PK/PD Principles for Decreasing the Development of Drug Resistance in Antimicrobials: a Road Map

Arnold Louie, M.D.
Institute for Therapeutic Innovation
University of Florida, Orlando, Florida
U.S.A.

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Combating Resistance

• Requires a concerted effort:

• In the clinic:
  – Effective infection control programs.
  – Strong antibiotic stewardship programs.

• On the farm: Reduce antibiotic use in livestock.

• On the benchtop: Identify new targets for anti-infective therapies that lack cross-resistance with existing medications.

• In the Lab and Clinic: Optimize the PD of drugs to suppress resistance. (This usually requires higher dosages. Toxicity?)
PK-PD for Suppressing Resistance

1. Impact of bacterial burden on antibiotic dose/exposure intensity. Describe the “inverted U” as it relates to resistance amplification/suppression.

2. Therapy duration and its impact on drug exposures for suppressing resistance.

3. Pharmacodynamic indices for suppressing resistance.

4. Effect of granulocytes in controlling the infection: Possible avenue for identifying a target for maximizing treatment efficacy for VAP?

(I will not be discussing how to choose the best in vivo or in vitro infection models for resistant suppression)
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1. Impact of bacterial burden on antibiotic dose/exposure intensity. The “inverted U” as it relates to resistance amplification/suppression.

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Bacterial burden in people with VAP

- Zaccard et al. conducted bilateral BALs to quantitate the bacterial burden in the lungs of 134 patients with suspected ventilator-associated pneumonia (VAP) due to *P. aeruginosa*, *Acinetobacter* spp., *Klebsiella* spp., and other GNRs.

- The mutation frequency for most antibiotics are between $10^{-5}$ to $8 \times 10^{-7}$ CFU.

- The higher the bacterial burden, the higher the probability pre-existing isolates with reduced susceptibilities to the drug are present at the infection site prior to the start of therapy.

<table>
<thead>
<tr>
<th>Quantitative culture range</th>
<th>Percentage of patients</th>
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<td>$\geq 3 \times 10^7$ CFU/mL</td>
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Zaccard et al. AAC 2011; 55: 1606.
Impact of bacterial burden on antibiotic exposures needed to achieve microbiological endpoints in a immunocompetent murine thigh model of \textit{P. aeruginosa}

Two bacterial inocula: $10^7$ and $10^8$ CFU/thigh   MF to 3x MIC: $2 \times 10^{-6}$ CFU.

The two bacterial challenge inocula are higher than the inverse of the MF value:
$10^7$ CFU/thigh: ~ 5 resistant CFU/thigh.  $10^8$ CFU/thigh: ~ 50 resistant CFU/thigh

Dose-range study over 24 hours for single-dose levofloxacin: 0 to 1,000 mg/kg IP.

Jumbe et al. J Clin Invest 2003;112:275
Peripheral (thigh) Compartment (C_p)

Central Blood Compartment (C_c)

Bacteria (X_{T/R})

\[
\frac{dC_a}{dt} = -k_a C_a
\]

\[
\frac{dC_c}{dt} = k_a C_a + k_{pc} C_p - k_{cp} C_c - k_e C_c
\]

\[
\frac{dC_p}{dt} = k_{cp} C_c - k_{pc} C_p
\]

\[
dC_c = k_{pc} C_p - k_{cp} C_c
\]

\[
dC_p = k_{cp} C_c - k_{pc} C_p
\]

\[
dC_a = -k_a C_a
\]

\[
\frac{dX_S}{dt} = K_{GS} x X_S x L - f_{KS}(C_c^{H\xi}) x X_S
\]

\[
\frac{dX_R}{dt} = K_{GR} x X_R x L - f_{KR}(C_c^{H\xi}) x X_R
\]

\[
L = (1 - (X_R + X_S)/\text{POPMAX})
\]

\[
f_{\psi\xi}(C_c^{H\xi}) = \frac{K_{max} \xi \cdot C_c^{H\xi}}{C_c^{H\xi50\psi} + C_c^{H\xi}}\]

\[
Y_1 = X_T = X_S + X_R
\]

\[
Y_2 = X_R
\]
The mathematical modeling outputs: Levofloxacin doses that amplify or suppress the growth of the levofloxacin-resistant *P. aeruginosa* subpopulations in mice infected with $10^8$ CFU/thigh.

