Intra-osteoblastic synergy of daptomycin with beta-lactams for *S. aureus* BJI

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Daptomycin in BJI: success and pitfalls

Increasingly used in staphylococcal BJI

(i) Acceptable bone diffusion at high concentration
(ii) Good tolerance
(iii) Targetting pathophysiologial pathways?

Good activity against bacteria embedded in biofilms

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ΔΔ ΔΔ log10 for 100,000 cells CFU of intracellular S. aureus after 24-h exposition

antibiotic
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BUT

Weak activity against staphylococcal intraosteoblastic reservoir

Daptomycin in BJI: success and pitfalls

Increasingly used in staphylococcal BJI

(i) Acceptable bone diffusion at high concentration
(ii) Good tolerance
(iii) Targetting pathophysiological pathways?
   - Good diffusion and activity within biofilms
   - Weak intracellular activity

Use of daptomycin in *S. aureus* BJI without enhancing the risk of relapse due to the intracellular reservoir requires to improve its intra-ostoblastic activity
Daptomycin synergy with betalactam antibiotics

**In vitro**  Synergy of daptomycin with betalactam antibiotics against MSSA and MRSA

*Mechanism: reduce the charge of the outer bacterial membrane which enhance daptomycin binding*

**In vivo**  Daptomycin-oxacillin synergy in experimental models (IE, foreign body infection)

**Clinical studies:** case reports of MRSA bacteremia +/- BJI (“rescue therapy”)

Objective: Assessing the efficacy of daptomycin in combination with oxacillin and daptomycin against intracellular MSSA and MRSA in an *ex vivo* model of human osteoblastic cell infection
MRSA strain LUG359 (COL strain) and its MSSA isogenic counterpart obtained by inactivation of the mecA gene by allelic replacement.
**Methods**

- **Infection**
  - MOI 100:1
  - 24h 37°C

- **Adhesion and Invasion**
  - 2h 37°C
  - Lysostaphin 10 µg/mL
  - 1h 37°C

- **Elimination of Extracellular Bacteria**
  - 24h 37°C
  - Antibiotics: Daptomycin, Oxacillin, Ceftarolin (alone or in combination at human bone concentration)

- **Evaluation of Cell Survival**
  - MTT

- **Quantification of Intracellular Bacteria**
  - Cell lysis
Results

**MSSA**

Proportion of intracellular bacteria at 24h compared to untreated cells (mean: 95%CI)

- Untreated cells
- Daptomycin
- Oxacillin
- Ceftaroline
- Daptomycin + Oxacillin
- Daptomycin + Ceftaroline

**MRSA**

Proportion of intracellular bacteria at 24h compared to untreated cells (mean: 95%CI)

- Untreated cells
- Daptomycin
- Oxacillin
- Ceftaroline
- Daptomycin + Oxacillin
- Daptomycin + Ceftaroline
Results

→ Confirmation of the weak activity of daptomycin against intracellular MSSA/MRSA
Results

→ Acceptable efficacy of oxacillin against intracellular *S. aureus* INCLUDING MRSA
Results

→ Superiority of the daptomycin-oxacillin combination compared to each molecule alone not observed for the daptomycin-ceftarolin combination


Complementary investigations

Intraosteoblastic *S. aureus*: partly intralysosomal = acidic pH

→ Evaluation of the impact of pH on antibiotic activity

Methods:
- MICs evaluations at pH 7 and pH 5
- Synergy evaluations at pH 7 and pH 5 (E-test, checkerboard)

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<td>pH 7</td>
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→ Weak intracellular activity of daptomycin might be partly due to its decreased activity at acidic pH

→ Intracellular restauration of oxacillin activity against MRSA is (at least partly) due to a major decrease in MICs at the intralysosomal acidic pH

→ No *in vitro* synergy was observed using these methods (partial results, not shown)
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Conclusions and perspectives

Local chemical conditions importantly impact the intracellular activity of antistaphylococcal molecules

**Perspective:** Evaluation of adjuvants modulating intracellular pH conditions for enhancing the ability of antimicrobials to eradicate the *S. aureus* intraosteoelastic reservoir leading to BJI chronicity and relapse
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