Treating biofilm infections by killing the messenger - quorum sensing

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The problem
Treatment

• (I) Early aggressive antibiotic treatment before the biofilm is formed
• (II) Chronic (life long) suppressive antibiotic treatment when biofilm have formed, if the infected area can not be removed

– User guide at Department of Clinical Microbiology Copenhagen
Quorum sensing and Biofilms

Cooperative behaviour among bacteria
QSI widens the therapeutic window
The opportunist *P. aeruginosa*

- 16% of nosocomial pneumonia cases
- 12% of hospital-acquired urinary tract infections
- 8% of surgical wound infections
- 80% of all large chronic wounds contain *P. aeruginosa*
- 10% of bloodstream infections
- 30% deaths in immunocompromised patients
- 38% deaths in intubated patients
- Associated with 60% of deaths under outbreaks in burn units
- Associated with 50% of deaths in the expanding AIDS population
- Cystic fibrosis patients are susceptible to a chronic pulmonary infection, which is responsible for high rates of illness and death
- CF is the most frequent severe genetic disease among Caucasians (1:4700 in Denmark)
QS regulates several hundred genes

Many other virulence factors
Rhamnolipids
Pyocyanin

Elastase
ToxA
Pyoverdine
Etc.
*P. aeruginosa* – quorum sensing in the grand scheme
Tobramycin sensitivity

0 μg tobra 10 μg tobra 20 μg tobra

-3 days old *P. aeruginosa ΔlasRrhlR* biofilms
-The biofilms were exposed to tobramycin for 48 h
-Biofilm viability assayed using LIVE/DEAD BacLight Bacterial Viability Kit.

*Pseudomonas aeruginosa* tolerance to tobramycin, hydrogen peroxide and polymorphonuclear leucocytes is quorum sensing dependent.

Microbiology. 2005 Feb;151:373-83
QS investigation *in vivo*:

The pulmonary mouse model

- Two groups of mice.
- On day 0, the mice are challenged with $10^7$ bacteria, imbedded in alginate beads, per lung.
- Groups of mice are sacrificed different days after the challenge and the lung content of bacteria determined by plating.
PAO1 vs. $\Delta\text{lasR rhlR}$

- Mortality
  - PAO1 – 73%
  - QS mutant ($\Delta\text{lasR rhlR}$) – 46%

Placebo versus garlic extract treated mice infected with PAO1

One s.c. injection per day for five days including two days of prophylaxis

Bacterial counts per lung

Days after bacterial challenge

### QS blocker activities in natural products

<table>
<thead>
<tr>
<th>Sample</th>
<th>QS blocker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bean sprout</td>
<td>+</td>
</tr>
<tr>
<td>Blackberry</td>
<td>-</td>
</tr>
<tr>
<td>Brown onion</td>
<td>-</td>
</tr>
<tr>
<td>Chamomile</td>
<td>+</td>
</tr>
<tr>
<td>Carrot</td>
<td>+</td>
</tr>
<tr>
<td>Coffee</td>
<td>-</td>
</tr>
<tr>
<td>Cranberry</td>
<td>-</td>
</tr>
<tr>
<td>Poison Ivy</td>
<td>-</td>
</tr>
<tr>
<td>Garlic</td>
<td>+</td>
</tr>
<tr>
<td>Gele Royal</td>
<td>-</td>
</tr>
<tr>
<td>Ginseng</td>
<td>-</td>
</tr>
<tr>
<td>Habanero</td>
<td>+</td>
</tr>
<tr>
<td>Honey (various sorts)</td>
<td>-</td>
</tr>
<tr>
<td>Leek</td>
<td>-</td>
</tr>
<tr>
<td>Mint-tea</td>
<td>-</td>
</tr>
<tr>
<td>Propolis</td>
<td>+</td>
</tr>
<tr>
<td>Raspberry</td>
<td>-</td>
</tr>
<tr>
<td>Red Chili</td>
<td>-</td>
</tr>
<tr>
<td>Spring onion</td>
<td>-</td>
</tr>
<tr>
<td>Tea Tree Oil</td>
<td>-</td>
</tr>
<tr>
<td>Water Lilly</td>
<td>-</td>
</tr>
<tr>
<td>Yellow pepper</td>
<td>+</td>
</tr>
<tr>
<td>Blood (plasma)</td>
<td>-</td>
</tr>
<tr>
<td>Stinging nettle</td>
<td>-</td>
</tr>
<tr>
<td>Anemone</td>
<td>-</td>
</tr>
<tr>
<td>Snowberry</td>
<td>-</td>
</tr>
</tbody>
</table>
Ajoene – the major bioactive QSI compound

