NEXT GENERATION SUSCEPTIBILITY TESTING: WHAT WORKS?

Improving antibiotic targeting *in vivo*

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Where are bacteria (in CF) ? (1/2)

1. in mucus/sputum!

2. in biofilms!

Hoiby et al, Future Microbiol. 2010; 5:1663-74

Where are bacteria (in CF)? (2/2)

3. inside the cells!

P. aeruginosa

S. aureus

B. cenocepacia


Cormet Boyaka et al, 2016; http://dx.doi.org/10.5772/64686
How to improve targeting of these bacteria?

How to improve targeting of these bacteria?

1. Mucus penetration
Administration by inhalation: for which drugs?

- Polymyxins
- Aminoglycosides
- Fluoroquinolones
- Beta-lactams

Biopharmaceutics Classification System

- Class I: high solubility, high permeability
- Class II: low solubility, high permeability
- Class III: high solubility, low permeability
- Class IV: low solubility, low permeability

Pulmonary concentration: inhalation >> IV/PO
Antibiotics by inhalation available today

- Colistin
  - Colobreathe®
  - Tobramycin
  - Tobi® Podhaler

- Nebulizer
  - Colistin
  - ColiFin®
  - Colistin® CF
  - Promixin®
  - Tobramycin
    - Bramitob®
    - Gernebcin®
    - Tobi®
  - Aztreonam
    - Cayston®

- Powder inhaler

- Cystic fibrosis
  - Autosomal recessive genetic disorder of CFTR gene
  - Clogging of airways due to mucus accumulation, decreased mucociliary clearance and inflammation
  - Chronic lung infections caused by S. aureus, P. aeruginosa, H. influenza
  - Microbial biofilms are resistant to immune response and antibiotic treatment

Antibiotics by inhalation: do they reach their target?

Tobramycin powder for inhalation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sputum values</th>
<th>Serum values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TIP 112 mg</td>
<td>TIS 300 mg</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>1,048</td>
<td>737</td>
</tr>
<tr>
<td>AUC$_\infty$ (µg · h/mL)</td>
<td>1,740</td>
<td>1,302</td>
</tr>
<tr>
<td>AUC$_{12}$ (µg · h/mL)</td>
<td>1,307</td>
<td>974</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (h)</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>2.2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

TIP was administered via the T-326 Inhaler and TIS was administered via PARI LC$^\circledR$ PLUS nebulizer.

Globally similar PK but easier use for TIP (powder) than for TIS (solution)
Delivery systems to increase drug concentrations

Antibiotics by inhalation: can we improve local concentrations?

Liposomal formulations

- Liposomal Formulation (DPPC Cholesterol)
- Aerosol Delivery (eFlow® Nebulizer)
- Neutral Charge of ARIKACE enables travel through mucus and biofilm
- Negative Charge
- Lung Mucus and Pa Biofilm Barrier

Conceptual diagram for illustration purposes

Among other factors, some bacteria secrete agents that disrupt liposomal encapsulation and release antibiotic.

Infection Site Treated with ARIKACE
- April 2010
- May 2010
- June 2010
- July 2010
- August 2010
- September 2010
- October 2010
- November 2010
- December 2010

Neutral Charge

Direct Access to Bacteria
Antibiotics by inhalation: can we improve local concentrations?

Liposomal formulations

PK in rats at equivalent doses

- Liposomal amikacin
- Tobramycin

efficacy in rats at equivalent doses

- Saline
- Amikacin: 4.6 ± 0.4
- Liposomal amikacin: 3.2 ± 0.4

Higher concentration in lung and better efficacy for liposomal formulation

Meers et al, JAC 2008; 61, 859–868
Approaches to enhance transport through CF sputum

A. PEGylated NPs
B. DNase
C. Mannitol

PEGylated NPs
Sputum

Biopolymers (DNA, mucin)

DNase

Drug

DNase

Mannitol

Approaches to enhance transport through CF sputum

Nanoparticles (AB + DNase)

Deacon et al, J Control Release 2015; 198:55-61

Higher penetration in mucus and better efficacy against strains non responding to free TOB
How to improve targeting of these bacteria?

