA, MRSA and VISA Strains in a Model of Human Macrophages

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REVISED ABSTRACT

Background: *S. aureus* is able to survive and thrive inside eukaryotic cells, which may contribute to recurrence or persistence of infections. Oritavancin, a new lipoglycopeptide with multiple modes of action, proved bactericidal against *Staphylococcus aureus* strains, including strains poorly susceptible to vancomycin. This study therefore examines the activity of oritavancin in a model of *S. aureus*-infected THP-1 macrophages allowing for 24-h exposure and detailed analysis of dose-response (AAC 2006; 50: 841-851), using strains with relevant resistance phenotypes.

Methods: MICs and MBCs were determined by microdilution in MH broth + 0.002 % Tween 80. Activity was measured as the changes in CFU from initial inoculum after incubation in MH broth + 0.002 % Tween 80 (extracellular) or with infected macrophages (intracellular) exposed to antibiotic concentrations up to 50 µg/mL. Emax and static concentration were calculated based on sigmoidal regressions.

Results:

Strain	Phenotype	MIC (µg/mL)	MBC (µg/mL)	Intracellular activity		
				Emax	Static concentration	
				Δlog from time 0	µg/mL	X MIC
ATCC25923	MSSA	0.06	0.25	-4.21	4.90	82
N4112910	HA-MRSA	0.03	0.06	-1.87	2.34	78
NRS192	CA-MRSA	0.12	0.25	-2.03	3.63	29
NRS18	VISA	0.5	1	-1.71	6.92	14
NRS126	VISA	0.25	0.5	-0.79	1.12	4

Bactericidal effect was observed in broth at 5 h (> -3.5 log) at 100 x MIC for all strains.

Conclusion: Oritavancin proved bactericidal in broth against all strains, irrespective of their resistance phenotype. Intracellularly, oritavancin showed activity on all strains, with an Emax ≤ -2 log achieved for MSSA and MRSA, and static effects obtained at lower x MIC for VISA.

INTRODUCTION

Staphylococcus aureus is a widespread pathogenic bacterium causing infections that are difficult-to-treat for several reasons, among which are:

- its capacity to survive and thrive inside intracellular compartments such as phago-lysosomes of phagocytic cells (1), where it is protected from immune defenses and antibiotics ;
- the development of resistance to most of the currently-used antibiotics, including vancomycin (2).

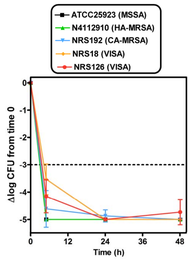
Oritavancin is a semi-synthetic lipoglycopeptide with multiple modes of action (3), characterized by a rapid concentration-dependent bactericidal activity against most gram-positive organisms, including vancomycin-resistant isolates (4).

AIMS OF THE STUDY

To compare the activity of oritavancin against the extracellular and intracellular forms of *S. aureus* displaying clinically-relevant resistance phenotypes (HA-MRSA, CA-MRSA and VISA) as compared to MSSA, using a pharmacodynamic model analysing dose-responses in detail (5).

RESULTS

Time-effect (extracellular; 100 x MIC)

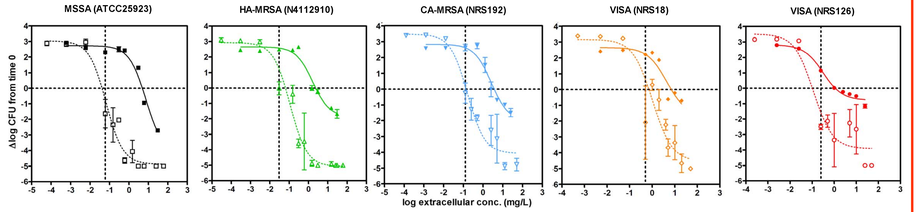


Change from initial inoculum (10^6 CFU/ml) after 5, 24 and 48 h of exposure to oritavancin in broth.