**Levofloxacin Effect: Mouse Thigh Infection Model**

Preventing Emergence of the Resistant Mutant Population

- 24h-AUC/MIC ratio of 157 suppresses the amplification of resistant mutants.
- 24h-AUC/MIC ratio of 52 maximally amplified the resistant subpopulation.

Jumbe et al. *J Clin Invest* 2003;112:275
Prospective Validation Experiment –

Immune normal mice were inoculated with $10^8$ CFU/thigh inoculum and were treated with these two levofloxacin exposures that had not been explicitly examined before.

Levofloxacin was dosed once-daily.

We also extended the study duration from 24h to 48h to further test the predictions.

The lines in the figures are NOT best-fit lines.

They are prospective prediction lines about which the data have been scattered.

Implications for drug development for resistance prevention

- **Bacterial burden does matter** when evaluating the efficacy of a compound for resistance suppression.

- **Mathematical modeling** of the dose-range data can be used to identify the drug exposures that are predicted to amplify or prevent the emergence of the drug-resistant microbial subpopulations.

- **Monte Carlo simulations** predicted that only 61.2% of people given levofloxacin 750 mg po QD would achieve the AUC/MIC target of 157 that would counterselect for resistance.
  - Higher dosages of levofloxacin would be needed to prevent resistance in a larger proportion of patients.
The exposure-response effect of antibiotics on the susceptible and resistant microbial populations differ.

Killing of the susceptible population is characterized by a monotonic function.

Killing of the less-susceptible population is described by an “Inverted U.”

Jumbe et al. J Clin Invest 2003; 112: 275

Tam et al. AAC 2007; 51: 744.
### PD indices for killing and resistance amplification

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Pathogen</th>
<th>PDI-killing</th>
<th>PD Index-intensities killing for:</th>
<th>resistance selection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garenoxacin</td>
<td>S. aureus</td>
<td>AUC/MIC</td>
<td>10 - 35</td>
<td>10 – 35</td>
<td>Tam et al. AAC 2007</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>Y. pestis</td>
<td>AUC/MIC</td>
<td>31 – 63.5</td>
<td>31 – 127</td>
<td>Louie et al. AAC 2011</td>
</tr>
<tr>
<td>Linezolid</td>
<td>B. anthracis</td>
<td>AUC/MIC</td>
<td>45 – 56</td>
<td>45 – 56</td>
<td>Louie et al. AAC 2008</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>S. aureus</td>
<td>AUC/MIC</td>
<td>14 – 71</td>
<td>0.5 – 40</td>
<td>Bowker et al. JAC 2009</td>
</tr>
<tr>
<td>Telavancin</td>
<td>Enterococcus</td>
<td>AUC/MIC</td>
<td>6 – 60</td>
<td>1 – 50</td>
<td>MacGowan AAC 2011</td>
</tr>
<tr>
<td>Ceftaroline</td>
<td>S. aureus</td>
<td>T&gt;MIC(%)</td>
<td>13 – 40</td>
<td>15 – 30</td>
<td>MacGowan AAC 2013</td>
</tr>
<tr>
<td>Doripenem</td>
<td>P. aeruginosa</td>
<td>T&gt;MIC(%)</td>
<td>14 – 42</td>
<td>12 – 37</td>
<td>Bowker et al. AAC 2012</td>
</tr>
<tr>
<td></td>
<td>A. baumannii</td>
<td>T&gt;MIC(%)</td>
<td>9 – 32</td>
<td>12</td>
<td>Bowker et al. AAC 2012</td>
</tr>
<tr>
<td>Ceftolozane/tazo</td>
<td>E. coli</td>
<td>T&gt;threshold(%)</td>
<td>87.5 (tazo)</td>
<td>75 – 98 (tazo)</td>
<td>VanScoy et al. AAC 2013</td>
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PK-PD for Suppressing Resistance

1. Impact of bacterial burden on antibiotic dose/exposure intensity. Describe the “inverted U” as it relates to resistance amplification/suppression.

