Activity of antibiotics against extracellular and intracellular forms of *Staphylococcus aureus*

*Pharmacodynamic studies in vitro using a model of human THP-1 macrophages*
I. INTRODUCTION
Staphylococcus aureus

Bacteria with the greatest pathogenic potential in human infections.

A major cause of nosocomial and community-acquired infections.
S. aureus infections

1. skin and soft tissue infections

impetigo

cellulitis

furuncle

erysipelas

abscess

S. aureus infections

impetigo

cellulitis

furuncle

erysipelas

abscess
S. aureus infections

2. food-poisoning
3. Deep-organ infections

- endocarditis
- pneumonia
- osteomyelitis
S. aureus infections

All these infections are difficult to treat:

• recurrence / persistence
  in relation with the intracellular character of S. aureus
  → selection of antibiotics based on pharmacokinetic / pharmacodynamics properties

• resistance to currently available antibiotics
  → need of drugs acting on multiresistant strains
S. aureus infections

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Intracellular infection by *S. aureus*

Normal process of phagocytosis

1) attachment to phagocyte membrane

2) ingestion (phagosome)

3) fusion lysosome and phagosome

4) bacterial destruction

5) digestion and release of microbial debris

Figure 7. Bacterial degradation by neutrophils.
Incomplete phagocytosis of *S. aureus*:

1) attachment to phagocyte membrane

2) ingestion (phagosome)

3) fusion lysosome and phagosome

4) bacterial destruction

5) digestion and release of microbial debris

Intracellular infection by *S. aureus*
Intracellular infection by *S. aureus*

Bacteria able to survive in different host cells: phagocytes and non-phagocytes

All these infections are difficult to treat:

- recurrence / persistence

  in relation with the intracellular character of *S. aureus*

  → selection of antibiotics based on pharmacokinetic / pharmacodynamics properties

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Antibiotic pharmacokinetics / pharmacodynamics

general concept

Dosage regimen → Concentration versus time in serum

Concentration versus time in serum → absorption, distribution, elimination

Concentration versus time in tissues and other body fluids → Pharmacologic or toxicologic effect

Concentration versus time at site of infection → Antimicrobial effect versus time

PHARMACOKINETICS

PHARMACODYNAMICS

Craig CID (1998) 26:1-10
intracellularly

Antibiotic pharmacokinetics / pharmacodynamics

All these infections are difficult to treat:

- recurrence / persistence
  
in relation with the intracellular character of \textit{S. aureus}
  
→ selection of antibiotics based on
  
pharmacokinetic / pharmacodynamics properties

- resistance to currently available antibiotics
  
→ need of drugs acting on multiresistant strains
The world first commercially available antibiotic appeared in 1941.

**S. aureus resistance**

1941: 
- Introduction of Penicillin
- Aminoglycosides, 1950
- Macrolides, 1952
- Glycopeptides, 1956
- Methicillin, 1960

1960: 
- Quinolones, Streptogramins, 1962

2000: 
- Oxazolidinones
## S. aureus resistance

Most worrying resistance phenotypes having emerged over time

<table>
<thead>
<tr>
<th>year</th>
<th>phenotype</th>
<th>first description</th>
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<tbody>
<tr>
<td>1960</td>
<td>HA-MRSA</td>
<td>England</td>
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<tr>
<td>1967</td>
<td>MDR HA-MRSA</td>
<td>Europe, Australia, Japan</td>
</tr>
<tr>
<td>1980</td>
<td>Genta-R MRSA</td>
<td>USA, Ireland, UK</td>
</tr>
<tr>
<td>1993</td>
<td>CA-MRSA</td>
<td>Australia</td>
</tr>
<tr>
<td>1997</td>
<td>VISA</td>
<td>Japan</td>
</tr>
<tr>
<td>2002</td>
<td>VRSA</td>
<td>USA</td>
</tr>
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</table>

S. aureus resistance

Percentage of MRSA resistance in Europe in 2004: S. aureus proportion of invasive isolates MRSA in 2004

Data from the European antimicrobial resistant surveillance system, EARSS.
Prevalence of resistance to other antibiotic classes

S. aureus resistance

S. aureus infections

All these infections are difficult to treat:

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  in relation with the intracellular character of S. aureus
  → selection of antibiotics based on pharmacokinetic / pharmacodynamics properties

• resistance to currently available antibiotics
  → need of drugs acting on multiresistant strains
## Need for new antistaphylococcal agents

### A lot of drugs in the pipeline ...

<table>
<thead>
<tr>
<th>recently brought on the Belgian market</th>
<th>on the market; not yet in Belgium</th>
<th>(late) stage of clinical development</th>
<th>investigational</th>
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</thead>
<tbody>
<tr>
<td>moxifloxacin</td>
<td>synercid</td>
<td>telavancin</td>
<td>CS-023/PZ-601</td>
</tr>
<tr>
<td>linezolid</td>
<td>daptomycin</td>
<td>oritavancin</td>
<td>MX-2401</td>
</tr>
<tr>
<td></td>
<td>tigecycline</td>
<td>dalbavancin</td>
<td>API-1252</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ceftobiprole</td>
<td>DK-619</td>
</tr>
<tr>
<td></td>
<td></td>
<td>icleaprim</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>retapamulin</td>
<td>new oxazolidinones</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WCK-771</td>
<td>new ketolides</td>
</tr>
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<td></td>
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<td>...</td>
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</tbody>
</table>

What will be your choice?