Bactericidal effect (- 3 log) : dotted line
Limit of detection : - 5 log.

- Bactericidal effect was observed after 5 h of exposure at 100 X MIC for all strains.

Concentration-effect (extracellular / intracellular; 24 h)



Dose-response curves for extracellular (open symbols; dotted lines) and intracellular (closed symbols; plain lines) activity, measured as the change in log CFU from initial inoculum after 24 h of exposure to oritavancin of bacteria in broth or of infected THP-1 macrophages.

Horizontal dotted line: static effect, vertical dotted line : MIC of oritavancin for the strain under study.

See Table below for the corresponding pharmacological parameters.

Extracellularly :

- A static effect was obtained for concentrations ≤ 1 µg/mL and close to the MIC for all strains except N4112910.
- Maximal effects did correspond to bactericidal effects (> 3 log decrease) against all strains.

Intracellularly :

- A static effect was obtained for concentrations ranging from 1 to 7 µg/mL. These values were 4- to 10-fold higher than extracellularly for MRSA and VISA, and 100 times for the MSSA strain.
- Maximal effects were much lower than extracellularly for all strains except for the MSSA strain.

MICs, MBCs and key pharmacological parameters for extracellular and intracellular activity of oritavancin, derived from dose-response curves after 24 h of exposure to oritavancin.
Cs: static concentration (µg/mL); EC50: concentration yielding half of the maximal effect (µg/mL); Emax: maximal effect (Δlog CFU from time 0).

Strain	Phenotype	MIC (µg/mL)	MBC (µg/mL)	Extracellular activity (24h)			Intracellular activity (24h)			
				Cs	EC50	Emax	Cs	Cs (x MIC)	EC50	Emax
ATCC25923	MSSA	0.06	0.25	0.04	0.07	-4.88	4.90	82	7.49	-4.21
N4112910	HA-MRSA	0.03	0.06	0.06	0.11	-5.07	2.34	78	1.64	-1.87
NRS192	CA-MRSA	0.12	0.25	0.14	0.16	-4.07	3.63	29	2.60	-2.03
NRS18	VISA	0.5	1	1.26	1.95	-5.31	6.92	14	4.51	-1.71
NRS126	VISA	0.25	0.5	0.10	0.11	-3.91	1.12	4	0.31	-0.79

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MATERIALS AND METHODS

- **Bacterial strains :** MSSA (ATCC25923), HA-MRSA (N4112910; clinical isolate from the Cliniques universitaires de Mont-Godinne), CA-MRSA (NRS192), VISA (NRS18) and VISA (NRS126).
- **Susceptibility testing:** MICs were determined by microdilution method in Mueller-Hinton broth + 0.002% Tween-80 (to avoid adsorption of the drug on plastic surfaces [6]). MBCs were determined as the concentration causing a 99.9% decrease from initial inoculum.
- **Kill curves in broth:** these experiments were performed as previously described (5), except that 0.002% Tween-80 was added to Mueller-Hinton broth.
- **Intracellular activity:** Infection of THP-1 macrophages was performed as previously described (5), with 1 h phagocytosis, followed by a washing with 50 µg/mL gentamicin (to eliminate extracellular bacteria) and reincubation in fresh medium containing oritavancin at concentrations ranging from 0.01 to 1000 x MIC (with a maximum set at 30 µg/mL to ensure drug solubility). Results are expressed as changes in CFU/mg protein from post-phagocytosis inoculum.

CONCLUSIONS

- Oritavancin proved bactericidal in broth, regardless of the phenotype of the strain.
- Intracellular activity was lower in all cases, with higher concentrations required to reach a static effect and maximal effects that were 2 to 3 log lower than extracellularly (except for the MSSA strain).
- No direct relationship could be established between MIC and pharmacological determinants of antibiotic efficacy, suggesting the importance of developing this type of more dynamic study to better evaluate the potential interest of new drugs against clinically-relevant isolates.

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