2. Biofilm penetration
Antibiotic PK/PD parameters in biofilms

**Pharmacokinetics**
- Diffusibility through the matrix
- Bioavailability within the biofilm
- Access to bacteria
- Efflux out of bacteria

**Pharmacodynamics**
- Bacterial responsiveness (metabolic activity of bacteria)
- Antibiotic expression of activity (local environment [O\(_2\), pH, ..])

**Nutrients & Oxygen**
- Catheter, bone, skin, cardiac valve, ..
Biofilm matrix: what is it made of?

Antibiofilm strategies ~ antibiotic penetration

Reversible-irreversible attachment
- Antiadhesion agents (e.g., mannosides, pilicides, and curlicides in inhibition of UPEC biofilms)
- Antibiofilm polysaccharides
- Signal transduction interference

Microcolony formation
- Lytic phages
- Silver nanoparticles
- EPS-degrading enzymes
- Antimicrobial peptides
- Antibiofilm polysaccharides
- Signal transduction interference
- DNAse I, Dispersin B
- Chelating agents

Biofilm maturation
- Lytic phages
- Silver nanoparticles
- EPS-degrading enzymes
- Antimicrobial peptides
- Antibiofilm polysaccharides
- Signal transduction interference
- DNAse I, Dispersin B
- Chelating agents

Dispersal
- c-di-GMP engineering to promote motility versus sessility
- Introduction of dispersing signals (e.g., D-amino acids/norspermidine in the case of B. subtilis)

Strategies to increase antibiotic penetration in biofilms

Inhibiting matrix biosynthesis

Enzymes involved in the synthesis of N-acetylglycosamine polymers of biofilm matrix in *S. aureus* and *P. aeruginosa* share homology with 1,3-β-D-glucan synthase

[Target for echinocandins in fungi]

Strategies to increase antibiotic penetration in biofilms

**Inhibiting matrix biosynthesis**

*S. aureus*

implanted catheter infection

**P. aeruginosa**

intraperitoneal infection

Echinocandins are synergistic with fluoroquinolones in vivo

Strategies to increase antibiotic penetration in biofilms

Degrading preformed matrix by enzymes

*P. aeruginosa* and tobramycin (512 mg/L)

Enzymes degrading matrix constituents \(\uparrow\) antibiotic activity in biofilms

*Alipour et al., JAC 2009: 64:317-25.*
Antibiotic PK/PD parameters in biofilms

N-acetyl-cysteine (anti-oxidant + reducing disulfure bridges)

Ciprofloxacin and *Pseudomonas* biofilms

NAC increases CIP activity against biofilms in vitro...

Zhao & Liu BMC Microbiology 2010; 10:140
Antibiotic PK/PD parameters in biofilms

N-acetyl-cysteine (anti-oxidant + reducing disulfure bridges)

... But does it work in patients? 900 mg 3 x/day for 24 weeks

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment effect (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV₁ (% pred)</td>
<td>4.4 (0.83, 7.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>FEV₁ (L)</td>
<td>0.15 (0.03, 0.28)</td>
<td>0.02</td>
</tr>
<tr>
<td>Sputum neutr. elastase activity (log&lt;sub&gt;10&lt;/sub&gt;)</td>
<td>0.21 (0.07, 0.48)</td>
<td>0.14</td>
</tr>
<tr>
<td>Sputum neutrophil count (log&lt;sub&gt;10&lt;/sub&gt;)</td>
<td>2.6 (12.1, 17.3)</td>
<td>0.73</td>
</tr>
<tr>
<td>Sputum IL-8 (log&lt;sub&gt;10&lt;/sub&gt;)</td>
<td>0.19 (0.03, 0.42)</td>
<td>0.09</td>
</tr>
<tr>
<td>Plasma IL-8 (log10)</td>
<td>−0.1 (−0.33, 0.14)</td>
<td>0.42</td>
</tr>
<tr>
<td>GSH in whole blood</td>
<td>64.2 (−177.6, 305.9)</td>
<td>0.60</td>
</tr>
<tr>
<td>Incidence of pulmonary exacerbation</td>
<td>−0.08 (−0.30, 0.14)</td>
<td>0.48</td>
</tr>
<tr>
<td>New use of antibiotics</td>
<td>0.08 (−0.14, 0.29)</td>
<td>0.50</td>
</tr>
<tr>
<td>CFQ-R respiratory domain</td>
<td>−0.34 (−6.3, 5.67)</td>
<td>0.91</td>
</tr>
<tr>
<td>CFRSD number of resp sx</td>
<td>−0.15 (−1.1, 0.8)</td>
<td>0.75</td>
</tr>
</tbody>
</table>

95% point wise confidence intervals (using t-distribution approximation) are included at each time point. Similar changes were measured in FEF 25–75% (see online supplement).