F. Van Bambeke, symposium on *S. aureus* – Brussels, 11-1-2007
II. AIM OF THE STUDY
• recurrence / persistence
  in relation with the intracellular character of S. aureus
→ selection of antibiotics based on
  pharmacokinetic / pharmacodynamics properties

  To develop an intracellular model allowing to compare
  the activity of antibiotics on a pharmacodynamic basis

• resistance to currently available antibiotics
→ need of drugs acting on multiresistant strains

  To evaluate the cellular pharmacokinetics
  and the intracellular activity towards multiresistant strains
  of a new antibiotic in development
III. METHODOLOGY
Setting-up of the intracellular model

Method

1) opsonization of *S. aureus* with human serum

2) phagocytosis of the bacteria by THP-1 macrophages (ratio 4 bacteria vs 1 macrophage)

3) elimination of extracellular *S. aureus* (gentamicin 100 X MIC).
   Rinse of infected macrophages (time-zero)

4) intracellularly infected macrophages, ready to test antibiotic activity

5) maintenance of gentamicin at its MIC during the whole incubation period for controls to avoid extracellular contamination
### Setting-up of the intracellular model

**cell line?**

THP-1 = many features of human monocytes/macrophages


<table>
<thead>
<tr>
<th>parameter</th>
<th>characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>morphological features</strong></td>
<td>morphology resembling that of monocyctic leukemia cells:</td>
</tr>
<tr>
<td></td>
<td>- diameter 12-14 µm</td>
</tr>
<tr>
<td></td>
<td>- moderate basophile cytoplasm</td>
</tr>
<tr>
<td></td>
<td>- small azurophiles granules, few vacuoles</td>
</tr>
<tr>
<td></td>
<td>- nuclei irregular in shape</td>
</tr>
<tr>
<td><strong>cytochemical features</strong></td>
<td>- positive for α-naphthyl butyrate esterase</td>
</tr>
<tr>
<td></td>
<td>- negative reaction with periodic acid-Schiff and Sudan black B</td>
</tr>
<tr>
<td></td>
<td>- diploid (46,XY) chromosome number</td>
</tr>
<tr>
<td><strong>surface antigens and receptors</strong></td>
<td>CD4, CD30, Factor X receptor, Factor Xa receptor, FcRI, FcRII,</td>
</tr>
<tr>
<td></td>
<td>GM-CSF-receptor, HDL receptor, LDL receptor, TNF receptor,</td>
</tr>
<tr>
<td></td>
<td>C3b receptor, LFA-1 receptor, Fibronectin receptor, Leu M1, Leu M2,</td>
</tr>
<tr>
<td></td>
<td>Leu M3, HLA-DR antigens, scavengers receptors</td>
</tr>
<tr>
<td><strong>secreted proteins</strong></td>
<td>- hormones, cytokines: TNF-α, IL-1, IL-1b, CSF-1, M-CSF,</td>
</tr>
<tr>
<td></td>
<td>erythrocyte differentiation factor, PDGF-1 and -2, thymosin B4,</td>
</tr>
<tr>
<td></td>
<td>killer T cell activating factor, monocyte chemotactic factor</td>
</tr>
<tr>
<td></td>
<td>- enzymes: lipoprotein lipase, lysozyme</td>
</tr>
<tr>
<td></td>
<td>- binding proteins: apoprotein E</td>
</tr>
<tr>
<td><strong>functional features</strong></td>
<td>- phagocytosis</td>
</tr>
<tr>
<td></td>
<td>- production of lysozyme</td>
</tr>
<tr>
<td></td>
<td>- capacity to restore the lymphocyte T mitogenic responsiveness</td>
</tr>
</tbody>
</table>
Setting-up of the intracellular model

bacterial strain?

ATCC 25923

- clinical isolate from 1976
- fully susceptible
- widely used as a standard for microbiological testing of antibiotics

useful to compare all antibiotics towards a single strain, but may differ in virulence with current strains …
Setting-up of the intracellular model

antibiotics?

