Inhaled nanoparticles and microparticles to treat pulmonary biofilms

7th NanoFar School

FREDERIC TEWES
Angers, June 2019
Inserm U1070
Pharmacology of antimicrobial agents

DOSE
Pharmacokinetics
Preclinical and clinical
- Colistin
- Burn
- Pediatrics
- Cystic Fibrosis
- Critically ill
- Lung transplant

Concentration
Plasma

Concentration
Tissues

Drug targeting
Aerosol delivery
- Micro & Nanoparticules

EFFECT
Microbiology
- Résistances Adaptations

PK-PD Modeling
Semi-mechanistic models, Bayesian approaches
Monte Carlo Simulations

Antibiotics aerosol for the treatment of lung infections
WHO PRIORITY PATHOGENS LIST FOR R&D OF NEW ANTIBIOTICS

Priority 1: CRITICAL

*Acinetobacter baumannii*, carbapenem-resistant

**Pseudomonas aeruginosa**, carbapenem-resistant

*Enterobacteriaceae* *, carbapenem-resistant, 3rd generation cephalosporin-resistant*
Chronic versus acute infections to *Pseudomonas Aeruginosa* (PA)

PA is an opportunistic pathogen that can cause invasive infections, such as acute pneumonia, in immune compromised hosts. It also causes chronic infections that are impossible to eradicate, such as the chronic lung infection in cystic fibrosis (CF) patients.

PA adapts to the host environment and undergoes changes which promote bacterial survival and evasion of host defenses.

**chronic infections**
- Persistent inflammation
- Low metabolic activity
- ATB resistance
- Low mobility (Flagellin ↓)
- ↓ host recognition
- bacterial biofilm

**acute infections**
- Planktonic bacteria (free swimming bacteria)
- High metabolic activity
- High virulence (T3SS, secreted proteases ↑)
- Can be eliminated by ATB and detected by host
Pulmonary biofilms

Biofilm: Bacterial aggregate in a matrix of exopolymeric substance (anionic polysaccharides (alginate), proteins & DNA)

Clusters of *P. aeruginosa* biofilms (<50-100 µm wide) are found embedded in the conducting airways mucus, often surrounded by immune cells such as polymorphonuclear Neutrophils (PMN)

Mucus of CF patient (anionic polymer)

Three barriers prevent the penetration of ATB

Biofilms contain regions with low metabolic activity, and this contributes to reduced susceptibility towards antibiotics

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Targeting area: the conducting zone

- Aerosol delivery vs IV or oral administration?

- Higher local conc. / Lower systemic conc.

Is it always true ?
Main factors controlling antibiotics lung conc.?
What is the objective?

Local Effect

Dissolution → Absorption

SYSTEMIC Effect
Systemic

**Biopharmaceutical Classification System**

- **I**: High solubility, High permeability
- **II**: Low solubility, High permeability
- **III**: High solubility, Low permeability
- **IV**: Low solubility, Low permeability

Diagram shows the correlation between volume required to dissolve the highest dose (mL) and permeability (1x10^-6 cm per s).
Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats: 1. Ciprofloxacin, Moxifloxacin, and Grepafloxacin

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Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats: 2. Colistin

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Biopharmaceutical Characterization of Nebulized Antimicrobial Agents in Rats: 3. Tobramycin

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1) *In-vitro* experiments transepithelial transport across Calu-3 cell monolayers

**apparent permeability → Papp**

Human lung epithelial cell line
Epithelial cells: thigh junctions
Efflux transport systems: P-gp
<table>
<thead>
<tr>
<th></th>
<th>Solubility (water, mg.mL(^{-1}))</th>
<th>Log P</th>
<th>Perreria</th>
<th>Papp ((\times 10^{-6}))</th>
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<tbody>
<tr>
<td>CIP</td>
<td>1.35</td>
<td>0.28</td>
<td>-0.81</td>
<td>0.7</td>
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<td>MOX</td>
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<td>5</td>
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<td>AZT</td>
<td>0.0429</td>
<td>-3.1</td>
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<td>0.0</td>
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<tr>
<td>TOB</td>
<td>53.7</td>
<td>-6.3</td>
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</table>
2) **Standardized in-vivo protocol in rats**

- **Routes of administration:**
  - Nebulization
  - IV infusion

- **Simultaneous sampling (5x6)**
  - Blood (intracardiac puncture) ➔ Plasma drug concentrations
  - BAL (1 mL NaCl 0.9% - 37°C)

- **LC-MS/MS assay** ➔ Epithelial lining fluid (ELF) concentrations
Class III: a) Colistin

Penn Century MicroSprayer IA-1B

(Gontijo et al., AAC 2014)
Class III: b) Tobramycin

IV

NEB

ELF

Plasma
Class II: Ciprofloxacin

Ciprofloxacin pulmonary delivery/ *P. aeruginosa*

- CIP has a good activity against *P. aeruginosa*
- → high permeability
- → rapid equilibrium between ELF and plasma

How can we maintain CIP in the lung after pulmonary administration?
CIP Pulmonary Permeability Control

Oral absorption of fluoroquinolones is significantly reduced by calcium-containing antacids, such as calcium carbonate.

