Hunting intracellular bacteria with antibiotics

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Traveling from one « Notre Dame » to the other …

... is not too disorienting!
A quite nice common experience ....

Restoration of Susceptibility of Methicillin-resistant Staphylococcus aureus to β-Lactam Antibiotics by Acidic pH

ROLE OF PENICILLIN-BINDING PROTEIN PBP 2a

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But what is the link between PBP2a and intracellular infections?

Insertion of Epicatechin Gallate into the Cytoplasmic Membrane of Methicillin-resistant Staphylococcus aureus Disrupts Penicillin-binding Protein (PBP) 2a-mediated β-Lactam Resistance by Delocalizing PBP2a

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A quite nice common experience ....

But what is the link between PBP2a and intracellular infections?

MRSA is everywhere ... including inside our cells!
The infected cell: a guided tour …

Listeria; cytosol

S. aureus; phagolysosomes
Intracellular killing of bacteria by host cell defence mechanisms

Phagosomes

Lysosomes

Phagolysosomes
Some bacteria can escape host cell defence mechanisms …

Phagosomes
- Salmonella spp.
- Brucella spp.

Phagolysosomes
- Legionella pneumophila
- Staphylococcus aureus

Lysosomes

Inclusions
- Chlamydia spp.

Early endosomes

Mycobacterium spp.

Cytosol
- Listeria monocytogenes
- Shigella flexneri
- Legionella pneumophila

Endoplasmic reticulum

Benefits of intracellular life

- protection
- invasion
- persistence
Benefits of intracellular life

invasion
Migration to the CNS

**Listeria:** from the gut to the CNS

- Direct invasion of endothelial cells
- Phagocyte-facilitated invasion
- Neural route

- Bone-marrow monocyte
- Adherence and transfer from monocytes to endothelial cells
- Intra-axonal labeling by anti-listeria antibodies

Benefits of intracellular life

persistence
**S. aureus** persistent infections

Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis

*Clement et al., J Infect Dis. (2005) 192:1023-8*
S. aureus persistent infections

Evidence of an intracellular reservoir in osteocytes (A,B), osteoblasts (C) and bone matrix of a patient with recurrent osteomyelitis

S. aureus persistent infections

Evidence of Small Colony Variants and of intracellular S. aureus after treatment failure * in patients with prosthetic joint infections

* Fluclox, CIP+ RIF, VAN + FEP

Benefits of intracellular life

protection
Failure to eradicate with antibiotics in vitro …
and treatment difficulties ...
How to hit intracellular bacteria with antibiotics?
Antibiotic properties for intracellular activity

PK / PD

- metabolism
- binding
- cooperation with host defences
- physico-chemical conditions
- bacterial responsiveness

- influx
- efflux

accumulation and bioavailability

Cellular pharmacokinetics ....

accumulation and distribution
Antibiotic accumulation and subcellular distribution

**diffusion**

- β-lactams: fast; ~ 1 x
- fluoroquinolones: fast
  - CIP, LVX: 4-10 x
  - MXF, GAR, GMF: 10-20 x
- linezolid: ~ 1 x
- lincosamides: 1-4 x
- tetracyclines: 2-4 x
- rifampin: 2-10 x
- synercid: 30-50 x

**endocytosis**

- aminoglycosides: slow; 2-4 x
- glycopeptides: slow
  - VAN: 8 x
  - TLV: 50 x
  - ORI: 150-300 x
- macrolides: fast
  - ERY: 4-10 x
  - CLR, ROX, TEL: 10-50 x
  - AZM: > 50 x
  - CEM-101: 350 x
- oxazolidinones: fast
  - RDZ: 10 x

**diffusion/segregation**
Can we simply predict intracellular activity based on MIC and antibiotic accumulation?

**MIC**
(L. monocytogenes)

**antibiotic accumulation**

activity on intracellular *Listeria* (5 h; 10 x MIC)

Importance of reaching intracellular bacteria ...

adapted from Carryn et al., AAC (2002) 46:2095-2103
Van Bambeke et al., AAC (2004) 48:2853-60
Barcia-Macay et al., AAC (2006) 50:841-51
Importance of optimizing time and concentration ...

ampicillin against *Listeria monocytogenes*

adapted from Lemaire et al., JAC (2005) 55:897-904
Cellular pharmacodynamics

cooperation with host defences

physico-chemical conditions

bacterial responsiveness
Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & S. aureus

Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & S. aureus


<table>
<thead>
<tr>
<th>model</th>
<th>$C_{stat}$ (x MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>extra</td>
<td>0.27</td>
</tr>
<tr>
<td>intra</td>
<td>0.63</td>
</tr>
</tbody>
</table>

relative potency
Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & S. aureus