- **Beta-lactams**: first choice for susceptible strains
- **Aminoglycosides**: highly bactericidal extracellularly
- **Rifampicin**: considered as first choice for intracellular infections
- **Macrolides, quinolones**: high cellular accumulation
- **Glycopeptides**: alternative for MRSA
- **Linezolid**: recently introduced, active on MRSA
**S. aureus** intracellular model

Bacteria multiply in **phagolysosomes** where **pH is acidic**

![Diagram of S. aureus intracellular model](image)

| extracell. [GEN] * | extracell. contamin. **
|-------------------|---------------------
| 0                | 17.2 ± 1.9          |
| 0.001            | 16.0 ± 1.0          |
| 0.01             | 0.013 ± 0.001       |
| 0.1              | 0.0026 ± 0.0003     |
| 1                | < 0.001             |

* x MIC  
** % of total bacteria in culture

Gentamicin prevents extracellular contamination

Membrane bond vacuole
General protocol

Assessment of antibiotic extracellular and intracellular activity

**EXTRACELLULAR**
- 10^6 CFU/ml
- Incubation over 24 h with antibiotics
- Colony counting (CFU/ml)

**INTRACELLULAR**
- 4 bacteria/cell
- 10^6 CFU/mg protein
- Collection and lysis of cells (sonication)
- CFU/protein content
  - Antibiotic concentration (microbiological, radiochemical, fluorimetric assays)
IV. RESULTS
First goal of this thesis

Pharmacodynamic Evaluation of the Intracellular Activities of Antibiotics against *Staphylococcus aureus* in a Model of THP-1 Macrophages

Maritza Barcia-Macay, Cristina Seral,† Marie-Paule Mingeot-Leclercq, Paul M. Tulkens, and Françoise Van Bambeke*

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Received 17 August 2005/Returned for modification 30 October 2005/Accepted 8 December 2005

Human macrophages

Fully susceptible strain
Antibiotics accumulate to variable levels in THP-1 macrophages

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Cellular accumulation</th>
<th>Extracellular concn (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azithromycin</td>
<td>37.8 ± 1.3</td>
<td>5</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>27.9 ± 1.3</td>
<td>2</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4.4 ± 0.1</td>
<td>250</td>
</tr>
<tr>
<td>Linezolid</td>
<td>0.5 ± 0.0</td>
<td>250</td>
</tr>
<tr>
<td>Penicillin V</td>
<td>1.2 ± 0.1</td>
<td>150</td>
</tr>
<tr>
<td>Nafcillin</td>
<td>2.6 ± 0.1</td>
<td>400</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1.0 ± 0.1</td>
<td>150</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>4.0 ± 0.1</td>
<td>250</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>7.4 ± 0.2</td>
<td>150</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>6.3 ± 0.1</td>
<td>100</td>
</tr>
<tr>
<td>Oritavancin</td>
<td>148.0 ± 12.0</td>
<td>25</td>
</tr>
<tr>
<td>Rifampin</td>
<td>17.6 ± 0.9</td>
<td>50</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5.1 ± 0.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>7.0 ± 0.6</td>
<td>4</td>
</tr>
<tr>
<td>Garenoxacin</td>
<td>9.1 ± 0.3</td>
<td>4</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>7.6 ± 0.3</td>
<td>4</td>
</tr>
</tbody>
</table>

**High level:**
- macrolides
- oritavancin
- rifampin

**Moderate level:**
- aminoglycoside
- old glycopeptides
- quinolones

**Low level:**
- linezolid
- \( \beta \)-lactams
influence of time on activity

GEN, RIF, ORI quickly cidal
OXA, MXF, ORI slowly cidal

• in all cases, intracellular activity << extracellular activity
• no direct relation between accumulation and intracellular activity
concentration-effect relationships

• sigmoidal relationships
concentration-effect relationships

- sigmoidal relationships
- Emax intra << Emax extra
concentration-effect relationships

- sigmoidal relationships
- $E_{\text{max}}$ intra $<< E_{\text{max}}$ extra

- $E_{\text{C50}}$ extra = $E_{\text{C50}}$ intra for OXA and MXF
- $E_{\text{C50}}$ extra $<$ $E_{\text{C50}}$ intra for GEN and ORI
intracellular killing is visible!
extracellular versus intracellular activity

Δ log CFU from time 0

extracellular

growth

killing

Poorly active against extra and intra S. aureus
extracellular versus intracellular activity

Active against extra S. aureus
extracellular versus intracellular activity

Best choice for activity against extra and intra S. aureus
model of infection of human macrophages by *S. aureus* over 24 h allowing for the study of

- influence of time and concentration on antibiotic activity
- relation between activity and accumulation

**intracellular activity << extracellular activity**

- no correlation with level of accumulation
- impairing effect of acidic pH on some antibiotics

**optimizing antibiotic efficacy**

- choice of the drug (active extra and intracellularly)
- optimization of exposure (time and concentration)
Second goal of this thesis

