Infectiology award:
Bacterial and cellular factors affecting antibiotic activity towards persistent infections

Françoise Van Bambeke
Louvain Drug Research Institute, UCL
Paul Ehrlich, the father of anti-infective chemotherapy

“The aim is to find chemical substances that have special affinities for pathogenic organisms and that, like “magic bullets”, go straight to their targets.”

“corpora non agunt nisi fixata”

Ehrlich’s “magic bullet” theory
Studying anti-infective pharmacology to improve antibiotic treatment
Specific lifestyles associated with persistent infections

Intracellular infection: an application of the Trojan horse strategy

Intracellular *Staphylococcus aureus*

*Seral et al, AAC 1993*
Specific lifestyles associated with persistent infections

Intracellular *Staphylococcus aureus*

*Carryn et al, Infect Dis Clin North Am., 2003*
Specific lifestyles associated with persistent infections

Biofilms: bacterial hibernation

Bauer, Siala et al, AAC 2013
Specific lifestyles associated with persistent infections

Biofilms: bacterial hibernation

Bauer, Siala et al, AAC 2013
Specific lifestyles associated with persistent infections

Intracellular infection: activity of the fluoroquinolone moxifloxacin on S. aureus

Lemaire et al, JAC 2011
Specific lifestyles associated with persistent infections

Intracellular infection: activity of the fluoroquinolone moxifloxacin on *S. aureus*

**Cs ~ intracellular bacteria:**
Measure of the « intracellular MIC »

- « Pharmacokinetic-related » parameter:
  accumulation in the infected compartment
  intracellular bioavailability

- influence of local environment on activity
  pH
  oxidant species

*close to the MIC even for antibiotics accumulating in cells*

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Lemaire et al, JAC 2011
Specific lifestyles associated with persistent infections

Intracellular infection: activity of the fluoroquinolone moxifloxacin on *S. aureus*

**Emax ~ intracellular bacteria:**
Measure of killing capacity

- **Pharmacodynamic-related** parameter:
  - mode of action of the drug
  - bacterial responsiveness
  - cooperation with host defenses

Lower than extracellularly, suggesting poor bacterial responsiveness and/or antibiotic expression of activity

*Lemaire et al, JAC 2011*
Specific lifestyles associated with persistent infections

Biofilm: activity of the fluoroquinolone moxifloxacin on *S. aureus*

Pharmacodynamic profile similar to that observed intracellularly, suggesting similar defeating factors.

*Bauer, Siala et al, AAC 2013*
Active efflux and **intrinsic/acquired resistance** to antibiotics

- **bacteria**
- **antibiotic**
- **patient**

low permeability + constitutive active efflux

**intrinsic resistance**
Azithromycin is inactive on *P. aeruginosa* ...

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<tr>
<th>medium</th>
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Active efflux and intrinsic/acquired resistance to antibiotics

Azithromycin is inactive on *P. aeruginosa* ...

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... but is successfully used in cystic fibrosis patients


Effectiveness and safety of macrolides in cystic fibrosis patients: a meta-analysis and systematic review

Yun Cai¹, Dong Chai¹, Rui Wang¹*, Nan Bai¹, Bei-Bei Liang¹ and Youning Liu²

Conclusions: Long-term use of azithromycin can improve lung function, especially for *P. aeruginosa*-colonized CF patients. There was no evidence of increased adverse events with azithromycin. More data are needed to verify the best azithromycin regimen and to evaluate other macrolides in CF patients.
Active efflux and intrinsic/acquired resistance to antibiotics

Azithromycin becomes active on *P. aeruginosa* when efflux systems are inactivated

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*Buyck et al, CID 2012*
Azithromycin becomes active on *P. aeruginosa* when cultivated in biological fluids

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<td>Bronchoalveolar lavage</td>
<td>PAO1</td>
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<td>CA-MHB/serum 50:50</td>
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*Buyck et al, CID 2012*
Active efflux and intrinsic/acquired resistance to antibiotics

Azithromycin does express its activity on *P. aeruginosa* in biological fluids by repressing the expression of its efflux transporters

Buyck et al, CID 2012
Active efflux and intrinsic/acquired resistance to antibiotics

- reduced concentration at the target site
- risk of suboptimal dosage
- risk of selection of resistance (target mutations)
Active efflux and intrinsic/acquired resistance to antibiotics

Efflux mechanisms are overexpressed during treatment

*Pseudomonas aeruginosa* isolated from patients in intensive care units suffering from hospital-acquired pneumonia during treatment with antibiotics

Riou et al, ECCMID 2010
Active efflux and modulation of cellular pharmacokinetics of antibiotics

- Reduced concentration inside the cells
- Modification of PK profile
- Alteration of activity against intracellular bacteria
Active efflux and modulation of cellular pharmacokinetics of antibiotics

Differential recognition of fluoroquinolones by macrophage efflux transporters

Michot et al., AAC 2004 & 2005
Active efflux and modulation of cellular pharmacokinetics of antibiotics

But similarity in recognition of fluoroquinolones by macrophage and bacterial efflux transporters

Substrates of efflux systems from macrophages and Gram-(+) bacteria

Poor substrates of efflux systems from macrophages and Gram-(+) bacteria

Dupont et al., ECCMID 2012
Studying anti-infective pharmacology to improve antibiotic treatment

- Bacteria
- Host cells
- Intracellular infection
- Antibiotic
- Efflux
- Patient

15 December 2013
Cooperation between procaryotic and eukaryotic efflux systems

Bacterial and eukaryotic efflux pumps cooperate to make intracellular *Listeria monocytogenes* resistant to ciprofloxacin

*Lismond et al., AAC 2008*
Cooperation between procaryotic and eukaryotic efflux systems

Moxifloxacin intracellular activity is not affected by eukaryotic / bacterial efflux pumps

Lismond et al., AAC 2008
Clinical implications of this work

1. Expression of activity is highly dependent on the environment
   - Growth medium can influence bacterial physiology
   - Intracellular bacteria / biofilms are refractory to antibiotics
   → importance of testing antibiotic activity in relevant media/models

2. Screening tests in routine laboratories may need to be revisited
   - Susceptibility changes during treatment
   - Low level resistance mechanisms (efflux) often escape detection in routine
     but are clinically meaningful
   → interest of basing dosages on PK/PD approaches and MIC determinations
   → importance of developing new diagnostic tools

3. Pertinent in vitro models may be helpful in preclinical evaluation of new drugs
   - Intracellular survival is considered as determinant in recurrence / persistence
   - Biofilms are associated to about 80% of infections
   → in vitro screening may help selecting drug candidates
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Coworkers over the years in the team - efflux

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15 December 2013