Setting-up appropriate models for the study of cellular activity of antibiotics

moxifloxacin & *S. aureus*

Quantitative comparison
~ models
~ drugs

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<th>$C_{stat} \times MIC$</th>
<th>$E_{max}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>extra</td>
<td>0.27</td>
<td>-3.86 (5.22 to 2.51)</td>
</tr>
<tr>
<td>intra</td>
<td>0.63</td>
<td>-2.77 (3.31 to 2.22)</td>
</tr>
</tbody>
</table>
in vitro vs in vivo

Linezolid & S. aureus

in vitro (macrophages)

in vivo (peritonitis)

Sandberg et al. JAC (2010) 65:962-973
What do these models tell us?
What do these models tell us?

comparison: 1 drug ~ different models of infection

Linezolid; THP-1 cells

- Cs close to the MIC
- Amplitude of the effect depending on intracell. growth

What do these models tell us?

**comparison : 1 drug ~ different bacterial strains**

Linezolid ; THP-1 cells & *S. aureus*

- Cs close to the MIC for all susceptible strains
- Resistant strains may show modified Emax

*Δ log CFU from time 0 vs Log concentration (x MIC)*

*Lemaire et al, AAC (2010) 54:2549-59*
What about intracellular potency?

comparison: 1 model ~ different drugs

THP-1; S. aureus

• Cs close or slightly higher than the MIC for all drugs
How to modulate intracellular potency?

➤ change pH!

THP-1; phagolysosomal *S. aureus*

**MIC at acidic pH**

*Baudoux et al, JAC (2007) 59:246-53*
How to modulate intracellular potency?

- change pH!

THP-1; phagolysosomal S. aureus

MIC at acidic pH x lysosomal accumulation

*Baudoux et al, JAC (2007) 59:246-53*
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But what is the link between PBP2a and intracellular infections?
MRSA vs. MSSA: intracellular activity of β-lactams

MRSA are as susceptible as MSSA to β-lactams when intracellular!

Lemaire et al., AAC (2007) 51:1627-32
MRSA vs. MSSA: extracellular activity of $\beta$-lactams

MRSA are as susceptible as MSSA in broth at acidic pH

Lemaire et al., AAC (2007) 51:1627-32
MRSA vs. MSSA: extracellular activity of $\beta$-lactams

Neutralization of lysosomes makes intracellular MRSA resistant to $\beta$-lactams!

Lemaire et al., AAC (2007) 51:1627-32

MRSA are inside [acidic] vacuoles

\[ \text{control} \quad \text{meropenem} \quad \text{cloxacillin} \]

\[ \Delta \log \text{CFU} (24h - 0h) \]

\[ 0 \quad 10 \quad \text{ammonium chloride (mM)} \]
PBP2a conformation is modified by acidic pH

**FIGURE 4.** Circular dichroic spectra of PBP 2a at pH 7.0 (left panel) and pH 5.5 (right panel) in the absence (open symbols) and in the presence (closed symbols) of oxacillin (30 μM) for 30 min at 25 °C. The thin dotted lines in each graph represent minima of PBP 2a molar ellipticity at 222 nm (vertical arrow on the abscissa) for each condition. The spectrum of oxacillin has been subtracted from all data points.
How to modulate intracellular potency?

→ increase concentration!

- intracellular potency
- accumulation in lysosomes

...of azithromycin are increased by P-glycoprotein inhibitors

*Seral et al., JAC (2003) 51:1167-73*
How to modulate intracellular potency?

- Increase concentration by modulating pH!

- Cellular accumulation
- Intracellular potency of delafloxacin are increased in medium at acidic pH

![Graph showing cellular accumulation and log cfu from time 0 against pH values and log concentration (mg/L)]

Lemaire et al., ICAAC (2010) A1-677
What about intracellular 

**efficacy**?

**comparison**: 1 model ~ different drugs

THP-1; S. aureus

- $E_{max}$ lower intracellularly
- highly variable depending on the drug

*Diagram showing $E_{max}$ for various drugs in THP-1 macrophages and broth.*

*Effect concentration*
How to modulate intracellular efficacy?

Comparison: 1 drug ~ S vs R strains

THP-1; S. aureus

- $E_{\text{max}}$ lower for a resistant strain

Baudoux et al., JAC (2010) 65:1228-36
How to modulate intracellular efficacy?

comparison: isogenic strains with different phenotypes

S. aureus: SCV vs normal phenotype

How to modulate intracellular efficacy?

Comparison: isogenic strains with different phenotypes

*S. aureus* – THP1

How to modulate intracellular efficacy?

