THINKING OUT OF THE BOX TO BEAT BIOFILMS

Activity of drug combinations against staphylococcal biofilms

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Disclosures

• Research grants for studies on biofilms (last 5 years) : Cempra, Melinta therapeutics

• Common research programs with OneLife, subsidized by the Region Wallonne, Belgium

• Research grants for studies on other topics (last 5 years): Debiopharm, GSK, Merlion pharmaceuticals, SMB

• Advisory board: Bayer
Staphylococcal biofilms ....
Staphylococcal biofilms ....a new point of view on how to cure them
Staphylococcal biofilms and human infections

Staphylococcal biofilms: why do they cause persistent infections? (1/2)

Otto, Microbiol Spectrum 2018; 6:GPP3-0023-2018
Staphylococcal biofilms: why do they cause persistent infections? (2/2)

attachment

Proliferation & Maturation

Detachment

host matrix proteins
to polymer surface
Surface hydrophobicity

to host matrix proteins
MSCRAMMs & other surface proteins

adhesive factors:
PIA
eDNA
Aap and other proteins

disruptive factors:
PSMs
Proteases
Nucleases

Antibiotics
Neutrophil
Macrophage

Otto, Microbiol Spectrum 2018; 6:GPP3-0023-2018
Staphylococcal biofilms: strategies currently under investigation

- Repurposed drugs (Thioridazine, Niclosamide)
- Phytochemicals: Ferulic acid, xanthohumol, plumbagin etc
- Nanoparticles (Silver, Zinc oxide & nitric oxide releasing nanoparticles)
- ECM degrading enzymes: Lysostaphin, CHAP, dispersin-B, neutrase etc
- Phages (K, DRA88)

**Drug combinations**
- Antibiotics (OXA+AZM/GEN+FA, RIF+CFZ/VAN etc)
- Antibiotic-phytochemical (OXA+ xanthohumol, CIP+reserpine etc)
- Antibiotic-repurposed drug (TOBRA and thioridazine)
- Antimicrobial peptides (LL-37, 17BIPHE2, KR-12, KE-18, citropin 1.1 etc)

**Alteration of surface morphology (MeCe, PEG)**
- ACL (GEN-EDTA, TMP+EtOH+EDTA; ML:8+Citrox)
- Chelators & sulphhydryl compounds (EDTA, TSC, DTT)
- Antimicrobial coating (SCAA)
- Antibodies (ClfA, ABC transporter, TA, PhnD)

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Laser shock waves
Photodynamic therapy (Ce6)
Ultra sound

Biofilm disruption & bacterial death
Mature biofilm
Initial attachment
Bacterial aggregation
S. aureus

Alteration of surface morphology (MeCe, PEG)
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Antibiotic combinations: in vitro models

MBEC (Calgary device)
MBEC - checkerboard

Antibiotic combinations: in vitro models

Metabolically • inactive • active

drug A

drug B
Antibiotic combinations: in vitro models

MBEC - checkerboard

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<th>MIC (mg L(^{-1}))</th>
<th>MBEC (mg L(^{-1}))</th>
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<td>Vancomycin</td>
<td>2</td>
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<td>Linezolid</td>
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<td>Rifampicin</td>
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<td>Dicloxacillin</td>
<td>0.125(^a)</td>
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<th>Vancomycin (25 mg L(^{-1}))</th>
<th>Daptomycin (130 mg L(^{-1}))</th>
<th>Linezolid (10 mg L(^{-1}))</th>
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<td>Vancomycin</td>
<td>256</td>
<td>*</td>
<td>256</td>
<td>512</td>
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<tr>
<td>Daptomycin</td>
<td>1024</td>
<td>&gt;2048</td>
<td>*</td>
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<td>&gt;1024</td>
<td>&gt;1024</td>
<td>&gt;1024</td>
<td>*</td>
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<tr>
<td>Tigecycline</td>
<td>8</td>
<td>N/A</td>
<td>N/A</td>
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But still >> clinically achievable concentrations!
Antibiotic combinations: from in vitro to in vivo models

Combinations do not seem more efficacious in vivo than drugs alone ...
Antibiotic combinations: in vitro models

Combinations in microplates at fixed concentrations (free human $C_{\text{max}}$)

Antibiotics at their human $fC_{\text{max}}$

- FUS
- DAP
- DAP+FUS
- LZD
- LZD+FUS
- VAN
- VAN+FUS
- MXF
- MXF+FUS
- RIF
- RIF+FUS
- DOX
- DOX+FUS

Percentage reduction in resorufin fluorescence

Combinations more effective at clinically achievable concentrations

Antibiotic combinations: in vitro models

Combinations in microplates at variable concentrations

Combined drug at $f_{C_{\text{min}}}$ alone
FUS alone [variable conc.]

