In vitro pharmacodynamic evaluation of intracellular activity of antibiotics alone or in combination against a small colony variant (SCV) of Staphylococcus aureus

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Small Colony Variant (SCV): a phenotype that facilitates persistent and recurrent infections

- **SCVs show a particular phenotype**
  - Growth: tiny, non-pigmented, non-hemolytic colonies
  - Reduced production of $\alpha$-toxin
  - Auxotrophic for haemin, thymidine, menadione…
  - Can revert to their normal phenotype

![Growth of SCV in comparison to normal isogenic phenotype on Columbia blood agar](image)


- **SCVs have been isolated in patients suffering from chronic, relapsing and difficult-to-treat infections**
  - Infection of skin (Darier’s disease), bone (osteomyelitis), device-associated infections …

SCVs are difficult to eradicate

- Reduced susceptibility to antibiotics
  - Slow growth
  - Metabolic defects


- More persistent within intracellular milieu ⇒ evasion host defenses and antibiotic actions

  Intracellular persistence assay within human keratinocyte with SCV and NP

  SCV, 48 h  NP, 48 h
How to target intracellular SCVs?

- **AB pharmacokinetic properties:**
  - Capacity to penetrate within host cells and to rejoin the infected compartment.

- **AB pharmacodynamic properties:**
  - Capacity to exert optimal bactericidal activity
    - in the intracellular environment
    - even against slowly growing bacteria
Aims of the study

• To compare the intracellular activity of a series of ATBs
  – against a stable mecA-negative, thymidine-dependent SCV variant, isolated from a CF patient, its isogenic normal phenotype (NP) and revertant counterparts.
  – In a model of THP-1 human macrophages

Selected antistaphylococcal agents:
  – First line antistaphyloccal agents: OXA, GEN, VAN, MXF, CLI, RIF.
  – New alternatives for resistant S. aureus: LZD, TGC, DAP, Q-D.
  – Investigational compounds: ORI, TLV.

• To examine the potential interest of combining ATBs against intracellular SCVs
Description of the model

Intracellular growth of SCV versus its NP counterpart

Subcellular localization

SCV and NP localized in acidic intracellular compartments
Intracellular activity:
SCV versus isogenic normal phenotype and revertant

THP-1; 24 h, antibiotics at Cmax

- Intracellular growth of NP and revertant is markedly increased
- Activity is higher against NP and revertant for all but LZD, ORI, TGC
- Enhancement is more pronounced for membrane-acting ATB: OXA, VAN, DAP, TLV
- ORI is the most active drug, with no difference against SCV or NP
Intracellular activity against SCV: time effect

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

- At 5 h, no marked change compared to initial inoculum
- At 24 h,
  - most antibiotics caused only a modest decrease in bacterial counts (< 1 log decrease)
  - an approx. 1 log decrease was reached for RIF, TGC, MXF
  - ORI achieved more than 2 log decrease
- At 72 h,
  - the activity of most ATBs (but TGC) increased (0.5-1 log)
  - ORI remained one of the most active drugs
Dose-response curves of the 3 most active antibiotics against extra- and intra-cellular SCV (24 h of exposure)

- **Extracellular activity:**
  - Highly bactericidal, concentration-dependent effects

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

**Gray zones:** clinically-relevant range of concentrations
Activity against extra- and intra-cellular SCV: dose-effect response

Curve analyses for all drugs

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Intracellular</th>
<th>Extracellular</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>$E_{\text{max}}$ (CI)</td>
<td>EC$_{50}$ (CI)</td>
</tr>
<tr>
<td>OXA</td>
<td>-0.39 (-0.52 to -0.27) A,a</td>
<td>0.83 (0.56 to 1.17) A,a</td>
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<tr>
<td>FA</td>
<td>-0.38 (-0.52 to -0.20) A,a</td>
<td>10.70 (7.77 to 14.72) B,a</td>
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<tr>
<td>CLI</td>
<td>-0.45 (-0.51 to -0.39) C,a</td>
<td>0.47 (0.39 to 0.57) A,a</td>
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<tr>
<td>GEN</td>
<td>-0.58 (-0.76 to -0.40) A,a</td>
<td>0.63 (0.41 to 0.97) A,a</td>
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<tr>
<td>RIF</td>
<td>-1.72 (-2.04 to -1.40) B,a</td>
<td>42.00 (29.42 to 61.66) C,a</td>
</tr>
<tr>
<td>VAN</td>
<td>-0.36 (-0.62 to -0.10) A,a</td>
<td>6.61 (3.77 to 11.61) D,a</td>
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<tr>
<td>LZD</td>
<td>-0.54 (-0.84 to -0.23) A,a</td>
<td>0.61 (0.36 to 0.97) A,a</td>
</tr>
<tr>
<td>Q-D</td>
<td>-0.60 (-0.76 to -0.44) A,a</td>
<td>0.25 (0.15 to 0.40) A,a</td>
</tr>
<tr>
<td>DAP</td>
<td>-0.50 (-0.64 to -0.35) A,a</td>
<td>4.17 (2.91 to 5.96) E,a</td>
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<tr>
<td>TGC</td>
<td>-1.11 (-1.26 to -0.95) C,a</td>
<td>0.80 (0.50 to 1.17) A,a</td>
</tr>
<tr>
<td>MXF</td>
<td>-1.32 (-1.45 to -1.19) C,a</td>
<td>2.49 (1.92 to 3.23) F,a</td>
</tr>
<tr>
<td>TLV</td>
<td>-0.35 (-0.53 to -0.17) A,a</td>
<td>0.52 (0.31 to 0.85) A,a</td>
</tr>
<tr>
<td>ORI</td>
<td>-0.43 (-0.56 to 0.30) na</td>
<td>41.61 (35.79 to 48.38) na</td>
</tr>
</tbody>
</table>

Reduced killing activities against intracellular SCV: lower $E_{\text{max}}$
Activity of antibiotic combination against intracellular SCV

At fixed concentrations with RIF or ORI (over the time)

- At $C_{\text{static}}$: 1.2 log after 24 h.
- At $C_{\text{max}}$: reaching the limit of detection (sterilize the infected macrophages)
Activity of combination RIF-ORI against intracellular SCV

Fractional maximal effect (FME) approach

- Analyse the nonlinear pharmacodynamics exhibited by antibiotics
- Examine the effect of combination for calculated and not arbitrarily chosen concentrations

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

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

FME is defined by the ratio observed effect/theoretical effect
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 concentrations to be tested alone and in combination:

$$ C_{xp} = \frac{FME \times EC_{50}}{1 - FME} $$

FME (for ATB alone or in combination) is then plotted versus concentrations

Interaction evaluation:
Additivity: effect equal to the theoretical sum of the effects of each ATB tested alone, FME=1
Synergy (FME > 1),
Antagonism: FME < best FME of ATB used alone
Indifference: FME between antagonism and additivity

Activity of combination RIF-ORI against intracellular SCV

Fractional maximal effect (FME) approach

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

- Most currently available anti-staphylococcal agents act only poorly on intracellular SCVs compared to its normal phenotype and revertant counterparts, unless upon prolonged exposure.

- None of the tested antibiotics is able to sterilize the infected macrophages, which may explain the difficulty of eradicating these organisms in CF patients.

- Among clinically-available antibiotics, RIF and MXF are the most active. Only ORI proves bactericidal activity towards both intracellular SCV and NP.

- Combination RIF-ORI is highly synergistic over a wide range of concentration ratios, reaching the limit of detection when used both at $C_{\text{max}}$.

- Our results suggest the interest of evaluating ATBs against intracellular SCV to select most appropriate therapies.
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