50 µM of CIP

Calu-3 cell layer

CIP Pulmonary delivery

CIP Pulmonary Permability Control

CIP-metal complex formation decrease its apparent permeability across pulmonary epithelium model.

The more stable the metal-CIP complex, the higher the CIP apparent permeability decrease.


Papp of CIP in conditions of 80% complexation. Cations concentrations were choose to complex 80% of CIP. Data are expressed as the percentage of the control Papp for CIP (means ± S.E.M., n = 8 to 12)
CIP Pulmonary Concentration Control

In vivo, the more stable the metal-CIP complex, the higher the extracellular concentration of CIP in the lung.

Mean unbound CIP normalized concentrations in ELF (black circle) and in plasma (open square) versus time after (A) IT nebulization of a CIP solution, (B) IT administration of CIP-Ca microparticles (F2a), or (C) IT administration of CIP-Cu microparticles (F2b). Dose = 0.35 mg/kg

In vitro antibacterial efficacy in vitro against Planktonic \textit{P. aeruginosa}

Antibacterial efficacy in vitro against Biofilm of *P. aeruginosa*

In vitro evaluation of the response of planktonic *P. aeruginosa* PAO1 strain (left side) and PA14 strain (right side) to various concentrations of CIP and CIP-Cu complex after 20 to 24 h.

High CIP Concentration Was Required to Avoid an Increase in *P. aeruginosa* Virulence

In vitro antibacterial efficacy in vitro against Biofilm of *P. aeruginosa*


In vitro evaluation of the response of biofilms of *P. aeruginosa*

When Treating CIP Susceptible P. aeruginosa Biofilms, High CIP Concentration Should Be Achieved in the Vicinity of the Biofilm as Low CIP Concentration Stimulates Biofilm Production.

fluorescein diacetate (FDA) hydrolysis
Antibacterial efficacy in vivo in a lung chronic infection model

Bioluminescent PAO1::p16Slux tagged by chromosomal integration of p16Slux was enmeshed in agar beads and instilled to rats to reproduce the lung pathology of cystic fibrosis patients with advanced chronic pulmonary disease. Infection was monitored by measuring the bioluminescence.
Antibacterial efficacy in vivo in rats

In vivo efficacy of CIP-Cu in a *P. aeruginosa* chronic lung infection model. Rats were treated on days 4 and 6 with CIP-Cu or CIP·HCl, and the lungs were harvested on day 8. Box and whisker plot of *P. aeruginosa* surviving colony forming units (CFU) per lung on day 8. Whiskers represent the minimal and maximal values (n = 4-6)

nose only exposure inhalation system (NOEIS)

CIP dose 0.35 mg/kg
TOBRAMYCIN against biofilms?

Interaction of the positive charges with the biofilm?
Effect of mucus on time-kill curves

Alginate beads dispersed in ASM to mimic in vivo chronic infections

Alginate beads
Bioluminescent PA

Artificial sputum medium (ASM) mimic the sputum of cystic fibrosis
- 5 g/L mucin from pig stomach
- 4 g/L salmon sperm DNA
- 0.5% (v/v) egg yolk emulsion
- 5.9 mg/L of DTPA
- Minerals and amino acids
- pH = 7

Kirchner, Sebastian, et al. Journal of visualized experiments: JoVE 64 (2012).
Effect of mucus on Tobramycin time-kill curves

Results in agreement with Müller et al.
Human airway mucus alters susceptibility of *P. aeruginosa* biofilms to tobramycin, but not colistin.

A significant reduction of tobramycin efficacy when *P. aeruginosa* biofilms were grown in the presence of mucus-like medium.

Biofilms in the presence of mucus-like medium respond differently to tobramycin.
PEGylation of Tobramycin Improves biofilm antimicrobial activity

Log-reduction of PA biofilms after treatment with TOB-PEG

Why nanoparticles to treat pulmonary biofilms?

- Improving diffusion
- Improving interactions with bacteria and overcome drug efflux pump
- Synergic effect with ATB (metal nanoparticles)

Lipid nanoparticles to deliver colistin and a terpenic adjuvant

Journal of Controlled Release Volume 190, 2014, Pages 607-623
Antimicrobial-resistant (AMR) infections currently claim at least 700,000 lives each year.

If the rise in resistance to ATB remains the same, The impact of the AMR by 2050 would lead to 10 million people dying every year.
Thank you!

- Julien Brillault
- Sandrine Marchand
- Barbara Lamy
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- ...
- Anne Marie Healy

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