Combined drug at $f_{C_{\text{max}}}$ alone
FUS alone [variable conc.]

Combined drug at $f_{C_{\text{min}}}$ + FUS [variable conc.]

Combined drug at $f_{C_{\text{max}}}$ + FUS [variable conc.]

Combinations more effective

Antibiotic combinations: in vitro models

Combinations in CDC bioreactor (mimicking human PK)

Combinations of FUS + LZD and DAP still more effective
...but not combinations with VAN?
Antibiotic combinations: in vitro models

Combinations in CDC bioreactor (mimicking human PK)

Biofilm grown in CDC reactor

Jorgensen et al, Path Dis, 2016;74: ftw019


Log CFU/bone fragment

Osteomyelitis
Staphylococcal biofilms: strategies currently under investigation

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Drug repurposing as a source of potentiators

... but often active at supratherapeutic concentrations and/or only in vitro

Drug repurposing as a source of potentiators: a possibly successful story?

What about the antifungal caspofungin?


Combining antibiotics with drugs having a complementary action

Fibrinolytic agents

Combining antibiotics with drugs having a complementary action

Fibrinolytic agents  catheters in vitro


Combining antibiotics with drugs having a complementary action

Fibrinolytic agents

catheters in vitro


Combining antibiotics with drugs having a complementary action

Fibrinolytic agents
catheters in vitro
catheters in vivo


Combining antibiotics with enzymes destroying the matrix

Dastgheyb et al, J Infect Dis. 2015;211:641–50


Siala et al., ECCMID 2018; O0081
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Mode of action of antimicrobial peptides against biofilms

Grassi et al, Front Microbiol. 2017;8:2409
An example with nisin ...

Nisin and derivatives thereof

Field et al, Front Microbiol. 2016;7:508
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Field et al, Front Microbiol. 2016;7:508
Mode of action of antibodies against biofilms

Antibodies against biofilms to destroy the matrix

Dimer of histone-like prot. stabilizing eDNA

Epitope targeted by Ab TRL1068

eDNA

In vitro (PEGs)

**Staphylococcus aureus**

Growth Control  TRL1068: 1.2 µg/mL

40 µm  10 µm

In vivo (endocarditis)

Heart Vegetation

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>vancomycin</th>
<th>vancomycin + TRL1068</th>
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<tr>
<td>log10 CFU/gram tissue</td>
<td>n=8</td>
<td>n=10</td>
<td>n=10</td>
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<tr>
<td>p</td>
<td>&lt;0.001</td>
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<tr>
<td>- 4.2 log</td>
<td></td>
<td>0.0 log</td>
<td>0.0 log</td>
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Xiong et al, Antimicrob Agents Chemother. 2017;60:61:e00904-17
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**Anti-persisters**

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**Biofilm disruption & bacterial death**

S. aureus
Antipersister compounds to help antibiotics eradicating bacteria in biofilms

Proteins degraded by ADEP4 (per metabolic pathway)

Conlon et al, Nature 2013;503: 365–70

targets ClpP, core unit of a major bacterial protease complex.

Biofilm in vitro
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Biofilms in vivo (thigh deep infection)

Conlon et al, Nature 2013;503: 365–70
Staphylococcal biofilms: which are the best weapons to combine?
Staphylococcal biofilms: which are the best weapons to combine?

- **Antibiotic combinations:**
  - Useful to prevent resistance
  - Frequent synergy in vitro
  - Sometimes synergy in vivo

- **Repurposed drugs as potentiatators:**
  - May accelerate development
  - But consider active concentrations vs. therapeutic concentrations

- **Peptides and antibodies:**
  - Promising, some are in clinical development
  - But ADME issues ..... 

- **Antipersisters:**
  - Highly effective
  - Currently only preclinical preliminary data available
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