Introduction

- *Pseudomonas aeruginosa* (PA) develop as biofilms in chronic pulmonary infections.
- Biofilms are aggregates of PA (50-100 μm) entrapped in a self-produced matrix of anionic polymers (alginate, DNA), often surrounded by patient mucus.
- The activity of cationic antibiotics “ATB” such as tobramycin (TOB) and colistin (COL) against these biofilms is low in-vivo because of their interactions with the matrix.

Purpose

- Develop an in-vitro model composed of anionic polymers found in-vivo in pulmonary PA biofilms to evaluate the efficacy of inhaled ATB used to treat chronic pulmonary infections.

Material & Methods

- A bioluminescent strain of PA (PA01-LUXCDEBA) was incorporated into large calcium alginate beads (φ = 1200 μm). These beads were dispersed in artificial sputum medium (ASM) to produce an in-vitro PA pulmonary biofilm model.
- The effectiveness of ATB (TOB and COL) was tested in the in-vitro PA pulmonary biofilm by measuring:
  - PA bioluminescence kinetics during 40H
  - Bacterial concentrations (log10 CFU / ml) after 40 h of exposure to ATB. These values were plotted as a function of ATB concentrations and modelled using the following inhibitor Emax model.

\[
CFU(t) = Emax \times \left(1 - \frac{C}{C_{50} + C}\right)
\]

- The index with the best fit and low value of C_{50} is more effective.
- The development of resistance to these ATBs among surviving PAs was evaluated by measuring the MIC.

Results

Above 10 times the MIC, the decrease in bioluminescence was greater and faster with COL compared to TOB.

Table 1: Parameter estimates for the Emax model plotted with different ATB tested in the in-vitro model of PA pulmonary biofilm. Data were expressed as mean ± SD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TOB</th>
<th>COL</th>
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<tr>
<td>CFU_{0} (PA)</td>
<td>3.52E+08 ± 4.4E+07</td>
<td>6.44E+08 ± 1.59E+08</td>
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<tr>
<td>C_{50} (μg/ml)</td>
<td>30.88 ± 3.26</td>
<td>11.07 ± 1.61</td>
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<td>η</td>
<td>4.26 ± 0.18</td>
<td>4.04 ± 0.18</td>
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Adaptation of PA to the presence of TOB

- Two phenotypes of colonies were observed.
- Normal and Small colony variant “SCV”. (Described in CF patients with chronic pulmonary infections).
- The bacteria of both types of colonies were bioluminescent.
- SCV appeared only after 48 h of plating on Müller-Hinton agar.
- SCV had similar MIC value compared to the MIC of bacteria before treatment with TOB.
- Percentage of small colonies relative to total number of colonies increased with TOB concentration.

Conclusion & Perspectives

- Bioluminescence measurements and colony counts show that COL was more effective than TOB on an in-vitro model of PA pulmonary biofilm, suggesting a better clinical efficacy of COL than TOB when treating these biofilms.
- PA may persist in biofilms even when exposed to high concentration of TOB without developing resistance to this antibiotic.

References

2. Magen Sonderholm et al, npj Biofilms and Microbiomes vol 4, 2018
4. S.D. Dinesh, Artificial sputum medium, Protoc. excil, 2010