Ajoene content in garlic bulbs: 600-700 μg/g
Ajoene and tobramycin treatments show synergy in vivo

Foreign-body infection biofilm model

25 μg ajoene g⁻¹ BW
every 24 h
2 days prophylactic treatment -
day 2 post-insertion
30 μg tobramycin g⁻¹ BW
every 24 h
post-insertion

P. aeruginosa, PAO1

(Christensen et al., J. Antimicrob. Chemother. 2012)
Fig. 1. Diagram of a medical biofilm. (A) Planktonic bacteria can be cleared by antibodies and phagocytes, and are susceptible to antibiotics. (B) Adherent bacterial cells form biofilms preferentially on inert surfaces, and these sessile communities are resistant to antibodies, phagocytes, and antibiotics. (C) Phagocytes are attracted to the biofilms. Phagocytosis is frustrated but phagocytic enzymes are released. (D) Phagocytic enzymes damage tissue around the biofilm, and planktonic bacteria are released from the biofilm. Release may cause dissemination and acute infection in neighboring tissue.

Biofilm bacteria

PMN (polymorphonuclear neutrophile leukocytes)
PMNs and *P. aeruginosa* biofilm

GFP tagged *P. aeruginosa*  

w/o PMNs  

2.5 h with PMNs stained with SYTO62

**PAO1**

**QS mutant (ΔlasR rhlR)**

ESCMID eLibrary by author
PMNs and Garlic treatment
This suggests that the difference in clearing between a QS functional and a QS deficient strain is caused by:

- Virulence inhibition
- Enhanced activity of the PMN’s
Flow cells

PMN Lekocytes

P. aeruginosa
PMNs versus biofilm bacteria

Footage from the top layers of the biofilms

Live from the Battlefield

\[ \text{O}_2^- + \text{hydroethidine} \rightarrow \text{2-hydroxyethidium (red fluorescence)} \]
\[ \text{2-hydroxyethidium} + \text{DNA (increased red fluorescence)} \]

Wild type

QS mutant (\(\Delta\text{lasRrhIR}\))
The shield against PMNs

- We purified and identified the toxin to be **rhamnolipid**, 2-O-α-L-Rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoic acid.

The rhamnolipid production is controlled by Quorum sensing (QS)

Jensen PØ, Bjarnsholt T, et al; Microbiology. 2007 May;153(Pt 5):1329-38
Rapid necrotic killing of polymorphonuclear leukocytes is caused by Quorum-Sensing-controlled production of rhamnolipid by *P. aeruginosa*.
Biofilms resist defense and antibiotics. Chronic infection develops, which may spark systemic infections.

No resistant bacterial biofilms. Bacteria eliminated by host defense system. No chronic infection established.
A Δ*rhlA* mutant is cleared rapidly from silicone implants.
The hypothesis

• Blocking of QS create an adequate immune response which destroy only the bacteria and not the fragile surrounding tissue.

• We envision that a treatment based on QSI drugs and antibiotics will enable clearance of bacterial biofilms from CF lungs, implants, chronic wounds and chronic otitis media etc.
However, is this clinical relevant

- QS regulation takes place the CF lung
- High amounts of free DNA possibly from PMNs
- High amount of necrotic PMNs in the chronic infected CF lung
- Biofilm mode of growth in the CF lung, chronic wounds, and chronic otitis media etc.
  - very tolerant
Acknowledgments

University of Copenhagen:
  Maria Alhede
  Kasper N Kragh
  Steffen R Eickhardt-Sørensen
  Anne K Nielsen
  Stephanie G Crone
  Majken Sønderholm
  Lasse Kvich
  Lene Bay
  Tim Holm Jakobsen

Michael Givskov
  Klaus Qvortrup
  Oana Ciofu
  Søren Sørensen
  Michael Kühl
  Mette Burmølle
  Hans Petter Hougen
  Mustafa Fazli

Rigshospitalet:
  Niels Høiby
  Peter Østrup Jensen
  Claus Moser
  Kim Thomsen
  Lars Christophersen
  Michael Tvede
  Claus B Andersen
  Preben Homøe

Others:
  Klaus Kirketerp-Møller
  Lise H Christensen
  Trine Rolighed Thomsen
  Claus Sternberg
  Christine R Hansen
  Tanja Pressler
  Mark Shirtliff
  Marvin Whiteley
  Steve Diggle
Funding Sources

- Human Frontier Science project
- Capital Region Research Foundation for Health Research
- Novo Nordisk A/S
- AdvanDx Inc.
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- www.coursera.org/course/bacteria