Deciphering the activity of antibiotics against intracellular *Staphylococcus aureus* with the help of PK/PD (pharmacokinetics/pharmacodynamics).

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From where do I come from?

"corpora non agunt nisi fixata"

"The goal is ... to find chemical substances that have special affinities for pathogenic organisms and that, like magic bullets, go straight to their targets"
Intracellular *S. aureus*: is it important?

*Brussels: atomium built for the universal exposition in 1958 (crystal structure of iron)*
Intracellular reservoir evidenced *in vivo*

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*
Intracellular reservoir evidenced \textit{in vivo}

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

\textit{Bosse et al., J Bone Joint Surg Am. (2005) 87:1343-7}
Intracellular reservoir evidenced *in vivo*

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

* Fluclox, CIP+ RIF, VAN + FEP

**S. aureus can survive and multiply in several cell types**

- Intracellular *Staphylococcus aureus*. A mechanism for the indolence of osteomyelitis.
  

- Intracellular persistence of *Staphylococcus aureus* small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease.
  

- Phagocytosis of *Staphylococcus aureus* by cultured bovine aortic endothelial cells: model for postadherence events in endovascular infections.
  

- Demonstration of intracellular *Staphylococcus aureus* in bovine mastitis alveolar cells and macrophages isolated from naturally infected cow milk.
  
S. aureus can survive and multiply in several cell types including phagocytic cells

PMN and macrophages
In vitro models:
a possible way for studying antibiotic activity towards intracellular *S. aureus*

*Brussels last week*
Setting up a model of intracellular infection over a 24 h period of time

- infection of macrophages (with opsonized bacteria)
  - Mouse (J774; 5 bact/cell)
  - Human (THP-1; 4 bact/cell)

- washing with GEN 50 µg/ml to eliminate extracellular bacteria

- incubation for up to 24 h with
  - GEN (0.5-1 x MIC)
  - antibiotic under study

Description of the model: how does *S. aureus* grow intracellularly?

Measuring the intracellular activity of antibiotics ...
Intracellular vs extracellular activity of antibiotics: PK – PD in action

- metabolism
- binding
- cooperation with host defences
- influx
- efflux
- accumulation and bioavailability
- physico-chemical conditions
- bacterial responsiveness

Intracellular vs extracellular activity of antibiotics: PK – PD in action

accumulation and bioavailability

Drug targeting is essential

*Belgian classical comic*
Extracellular vs intracellular activity at Cmax

THP-1; 24 h, ATCC25923, antibiotics at Cmax

Extracellular vs intracellular activity at Cmax

THP-1; 24 h, ATCC25923, antibiotics at Cmax

Pharmacodynamic relationships: time-effects at Cmax

Slower killing rate intracellularly

Pharmacodynamic relationships: concentration-effects at 24 h

Concentration-dependent killing; lower Emax intracellularly

Intracellular killing is visible for antibiotics working on cell wall

Any relationship between activity and accumulation?

THP-1; 24 h, ATCC25923, antibiotics at Cmax

Smart choice of antibiotics based on balanced extra- / intra- activity

Adapted from Van Bambeke et al., Curr Opin Drug Discov Devel. (2006) 9:218-30
Do resistant strains escape antibiotics intracellularly?

Art Nouveau in Brussels
Intracellular vs extracellular activity of antibiotics: PK – PD in action

bacterial responsiveness

MSSA, MRSA, (VISA, VRSA)

a lipoglycopeptide shows bimodal effects towards Vanco-S strains…

… because of dual mode of action?

based on Higgins et al AAC (2005) 49: 1127-34

(MSSA, MRSA), VISA, VRSA

A lipoglycopeptide shows unimodal effects towards Vanco-I/R strains…

… because only one mode of action left?

SCV isolated from a cystic fibrosis patient


Thymidine dependent

Normal Phenotype

Spontaneous revertants

SCV

Thy
Intracellular activity, SCV vs normal phenotype

THP-1; 24 h, antibiotics at Cmax

Nguyen et al, RICAII 2007, poster 325
Intracellular activity, SCV over time

THP-1; SCV, antibiotics at Cmax for up to 3 days

Nguyen et al., ICAAC 2007, poster A1437
Intracellular activity, thymidine supplementation

THP-1; SCV, antibiotics at Cmax for up to 3 days

Thymidine supplementation restores intracellular growth but does not affect the activity of most antibiotics.
Dose-response curves of the 3 most active antibiotics against extra- and intra-cellular SCV (24 h of exposure)

- **Extracellular activity:**
  - all drugs show concentration-dependent bacteridal effects

- **Intracellular activity:**
  - RIF and MXF show markedly reduced activity
  - ORI shows a bimodal effect with maximal activity ≈ 3 log

