



# Oritavancin accumulates to high levels in the lysosomes of macrophages by endocytosis

F. Van Bambeke, H. Chanteux, D. Tyteca, M.P. Mingeot-Leclercq, P.M. Tulkens

Pharmacologie Cellulaire et Moléculaire, Université catholique de Louvain - Brussels - Belgium



A-1169

### Mailing address:

F. Van Bambeke  
Pharmacologie cellulaire et moléculaire  
UCL 73.70 av. Mounier 73  
1200 Brussels - Belgium  
vanbambeke@facm.ucl.ac.be

**Background :** Oritavancin (ORI) is a new glycopeptide antibiotic which (i) accumulates to exceptionally high levels (up to 300-fold) in eucaryotic cells, and (ii) shows a very slow efflux from cells (ICAAC 2001 #2069). ORI is very active against intracellular infection caused by lysosomal bacteria like *S. aureus* (ICAAC 2002 #A483). This triggered us to examine the mode of entry and the subcellular localization of ORI.

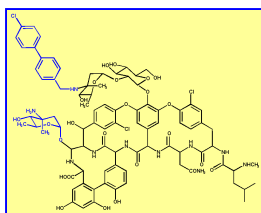
**Methods:** J774 mouse macrophages were incubated with ORI and its kinetics of uptake and subcellular localization (using isopycnic centrifugation of cell homogenates prepared in isotonic sucrose) examined in comparison with chloroquine (CHL, a cationic amphiphilic drug that accumulates in cells and lysosomes by proton-trapping) and horseradish peroxidase (HRP; a protein entering cells by mixed fluid-phase and adsorptive endocytosis).

**Results:** The accumulation kinetics of ORI and HRP were linear for 4 h whereas CHL reached a plateau after 2 h. ATP-depletion caused a reduction in the accumulation of ORI, CHL and HRP to less than 30 % of control values. Monensin, an agent collapsing intracellular pH gradients and favoring regurgitation from endocytic vesicles, abolished CHL accumulation, and reduced ORI and HRP accumulation to 30 % of control values. Cellular fractionation studies showed that ORI was associated with the lysosomes (using N-acetyl-beta-hexosaminidase as marker), whereas HRP was found both in lysosomes and at the cell surface (adsorptive endocytosis). CHL was distributed equally between cytosol and lysosomes.

**Conclusion:** ORI accumulation kinetics and subcellular localization are suggestive of an entry in cells by endocytosis with little or minimal binding to the pericellular membrane. This mechanism drives the drug to lysosomes, which may explain its high activity towards intracellular *S. aureus*.

## INTRODUCTION

Oritavancin is a new glycopeptide antibiotic which differs from vancomycin by an lipophilic side chain and an additional aminated sugar (highlighted in blue).<sup>1</sup> These features confer to the molecule a lipophilic and ionizable character.



We showed previously that oritavancin accumulates to exceptional levels in cultured macrophages (up to 300-fold in 24 hours) and is very active against intracellular *S. aureus* (see A-1174), a bacteria which multiplies in the lysosomes, but not against *L. monocytogenes* which infects the cytosol.<sup>2-3</sup>

## AIM OF THE STUDY

- to study the mechanism of accumulation of oritavancin, using as comparators
  - chloroquine, a cationic amphiphile which accumulates in acidic compartments (lysosomes) by diffusion and proton trapping of its cationic form,<sup>4</sup>
  - HRP (horseradish peroxidase) which accumulates in the lysosomes by endocytosis.<sup>5</sup>
- to determine the subcellular localization of oritavancin.

## ACCUMULATION STUDIES

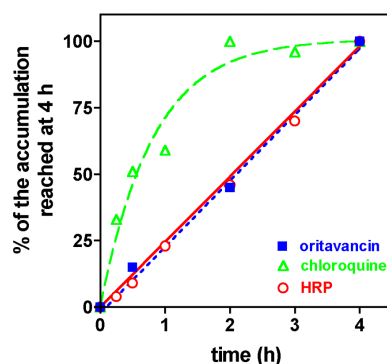
**Methods:** J774 mouse macrophages at confluency were incubated with oritavancin, chloroquine, or HRP, washed in NaCl 0.9 % (oritavancin, chloroquine) or with a procedure allowing to eliminated adsorbed HRP.<sup>5</sup> Cell content in oritavancin was measured by scintillation counting; in chloroquine, by fluorimetry ( $\lambda_{exc}$ , 335 nm;  $\lambda_{em}$ , 378 nm); in HRP by enzymatic activity.<sup>4-5</sup> Cell content was expressed by reference to the protein content.