2. Therapy duration and its impact on drug exposures for suppressing resistance

3. Pharmacodynamic indices for suppressing resistance

4. Effect of granulocytes in controlling the infection: identifying a potential target for maximizing treatment efficacy for VAP?
• Therapy duration is one of the great understudied areas of Infectious Diseases.

• There are only a few infectious diseases for which duration of therapy has been established based on firm scientific data – Strep throat, endocarditis, UTI, and some STDs.

• But what is known about the impact of duration of antibiotic therapy on the amplification or suppression of the antibiotic-resistant subpopulations?
Suppressing Resistance – Therapy Duration

We conducted a 10 day dose-range study with the fluoroquinolone garenoxacin vs S. aureus in a HFIM.

Suppressing Resistance – Therapy Duration

- We modeled the dose-ranging data to ascertain the drug exposure that would suppress resistance amplification after 2 days of garenoxacin therapy and after 10 days of therapy.
- We also modeled the time-to-failure for the less intense exposure.
- Model predictions:
  1. A daily administered AUC/MIC ratio of 100 would suppress resistance for at least 2 days.
  2. A daily administered AUC/MIC ratio of 280 would suppress resistance for the duration of the 10-day study.
  3. Time to failure due to resistance amplification for the daily-dosed AUC/MIC ratio of 100 would be 5 days.
- A prospective validation study was conducted to validate these predictions.
Suppressing Resistance – Validation Study

- No-treatment control provided no selective pressure. The resistant population increased in parallel to the total population.

- The AUC/MIC ratio of 100 given once daily for 10 days failed after day 5.

- The AUC/MIC ratio of 280 eliminated the less-susceptible subpopulation by day 4 and resulted in a progressive reduction in the total population for the entire 10-day treatment period.

- With longer treatment duration, the drug exposure needed to suppress the resistant subpopulation increased 28-fold.

Extended infusion of beta-lactam drugs is now in vogue for killing the drug-susceptible infecting bacterium.

Felton et al. evaluated 0.5 h bolus vs 4 h extended infusions of 3 to 17g piperacillin (with tazobactam) given q8h on resistance amplification in *P. aeruginosa* in a 5 day *in vitro* HFIM experiment.

- High inoculum (8x10^8 CFU/mL): all arms failed with resistance.
- Low inoculum (4x10^5 CFU/mL):
  - 0.5 h bolus dosing every 8 h: trough/MIC ratio for piperacillin of 3.4 was needed to suppress resistance.
  - 4 h extended infusions every 8 h: trough/MIC ratio of 10.4 was required.

Felton et al. AAC 2013; 57: 5811.
Felton et al. evaluated 0.5 h bolus vs 4 h extended infusions of 3, 9, & 17 g of piperacillin (with tazobactam) given every 8 h on resistance amplification in *P. aeruginosa* in a 5 day *in vitro* HFIM study.

- High and low starting inocula: 4x10^5 and 8x10^8 CFU/mL (VAP)

**Results:**

- High inoculum: all regimens failed due to resistance amplification.
- Low inoculum: In order to suppress resistance, **modeling of the results:**
  
  - 0.5 h bolus dosing every 8 h: trough/MIC ratio of 3.4 was needed
  - 4 h extended infusions every 8 h: trough/MIC ratio of 10.4 was required.