Conrad et al., J. Cystic Fibrosis 2015; 14:219–227
How to improve targeting of these bacteria?

3. Intracellular targeting

Agent antibactérien
PK/PD parameters against intracellular bacteria

Antibiotic accumulation and subcellular distribution

diffusion → possible re-distribution → confined in vacuoles → endocytosis

### β-lactams: fast; ~ 1 x
- fluoroquinolones: fast
  - CIP, LVX: 4-10 x
  - MXF, GAR, GMF: 10-20 x

### linezolid: ~ 1 x
- lincosamides: 1-4 x
- tetracyclines: 2-4 x
- rifampin: 2-10 x
- synercid: 30-50x

### aminoglycosides: slow; 2-4 x
- glycopeptides: slow
  - VAN ~ 8 x
  - TLV ~ 50 x
  - ORI ~ 150-300 x

### macrolides: fast
- ERY: 4-10 x
- CLR, ROX, TEL: 10-50 x
- AZM, SOL: > 50 x

### some oxazolidinones: fast
- RDZ: 10 x

mainly in vacuoles slow release

diffusion/ segregation
Strategies to increase antibiotic cellular concentrations

- pharmacokinetics
  - influx
  - efflux
  - metabolism binding
  - accumulation and bioavailability

- pharmacodynamics
  - cooperation with host defenses
  - bacterial responsiveness
  - physico-chemical conditions
Strategies to increase antibiotic cellular concentrations

Influx and pH gradient

Neutral/zwitterionic molecules are more diffusible

If extracellular pH ↓:
• ↑ uptake of acidic molecules
• ↓ uptake of basic molecules

Pezzulo et al, Nature 2013; 487: 109–113

pH of airway surface in pigs

Pezzulo et al, Nature 2013; 487: 109–113
Strategies to increase antibiotic cellular concentrations

**β-lactams**

Neutral/zwitterionic molecules are more diffusible → Masking the charges to change diffusibility

Ampicillin prodrug: accumulation and activity

Chanteux et al., JAC 2003; 52:610-15
Increasing accumulation by improving diffusibility

fluoroquinolones

Neutral/zwitterionic molecules are more diffusible → accumulation lower for most fluoroquinolones at acidic pH

Increasing accumulation by improving diffusibility

**fluroquinolones**

Neutral/zwitterionic molecules are more diffusible
→ accumulation higher for acidic fluoroquinolones (delafloxacin/finafloxacin)

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Strategies to increase antibiotic cellular concentrations

Inhibition of efflux

Ciliated cells
Goblet cells
Basal cells
Macrophages

- P-gp
- MRP1
- MRP2*
- BCRP*
- PEPT1-2
- OCT1
- OCT2
- OCT3
- OCTN1
- OCTN2

Fluoroquinolones
Macrolides
β-lactams
Rifampin

* Conflicting data

Adapted from
Bosquillon, J Pharm Sci, 2010; 2240-55;
Van Bambeke et al, JAC 2003; 51:1067-1077
Strategies to increase antibiotic cellular concentrations

Inhibition of efflux

Calu-3 cell

Pgp activity modulates fluoroquinolone concentrations to different extents

Adapted from
Brillault et al, AAC 2010; 54: 543–5
Strategies to increase antibiotic cellular concentrations

Modulation of distribution: use of delivery systems

gentamicin (GEN) + surfactant (AOT [bis(2-ethylhexyl) sulfosuccinate sodium salt]) + poly(D,L-lactide-co-glycolide) (PLGA)

Delivery systems can help delivering antibiotics to different subcellular compartments

Take home messages

- Antibiotic access to bacteria is made difficult in CF lung by mucus/specific modes of life
- Enzymes and NAC already used in the clinics but no direct demonstration of their adjuvant efficacy towards infections
- Many strategies evaluated in vitro still lacking in vivo and/or clinical evaluation
- A lot of work needed ....

https://loonylabs.org/2015/01/04/antibiotic-resistance-2/
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