Use combinations: Fractional maximal effect (FME) approach

- Handle the nonlinear pharmacodynamics exhibited by antibiotics
- Analyse the combinations with calculated and not arbitrarily chosen concentrations

Effect (E): decrease of inoculum after 24 h. Sigmoid $E_{\text{max}}$ model $\Rightarrow E_{\text{max}}, EC_{50}$

$$E = \frac{E_{\text{max}} \cdot C^n}{EC_{50}^n + C^n}$$

ATBs (A et B) are combined to a FME = 1.
5 pairs: 0.1 FME$_{A}$ + 0.9 FME$_{B}$, 0.3 FME$_{A}$ + 0.7 FME$_{B}$, 0.5 FME$_{A}$ + 0.5 FME$_{B}$, 0.7 FME$_{A}$ + 0.3 FME$_{B}$, 0.9 FME$_{A}$ + 0.1 FME$_{B}$

Corresponding concentration to be tested alone and in combination:

Desbiolles et al, AAC (2001) 45: 3328-33
How to modulate intracellular efficacy?

Fractional maximal effect (FME) approach: RIF – ORI vs SCV

RIF-ORI combination is highly synergistic over a wide range of concentration ratios

FME > 1: synergistic; = 1: additive

Nguyen et al, AAC (2009) 53:1443-49
Conclusion: what do these models tell us?

"You are completely free to carry out whatever research you want, so long as you come to these conclusions."
Conclusion: what do these models tell us?

• intracellular drug relative potency (Cs) = intracellular « MIC »
  ✓ close or slightly higher than MIC in broth even for drugs with high accumulation
  ✓ reflect of 
    drug concentration in the infected compartment
    influence of environment on intrinsic activity
    bioavailability

• drug efficacy
  ✓ lower than extracellularly
  ✓ highly variable depending on
    the drug
    the bacteria
  ✓ reflect of change in
    bacterial responsiveness ?
    metabolism ?
    persisters ? SCV ?
    bacterial growth rate ?
Perspectives : what to do with these data?

"First of all, I'd like to thank the bacteria..."
Use of these models to suggest new therapeutic options

**Listeria in vitro**

| control | ampicillin | meropenem | gentamicin | azithromycin | moxifloxacin |

**Listeria in vivo**

Bacterial counts in CSF of rabbits during the study period

<table>
<thead>
<tr>
<th>Group</th>
<th>16 h after induction of meningitis(^a)</th>
<th>End of treatment(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5.340 ± 0.717 (n = 12)(^b)</td>
<td>6.334 ± 0.634 (n = 10)</td>
</tr>
<tr>
<td>M</td>
<td>5.375 ± 0.356 (n = 11)</td>
<td>3.830 ± 0.518 (n = 9)</td>
</tr>
<tr>
<td>A2</td>
<td>4.428 ± 0.810 (n = 5)</td>
<td>3.520 ± 0.840 (n = 5)</td>
</tr>
</tbody>
</table>

\(^a\) log\(_{10}\) cfu/mL.  
\(^b\) Number of rabbits.

Use of these models to position new molecules

Guidance for Industry
Microbiological Data for Systemic Antibacterial Drug Products — Development, Analysis, and Presentation

DRAFT GUIDANCE

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

September 2009
Clinical Antimicrobial

C. Intracellular Antimicrobial Concentration Assessment

The ability of an antibacterial drug product to achieve significant intracellular concentrations may have clinical importance when the target organism can reside within the cell (e.g., *Listeria, Chlamydophila, Legionella*). In situations where the antimicrobial drug product is intended to treat infections caused by microorganisms that reside within the cell, sponsors should provide data on the drug product’s ability to penetrate into host cells and demonstrate the drug product’s activity inside the cell against target microorganisms.
Use of these models to position new molecules

New on the US market!

Use of these models to position new molecules

SA 238 (LNZ-S)  
SA 238L (LNZ-R)  

\[ \Delta \log \text{CFU from time 0} \]

Log extracell. conc. (mg/L)

-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0 2.5 3.0

-2 -1 0 1 2

LNZ RX-1741

2 protonable aminated functions

completed Phase II for radezolid

Improved binding to the target
Increased cellular accumulation

Lemaire et al, AAC (2010) 54:2549-2559
Use of these models to position new molecules

Solithromycin - fluoroketolide

Improved binding to the target
High cellular accumulation

entering Phase II

Use of these models to position new molecules

But failed at FDA …
Yet, ceftaroline received a positive advice on Sept. 7!

Which drug is going to win the battle against intracellular bacteria?
Our intracellular PK/PD team over the years ...

S. aureus

C. Seral
M. Barcia-Macay
S. Lemaire
H.A. Nguyen
A. Olivier
P. Baudoux
L. Garcia
Y. Ouadhriri
S. Cerryn
S. Vandevelde
A. Lismond

L. monocytogenes

P. aeruginosa

J. Buyck
G. De Laminne

09/09/2010 University of Notre Dame