Felton et al. AAC 2013; 57: 5811.
• Overall, the data suggests that to minimize resistance amplification:
  – antibiotics should be given at higher dosages for a shorter duration of time.
  – Piperacillin-tazobactam, should be given as bolus doses and not as extended infusions.
Clinical Correlation

• Prospective, randomized, double-blinded clinical trial comparing 8 vs 15 days of antibiotic therapy for VAP.
  – VAP dx’ed by quantitative cultures of BAL specimens
  – Findings:
    • No differences in overall mortality (18.8% vs 17.2%).
    • More antibiotic free days with 8 days of therapy ($p < 0.001$).
    • For VAP caused by non-fermenting GNRs, including \textit{P. aeruginosa}, more recurrence was observed with shorter treatment (8 vs 15 days of therapy: 40.6% vs 25.4%, difference 15%, 90% CI, 3.9% to 26.6%).
    • But 8 days of treatment had less recurrences due to multidrug-resistant bacteria than 15 days of treatment (42.1% vs 62.3%, respectively, $p = 0.04$).

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Suppressing Resistance - Pharmacodynamic Drivers

• For many antibiotics, the PD indices for killing the parent isolate and for suppressing resistance differ.
  – Minimizing resistance amplification requires antibiotics to be given using a different schedule of administration compared with regimens aimed that maximizing the killing of the parent isolate.
  – However, the higher dosages needed to suppress resistance often

• Examples:
  1. Rifampin for *Mycobacterium tuberculosis*
  2. Vancomycin for *S. aureus*
Rifampin suppressing resistance—pharmacodynamic drivers for *M. tuberculosis*

Cell kill is AUC/MIC-driven

Resistance suppression is Cmax/MIC-driven (but the exposure is not achievable in humans)

Gumbo et al. AAC 2007;51:3781.
Vancomycin PD for killing and resistance prevention (10-day HFIM)

PD index for killing the susceptible-bacteria: AUC/MIC or Cmax/MIC

PD index for suppressing resistant subpopulations: Cmax/MIC ratio.

Resistance amplification and suppression follows the “inverted U” paradigm.

Ramos-Martin et al. JAC 2016; 72: 992
### PD indices for killing and resistance suppression

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<td>T&gt;threshold</td>
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What about the role of the innate immune system?

- Inoculum range study for *P. aeruginosa* in immune normal mice.
  - Thigh infection model
  - Pneumonia model
- A Michaelis-Menten model was fit to the data:
  - For the thigh model, the $K_m$ or the bacterial burden that half saturates neutrophil function, was $4.3 \times 10^6$ CFU/g.
  - For the pneumonia model, the $K_m$ was $2.15 \times 10^6$ CFU/g.

Drusano et al. AAC 2010; 54: 4368.
Drusano et al. AAC 2011; 55: 2693.
WBC and Plazomicin vs *P. aeruginosa*

Higher humanized plazomicin dosages given from 2 - 26 hr, reduced the *P. aeruginosa* burden to < 10⁵ CFU/g. This restored granulocyte function, allowing the neutrophils to kill an additional 1 – 1.5 log CFU/g of bacteria (without antibiotic) between the 26 and 50 hr time points.


No survivors at 50hrs

<table>
<thead>
<tr>
<th>Infect Mice</th>
<th>Plazomicin Treatment</th>
<th>No Antibiotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>0h</td>
<td>2h</td>
<td>26h</td>
</tr>
<tr>
<td>50h</td>
<td></td>
<td></td>
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With the humanized plazomicin regimens, no drug would be detected in serum by hour 26.
WBC and Plazomicin vs *P. aeruginosa*

Plazomicin dosages that reduce the bacterial burden to \( \leq 10^5 \) CFU/g in concert with WBCs prevented the amplification of the less-susceptible *P. aeruginosa* isolates.

Together with the Qcx data by Zaccard, this suggests a \( \geq 2 - 3 \) log CFU/g reduction is needed to achieve this endpoint over \( ? \) