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

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INTRODUCTION

Oritavancin is a new glycopeptide antibiotic which differs from vancomycin by an lipophilic side chain and an additional aminated sugar (highlighted in blue). 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 a lysosomal infection caused by Salmonella aureus (see A-1174), a bacteria which multiplies in the lysosomes, but not against L. monocytogenes which infects the cytosol. 1-2

AIM OF THE STUDY

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

METHODS

ACCUMULATION STUDIES

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. 5 Cell content in HRP by enzymatic activity. 4,5 Cell content was expressed in reference to the protein content.

RESULTS:

Table: Accumulation of oritavancin, chloroquine and HRP

<table>
<thead>
<tr>
<th>Agent</th>
<th>Concentration (mg/L)</th>
<th>Content (mg/g prot)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oritavancin</td>
<td>20</td>
<td>55</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>20</td>
<td>42.4</td>
</tr>
<tr>
<td>HRP</td>
<td>2000</td>
<td>15.7</td>
</tr>
</tbody>
</table>

* 2 h incubation

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 - segregation (chloroquine)
• oritavancin enters in cells by endocytosis
• the accumulation of oritavancin is markedly reduced
  - by ATP-depletion, which is associated 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 previously.
• the sedimentable fraction of control cells or of cells incubated during 2 hours with one of these agents.

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REFERENCES


PHARMACOLOGIE CELLULAIRE ET MOLECULAIRE

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