Activity of antibiotics against intracellular S. aureus

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Intracellular *S. aureus*: is it important?
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 *in vivo*

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

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

Evidence of Small Colony Variants and of intracellular \textit{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

Mechanisms of *Staphylococcus aureus* invasion of cultured osteoblasts.

Invasion of human keratinocytes by *Staphylococcus aureus* and intracellular bacterial persistence represent haemolysin-independent virulence mechanisms that are followed by features of necrotic and apoptotic keratinocyte cell death.

In vitro ability of *Staphylococcus aureus* isolates from bacteraemic patients with and without metastatic complications to invade vascular endothelial cells.

*Staphylococcus aureus* invasion of bovine mammary epithelial cells.
**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 way for studying antibiotic activity
towards intracellular S. aureus
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 µg/ml (MIC)
  - antibiotic under study

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

Measuring the intracellular activity of antibiotics . . .

Very complicated?

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

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

What about resistant strains?
intracellular vs extracellular activity of antibiotics: PK – PD in action

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

Intracellular activity, SCV vs normal phenotype

THP-1; 24 h, antibiotics at Cmax

Nguyen et al, RICAi 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 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
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

**Graphs**

- **Left graph**:
  - Vancomycin MIC (mg/L) vs. μg vancomycin/mg protein
  - HMC546 MIC: 1.25 mg/L
  - HMC547 MIC: 2 mg/L
  - HMC548 MIC: 3.5 mg/L
  - HMC549 MIC: 4 mg/L

- **Right graph**:
  - Daptomycin MIC (mg/L) vs. μg daptomycin/mg protein
  - ATCC25923 MIC: 0.125 mg/L
  - NRS126 MIC: 0.5 mg/L
  - HMC546 MIC: 1 mg/L
  - HMC549 MIC: 4 mg/L

**References**

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 EC$_{50}$

lower intracellular Emax

no effect of resistance phenotype

Cellular factors affecting antibiotic intracellular activity
Intracellular vs extracellular activity of antibiotics: PK – PD in action

acid pH of lysosomes
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 β-lactams

MRSA are as susceptible as MSSA in broth at acidic pH

MRSA vs MSSA: extracellular activity of β-lactams

Neutralization of lysosomes makes intracellular MRSA resistant to β-lactams!

MRSA are inside [acidic] vacuoles

Efflux pumps
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

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

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

How are all these parameters inter-connected?

Still a lot of work ahead …
Our "Staph" team

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