Etude de l’activité d’antibiotiques vis-à-vis des formes intracellulaires de *Staphylococcus aureus* et *Legionella pneumophila*

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What makes bacteria so difficult-to-treat?

- Rising resistance to antimicrobials … reaching the limit of clinical application …
- Difficulty in eradicating intracellular forms, which probably results in recurrences, relapses and selection of drug-resistant organisms.

Routine evaluation of antibiotic activity is usually performed on extracellular infections.
Intracellular bacteria

S. aureus

The intracellular environment:

• delays the growth rate of this organism
• protects bacteria from the lethal action of most antimicrobials

Treatment of intracellular infections

An effective treatment requires antibiotics that efficiently accumulate within the infected intracellular compartment, and express activity therein.
Aim of the study

Evaluation of the activity of antibiotics against bacteria sojourning intracellularly in distinct subcellular compartment, such as

- *L. pneumophila* (phagosomal model of infection)
- or *S. aureus* (phagolysosomal model of infection)

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Accumulation (Cc/Ce)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>+++ (&gt; 40)</td>
</tr>
<tr>
<td>Clindamycin, tetracycline,</td>
<td>++ (5 to 20)</td>
</tr>
<tr>
<td>fluoroquinolones</td>
<td></td>
</tr>
<tr>
<td>Linezolid</td>
<td>+ (1 to 2)</td>
</tr>
</tbody>
</table>
Intracellular *L. pneumophila*

- Intracellular replication of *Legionella pneumophila*

- Intracellular multiplication of *Legionella pneumophila* in cultured human embryonic lung fibroblasts

- Cell-mediated immunity in Legionnaire’s disease
  Horwitz, J Clin Invest (1983), 6:1686-97

- Adhesion, penetration and intracellular replication of *Legionella pneumophila*: an in vitro model of pathogenesis

- Intracellular survival and expression of virulence determinants of *Legionella pneumophila*
  Hacker et al, Infection (1991), 4:S198-201

- Modulation of caspases and their not-apoptotic functions by *Legionella pneumophila*
  Amer AO, Cell Microbiol (2009), in press
Intracellular *S. aureus* is a reality ...
Intracellular *S. aureus*

**PULMONARY INFECTIONS ASSOCIATED WITH CYSTIC FIBROSIS**  
*Jarry and Cheung, Infect Immun, 2006*

**COMPLICATED SKIN INFECTIONS**  
*Mempel et al, Br J Dermatol., 2002*

**RECURRENT RHINOSINUSITIS**  
*Clement et al, J Infect Dis, 2005*

**ENDOCARDITIS**  
*Sinha and Herrmann, Thromb Haemost, 2005*

**OSTEOMYELITIS**  
Wright and Nair, Int J Med Microbiol; 2009*
**S. aureus** reprograms its transcriptome once it reaches the cellular environment...

- **Down-regulation of major metabolic pathways** (cell division, nutrient transport, ...)
- **Up-regulation of bacterial genes contributing to oxidative stress protection**
- **Down-regulation of toxin genes known to affect host cell integrity** (i.e. *hla*)

Human lung epithelial cells

Garzoni et al, BMC Genomics, 2007
Intracellular lifestyle of *S. aureus* and *L. pneumophila*

- *L. pneumophila*
- *S. aureus*

Phagosomes

ENDOPLASMIC RETICULUM-DERIVED VESICLES

~ pH 6.1

Phagolysosomes (pH 5-5.5)
Methods

THP-1 macrophages

*L. pneumophila*  
Opsonized *S. aureus*

phagocytosis

Removal of extracellular bacteria (washings)

Incubation with antibiotics  
(equivalent to human Cmax)

At 24h (SA) and 48 h (LP): removal of extracellular bacteria/antibiotics and cell lysis

CFU counting and assay of protein content

*Lemaire et al, JAC, 2005; Barcia-Macay et al, AAC, 2006*
Bacterial growth

The intracellular environment delays the growth of both bacteria
### Intrinsic activities (MICs)

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Human Cmax (mg/L)</th>
<th>S. aureus ATCC 25923*</th>
<th>L. pneumophila ATCC 33153**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>pH 7.4</td>
<td>pH 5.5</td>
</tr>
<tr>
<td>azithromycin</td>
<td>0.5</td>
<td>0.5</td>
<td>256</td>
</tr>
<tr>
<td>tetracycline</td>
<td>5</td>
<td>0.5</td>
<td>N.D.</td>
</tr>
<tr>
<td>clindamycin</td>
<td>20</td>
<td>0.125</td>
<td>4</td>
</tr>
<tr>
<td>linezolid</td>
<td>20</td>
<td>1-2</td>
<td>1-2</td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>4</td>
<td>0.125</td>
<td>0.5</td>
</tr>
<tr>
<td>finafloxacin</td>
<td>10</td>
<td>0.06</td>
<td><strong>0.01</strong></td>
</tr>
<tr>
<td>moxifloxacin</td>
<td>4</td>
<td>0.03</td>
<td><strong>0.125</strong></td>
</tr>
</tbody>
</table>

* Mueller Hinton broth (pH 7.4);
** Buffered yeast extract supplemented with a-ketoglutarate (pH 6.9)
Intracellular activity of antibiotics against *S. aureus*

- Azithromycin fails to reduce the intracellular foci (probably in relation with the deleterious effect of acidic pH on its antibacterial activity)

- Poor intracellular activity of tetracycline, clindamycin, linezolid and finafloxacin

- Higher reduction of the intracellular load obtained for ciprofloxacin- and moxifloxacin-treated cells
Intracellular activity of antibiotics against *S. aureus*

Increasing the incubation time (to 72 h) is associated with:

- a slight increased of clindamycin and linezolid activities

- a true bactericidal effect (≥ 3 log cfu decrease) of moxifloxacin against the intraphagocytic forms of *S. aureus*
Intracellular activity against *L. pneumophila*

- Azithromycin fails to reduce the intracellular foci
- Poor intracellular activity of tetracycline, clindamycin, linezolid and ciprofloxacin
- Higher reduction of the intracellular load obtained for moxifloxacin- and finafloxacin-treated cells
Dose-response activities against *L. pneumophila*

- Both antibiotics exerted concentration-dependent activities against intraphagocytic *L. pneumophila*

- Pertinent pharmacological descriptors of antibiotic activity for finafloxacin:
  - Static concentr. : 0.06 mg/L
  - EC$_{50}$: 0.33 mg/L
  - Emin : 0.9 log CFU
  - Emax: - 2.7 log CFU
Discussion and perspectives
Conclusions

• Despite of their marked cellular accumulation,
  - azithromycin fails to prove efficacy against intracellular organisms
  - tetracycline and clindamycin show only poor intracellular activity

• Potential advantages of moxifloxacin (for intracellular *S. aureus*) and finafloxacin (for intracellular *L. pneumophila*)
Conclusions

• Absence of direct correlation between the cellular accumulation of antibiotics and the expression of antibiotic efficacy

• Routine evaluation of antibiotic efficacy (which is useful to predict the therapeutic outcome when dealing with extracellular bacteria), does not allow foreseeing efficacy against intracellular organisms

• It is crucial to critically examine the cellular pharmacodynamics of antibiotics against intracellular pathogens
Thank you for your attention!
Thanks to …

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• FRS-FNRS (Belgique)