Gray zones: clinically-relevant range of concentrations
Intracellular activity of combinations against SCV

THP-1; SCV, antibiotics at Cmax for 3 days

Poorly active AB + RIF

Combinations between the most active AB

Slightly less active than RIF alone

Combinations with ORI are synergistic

Nguyen et al., ECCMID 2008, poster 1059
Activity of combinations with ORI against intracellular SCV

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:

Activity of RIF-ORI combination against intracellular SCV

Fractional maximal effect (FME) approach

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

FME > 1: synergistic; = 1: additive
VISA and DAP-resistant strains isolated from a patient with endocarditis


Reduced susceptibility associated with

increased amount of bound vancomycin

decreased amount of bound daptomycin

Intracellular activity against VISA and DAP-resistant strains isolated from a patient with endocarditis

- Higher intracellular EC$_{50}$
- No effect of resistance phenotype
- Higher intracellular Emax
- No effect of resistance phenotype

Cellular factors affecting antibiotic intracellular activity

Brussels Grand-Place
Flower carpet
Intracellular vs extracellular activity of antibiotics: PK – PD in action

physico-chemical conditions

acid pH of lysosomes

Famous Belgian bier
MRSA vs MSSA: intracellular activity of β-lactams

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

MRSA vs MSSA: extracellular activity of $\beta$-lactams

MRSA are as susceptible as MSSA in broth at acidic pH

MRSA vs MSSA: extracellular activity of $\beta$-lactams

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

MRSA are inside [acidic] vacuoles

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.

Lemaire et al., JBC (2008) 283:12769-76
Efflux pumps

*Manneken Pis, who saved Brussels from fire by urinating on a burning fuse*
Intracellular vs extracellular activity of antibiotics:
PK – PD in action

P-gp as a cellular mechanism of resistance to intracellular efficacy of antibiotics

- intracellular activity
- accumulation in lysosomes

of azithromycin are increased by P-glycoprotein inhibitors

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P-gp as a cellular mechanism of resistance to intracellular efficacy of antibiotics

- intracellular activity
- accumulation in lysosomes

of **daptomycin** are increased upon P-glycoprotein inhibition or under-expression

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But again targeting the infected compartment is important ....

- intracellular activity
- accumulation in lysosomes

of ciprofloxacin are NOT increased by MRP inhibitors

Cell metabolic state

Belgian gastronomy
Intracellular vs extracellular activity of antibiotics: PK – PD in action

Cooperation between fluoroquinolones and PMA against *Listeria monocytogenes*

PMA increases the cellular concentration of MXF but not of LVX

PMA
- reduces the cellular growth of *S. aureus*
- increases the intracellular activity of MXF against *Listeria* only

*Van de Velde et al., JAC (2008) 62:518-21*
How can these models help the clinican?

Hôpital Notre Dame à la Rose, Lessines
In vitro models to predict failure/success?

- SCV isolated from a patient
  - with complicated prosthetic vascular graft infection and bacteraemia,
  - unsuccessfully treated successively with
    - cotrimoxazole (SMX/TMP),
    - minocycline (MIN),
    - a combination of vancomycin and rifampin (VAN-RIF)
    - a combination of linezolid and rifampin (LNZ-RIF)

- thymidine-auxotrophic MRSA, growing as tiny, non-pigmented and non-hemolytic colonies on Columbia blood agar.

- resistant to OXA, SXT, CLI, LIN, ERY, quinupristin and TET.
In vitro models to predict failure/success?

Nguyen et al., ECCMID (2008) - A970
In vitro models to predict failure/success?

combinations received by the patient

\[ \Delta \log \text{from initial inoculum} \]

\[ \text{Time (h)} \]

combination at C\(_{\text{static}}\)

- LNZ
- RIF
- VAN
In vitro models to predict failure/success?

combinations received by the patient

Δ log from initial inoculum

Time (h)

0 6 12 18 24

-4 -3 -2 -1 0 1 2 3 4

LNZ RIF VAN RIF-LNZ RIF-VAN

Nguyen et al., ECCMID (2008) - A970
In vitro models to predict failure/success?

Combinations received by the patient

Nguyen et al., ECCMID (2008) - A970
In vitro models to predict failure/success?

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- bacterial responsiveness

Still a lot of work ahead to fully understand …

Magritte, Belgian surrealism
Our "Staph" team

In collaboration with:

- Y. Glupczynski, cliniques universitaires de l’UCL à Mont-Godinne, Yvoir, Belgium
- A. Vergison, O. Denis, M. Struelens, Hôpital Erasme, ULB, Brussels, Belgium
- P. Appelbaum, Hershey Medical Center, Hershey, PA, USA
We wish you a Happy New Year!