Pharmacodynamic evaluation of the intracellular activity of tobramycin, doripenem, levofloxacin, and colistin towards Pseudomonas aeruginosa (PAO1) after phagocytosis by human THP-1 macrophages.

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Introduction (1/2)

- *Pseudomonas aeruginosa*, an important human pathogen
  - Gram-negative bacillus
  - Opportunistic human pathogen
    - respiratory system infections
    - chronic infection in CF patients
  - (multi)resistance to antibiotics
  - Intracellular survival
    - ~ 50% of strains demonstrate measurable internalization
      (Engel, 2003)
Introduction (1/2)

- **Pseudomonas aeruginosa**, an important human pathogen
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Introduction (2/2)

• **Current view of the internalization pathway**

  ![Diagram of internalization pathway]

  - **Internalization Raft:**
    - CFTR
    - Caveolin-1
    - Cholesterol

  - Cytoskeletal and membrane changes

  Adapted from:
  - Kannan et al., 2008
  - Bajmoczi et al., 2009

• **Potential role for intracellular reservoir?**
  May constitute a source for chronic infection
Aims of the study

• To develop a model of intracellular infection by *P. aeruginosa* over a 24 h period to allow intracellular growth

• To study in this model the activity of antibiotics representative of the main classes currently used in the clinics

• To compare pertinent pharmacological descriptors of antibiotic activity (maximal efficacy, relative potency) against both extracellular and intracellular forms of *P. aeruginosa*
Experimental procedure

- **Model:**
  - THP-1 cells: Human acute monocytic leukemia cell line
  - PAO1 strain

- **Opsonization** (45', 37°C)
  - 9 mL RPMI + 1 mL human serum

- **Phagocytosis** (2 h)
  - MOI 10

- **Extracellular Wash**
  - GEN 100 µg/ml (1 h)

- **Incubation** (with ATB)
  - (T0, T5 and T24 h)

- **Time 0**
  - 5 to 7x10^5 CFU/mg prot.
Setting-up the model

- **Intracellular localisation of PA:**
  - Confocal imaging

**Blue:** nucleus staining by TO-PRO 3,
**Red:** actin staining by Rhodamin-phalloïdin,
**Green:** *Pseudomonas* specific staining by a FITC-labeled antibody
Results

- **Definition of pharmacodynamic parameters:**
  - **Gentamicin** as an example

<table>
<thead>
<tr>
<th></th>
<th>MIC</th>
<th>$E_{\text{max}}$</th>
<th>$C_{\text{static}}$($\times$MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular</td>
<td>0.5</td>
<td>$-3.95 \pm 0.41$</td>
<td>0.96</td>
</tr>
<tr>
<td>Intracellular</td>
<td>-</td>
<td>$-1.62 \pm 0.19$</td>
<td>22.95</td>
</tr>
</tbody>
</table>
Summary

• **Intracellular *Pseudomonas aeruginosa***

- **$E_{\text{max}}$** for all antibiotics
- **$C_{\text{stat}}$** for GEN, TOB, and CST; ~ for DOR, LVX

- **$E_{\text{max}}$** ↓ for all antibiotics
- **$C_{\text{stat}}$** ↑ for GEN, TOB, and CST; ~ for DOR, LVX
Conclusion

• *P. aeruginosa* is able to invade and survive within human THP-1 cells

• All antibiotics tested show reduced efficacy but to different extents

• This lower activity may contribute to persistence or recurrence of infection.

• Fluoroquinolones seem of interest for further investigation
Acknowledgments

• **Financial support:**

![Logo](image1)

• **FACM Team:**

![Photo of team](image2)
Thank you for your kind attention