Comparative study of the membrane permeabilization induced by oritavancin (ORI) *vs.* vancomycin (VAN) using a model of negatively-charged liposomes.

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ABSTRACT

Background: Novel lipoglycopeptides, such as ORI, exert a marked and rapid bactericidal effect against gram-positive bacteria. While ORI inhibits the biosynthesis of peptidoglycan like conventional glycopeptides, permeabilization of the bacterial membrane has also been shown to contribute to its antibacterial properties, owing to the presence of a 4'-chlorobiphenylmethyl group on the molecule that is absent from vancomycin. We have examined the effect of ORI *vs.* VAN on the release of a fluorescent tracer from liposomes with compositions containing zwitterionic and negatively charged phospholipids.

Methods: Permeabilization of liposomes was assessed by measuring the release of the fluorescent dye calcein encapsulated at a self-quenching concentration in phospholipid vesicles (liposomes) made of phosphatidylcholine (PC; zwitterionic), phosphatidylethanolamine (PE; zwitterionic) and phosphatidylglycerol (PG; negatively-charged; abundant in *S. aureus* membranes). Large unilamellar liposomes (5 μM phospholipids) were prepared in 20 mM Tris-HCl - 200 mM NaCl-pH 7.4 by extrusion in the presence of calcein and purified by centrifugation. They were then incubated in the same medium in the presence of ORI or VAN (0.5 μM; 0.82 or 0.71 mg/L) at 37 ºC for up to 60 min with continuous monitoring of the change in fluorescence signal.

Results: The table shows the release of entrapped calcein from liposomes over time.

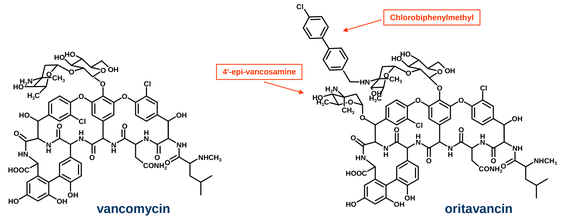
Composition and property	Release of calcein (% of total) vs. time					
	10 min		30 min		60 min	
	VAN	ORI	VAN	ORI	VAN	ORI
PE:PC (6:4, mol/mol)	-17 ± 1	19 ± 3	-11 ± 1	35 ± 5	-15 ± 1	43 ± 2
PE:PG (6:4, mol/mol)	-5 ± 2	35 ± 5	-8 ± 2	67 ± 3	-6 ± 1	75 ± 3

Conclusion: In contrast to VAN, which did not permeabilize liposomes (whether neutral or acidic), ORI caused a marked release of calcein, which was more pronounced from liposomes containing PG. This suggests that, in addition to hydrophobic interactions, electrostatic binding forces also play a role in the permeabilization process induced by ORI.

INTRODUCTION

Oritavancin is a semi-synthetic lipoglycopeptide characterized by an additional 4'- epi-vancosamine and a chlorobiphenylmethyl side chain relative to vancomycin.

In contrast to glycopeptides such as vancomycin and teicoplanin, oritavancin shows a marked and rapid bactericidal effect, suggestive of a new mode of action. The presence of the lipophilic side chain can favor the interaction of oritavancin with membrane lipids (1), which could result in alterations of membrane properties.



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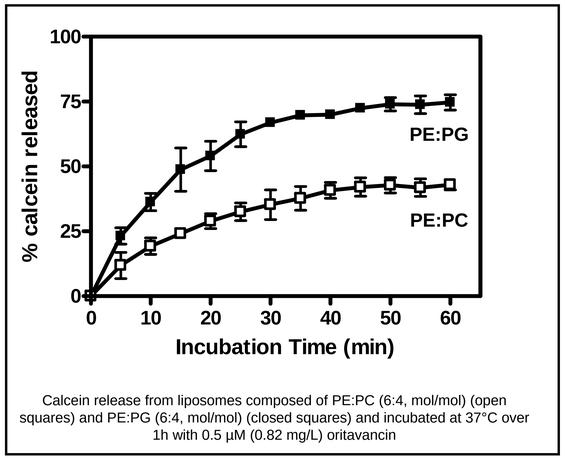
RESULTS