Evaluation of the extracellular and intracellular activities (human THP-1 macrophages) of telavancin versus vancomycin against methicillin-susceptible, methicillin-resistant, vancomycin-intermediate and vancomycin-resistant Staphylococcus aureus

Maritza Barcia-Macay, Sandrine Lemaire, Marie-Paule Mingeot-Leclercq, Paul M. Tulkens and Françoise Van Bambeke*

Unité de Pharmacologie cellulaire et moléculaire, Université catholique de Louvain, B-1200 Brussels, Belgium

Received 26 July 2006; returned 29 August 2006; revised 15 September 2006; accepted 25 September 2006
Telavancin, a new glycopeptide

Hemi-synthetic derivative of vancomycin, with new mode of action and new pharmacokinetic profile

- permeabilization of bacterial membrane
- prolonged half-life
- dimerization and cooperative binding to peptidoglycan precursors
- shortening of half-life
**MIC and MBC against S. aureus with different resistance phenotypes**

- More active than VAN against VISA and VRSA
- Bactericidal against all strains

<table>
<thead>
<tr>
<th>phenotype</th>
<th>strain</th>
<th>vancomycin</th>
<th></th>
<th>telavancin</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>MIC</td>
<td>MBC</td>
<td>MIC</td>
<td>MBC</td>
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<td>MSSA</td>
<td>ATCC25923</td>
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<td>1</td>
<td>0.5</td>
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<td>1</td>
<td>0.5</td>
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<td>ATCC33591</td>
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<td>4</td>
<td>0.5</td>
<td>1</td>
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<td>ATCC43300</td>
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<td>0.5</td>
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</tr>
<tr>
<td>VISA</td>
<td>NRS23</td>
<td>4</td>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>NRS52</td>
<td>4</td>
<td>4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>VRSA</td>
<td>VRS1</td>
<td>&gt;128</td>
<td>&gt;256</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>VRS2</td>
<td>16</td>
<td>64</td>
<td>2</td>
<td>8</td>
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</tbody>
</table>
Influence of time on EXTRACELLULAR activity

Telavancin is more rapidly cidal than vancomycin

Telavancin is more rapidly cidal than vancomycin
Influence of concentration on EXTRACELLULAR activity

VAN vs TLV – MSSA ATCC 25923

- at 3 h, TEL shows bimodal conc-dependent effects
- at 24 h, both drugs are bactericidal at high concentrations
EXTRACELLULAR activity of telavancin: comparison of different strains

TLV versus MSSA, MRSA, VISA, VRSA

at 3 h, TEL shows bimodal conc-dependent effects towards MSSA and MRSA
INTRACELLULAR activity of vancomycin and telavancin towards different strains

VAN and TLV versus MSSA, MRSA, VISA, VRSA

TEL shows bimodal conc-dependent effects towards MSSA / MRSA
VAN is only static intracellularly
Why bimodal effects for telavancin?

VAN and TLV: inhibition of peptidoglycan synthesis

In MSSA and MRSA, telavancin can exert multiple modes of action

TLV: membrane permeabilization

LOG CONCENTRATION (µG/ML)

Δ LOG CFU FROM TIME 0

Higgins et al., AAC (2004) 49:1127-34
Telavancin cellular pharmacokinetic data rationalizing its intracellular activity

(studies with J774 macrophages)
subcellular distribution of telavancin

Same distribution as a lysosomal enzyme

High concentration in the compartment where *S. aureus* sojourns!
**Conclusion**

Model of infection of human macrophages by *S. aureus* over 24 h applicable to multiresistant strains

**Vancomycin**
- slowly bactericidal extracellularly (MSSA and MRSA)
- poorly or not active on VISA and VRSA
- static intracellularly

**Telavancin**
- bactericidal extra- and intracellularly, including against resistant strains
- bimodal effect against MSSA and MRSA could be related to multiple modes of action
- high accumulation in the infected compartment
V. GENERAL CONCLUSION:
can we do better?
Limitations of the model and perspectives for future work

Constant concentrations
(pharmacokinetic variations not taken into account):
- develop dynamic models

Protein binding
(free fraction is active and able to accumulate)
- develop in vivo models

Phagocytic cells
(S. aureus also infects non phagocytic cells
where its fate may be different)
- develop models of infection in non-phagocytic cells

Testing of antibiotics alone
(combinations often used in the clinics to cope with resistance)
- testing of drug combinations
THANK YOU!
THANKS TO:

Prof. M.-P. Mingeot-Leclercq
Dr. C. Seral (Spain)
Prof. J.-P. Hervey
Prof. V. Préat
Prof. M. Delmée
Prof. Y. Glupczynski
Prof. B. Gallez
Prof. A. Pascual (Spain)
Prof. M. Struelens (ULB)

Theravance (USA)
HURRA FACM !!!

Special thanks to O. Meert and M.-C. Cambier
THANKS TO YOU ALL