### Results:

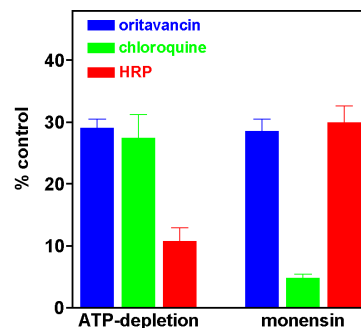
#### cellular accumulation of the studied agents

agents	extracellular concentration mg/L	cellular content * mg/g prot	accumulation
oritavancin	20	5.5	55
chloroquine	20	42.4	424
HRP	2000	15.7	2

\* 2 h incubation



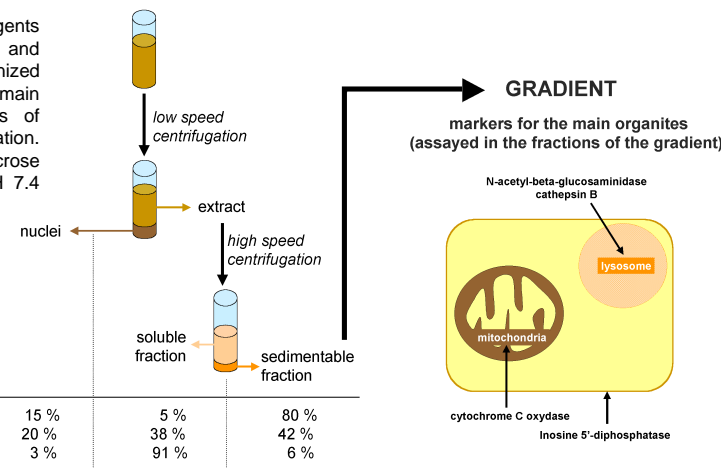
LEFT : Kinetics of accumulation for oritavancin, chloroquine and HRP in J774 macrophages expressed in % of the value reached at 4 h.  
 RIGHT: influence of ATP-depletion (as a mean to inhibit all active processes) and of monensin (as an agent collapsing pH gradients between cell compartments and stimulating regurgitation) on the accumulation of oritavancin, chloroquine and HRP after a 2 h incubation.



## SUBCELLULAR LOCALIZATION STUDIES

**Methods:** Cells incubated with one of the agents under study were washed in NaCl 0.9 % and collected in sucrose 0.25 M. Cells were homogenized with a Dounce grinder. Separation of the main subcellular organelles was made by means of combined differential and isopycnic centrifugation. Cell homogenates were then fractionated in sucrose 0.25 M - 1 mM EGTA - 3 mM imidazole pH 7.4 following the procedure described in the schema.

The sedimentable fraction was then deponed at the top of a linear sucrose gradient (density 1.1 - 1.2) and was separated in 12 fractions by ultracentrifugation. Enzymatic markers of the main organelles were assayed in each fraction, as well as oritavancin, chloroquine and HRP



## CONCLUSIONS

- the kinetics of accumulation of oritavancin is similar to that of a marker of endocytosis (HRP) but slower than that of an agent trapped in lysosomes by diffusion - ségrégation (chloroquine) → oritavancin enters in cells by endocytosis
- the accumulation of oritavancin is markedly reduced
  - by ATP-depletion, which is coherent with the energy-dependent character of this process
  - by monensin, probably in relation with its stimulating effect on regurgitation
- cell-associated oritavancin is truly intracellular and not simply adsorbed at the cell membrane as observed for HRP.
- sedimentable oritavancin shows a distribution similar to that of lysosomal enzymes → oritavancin is mainly localized in the lysosomes.

→ oritavancin enters cells by endocytosis, a process which may be facilitated by an interaction with the membrane thanks to its lipophilic and ionizable character.  
 → oritavancin is driven from endosomes to lysosomes where it accumulates in large amounts. This explains why this antibiotic is very active against a lysosomal infection caused by *S. aureus* but not against a cytosolic infection due to *L. monocytogenes*.

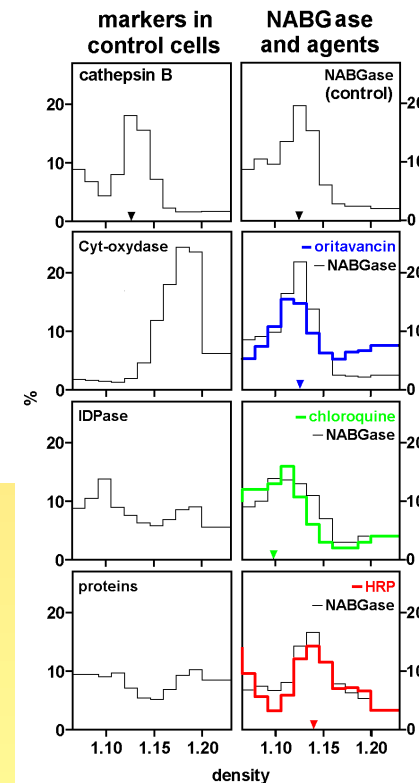
## REFERENCES

- Cooper *et al.* J. Antibiot. (1996) 49: 575-581
- Van Bambeke *et al.* ICAAC (2001) # 2069
- Seral *et al.* ICAAC (2002) # 483.
- Chanteux *et al.* Pharm Res. (2003) 20: 624-31
- Tyteca *et al.* Exp Cell Res. (2002) 281: 86-100

## ACKNOWLEDGMENTS

The authors thank N. Aguilera, M.C. Cambier, and F. Renoid for their expert technical assistance.

## Results



LEFT: distribution pattern of the markers of the main subcellular compartments, upon ultracentrifugation on a linear sucrose gradient.  
 RIGHT: distribution pattern of NABGase and of oritavancin, chloroquine, or HRP in the sedimentable fraction of control cells or of cells incubated during 2 hours with one of these agents. The arrow heads point to the median density of the NABGase distribution. Results are expressed in percent of the total amount recovered in the 12 fractions.