Permeabilization of liposomes by oritavancin

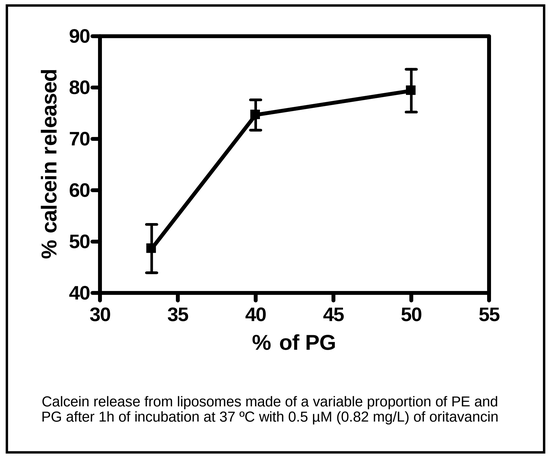
A) Influence of time and of membrane composition



Calcein release from liposomes composed of PE:PC (6:4, mol/mol) (open squares) and PE:PG (6:4, mol/mol) (closed squares) and incubated at 37°C over 1h with 0.5 μM (0.82 mg/L) oritavancin

Release of calcein occurs rapidly and is faster with liposomes containing a negatively-charged phospholipid such as phosphatidylglycerol (PG).

B) Influence of phospatidylglycerol (PG) content in the membrane



Calcein release from liposomes made of a variable proportion of PE and PG after 1h of incubation at 37 ºC with 0.5 μM (0.82 mg/L) of oritavancin

Enhancement in phospatidylglycerol (PG) content increases the susceptibility of liposomes to permeabilization by ORI.

Permeabilization of liposomes by vancomycin

Vancomycin showed no effect when used at the same molar concentration as oritavancin, regardless of the composition of the liposomes.

AIM OF THE STUDY

- To examine the effect of oritavancin on membrane permeability of liposomes by measuring the release of a fluorescent marker.
- To assess the importance of membrane composition, and of its content in phosphatidylglycerol in particular, as this phospholipid is abundant in the plasma membrane of *S. aureus* (2).
- To compare oritavancin to vancomycin in this context.

METHODS

- **Liposomes:** PE (phosphatidylethanolamine; zwitterionic), PC (phosphatidylcholine; zwitterionic) and PG (phosphatidylglycerol; anionic) were dissolved in chloroform:methanol (2:1, v/v), mixed at the desired ratio in a round flask and evaporated in a rotavapor. The dried films were resuspended (vortex and sonication) with buffer containing calcein at a self-quenching concentration to form Multilamelar Large Vesicles (MLV). MLV were extruded by forcing the preparation 10 times through 100 nm filters to obtain Large Unilamelar Vesicles (LUV). The non-trapped calcein was eliminated by column chromatography using a G-50 Sephadex® gel.
- **Fluorescence measurements:** LUV with entrapped calcein were resuspended at 5 μM with 20 mM TRIS-HCl - 200 mM NaCl, pH 7.40 and put in contact with the antibiotic under study at 37ºC with continuous stirring. Permeabilization of liposomes was monitored by measuring the increase of the fluorescence signal due to the dilution of the marker in the external medium. Maximum fluorescence release (100%) was obtained by disrupting the liposomes with Triton X-100 (3) .

CONCLUSIONS

- Oritavancin caused a marked permeabilization of liposomes which was not observed for vancomycin.
- This effect was increased if the membrane contained phosphatidylglycerol, a negatively-charged phospholipid abundant in the membrane of bacteria.
- This model may serve to better assess the bactericidal mechanism of oritavancin and other lipoglycopeptides.

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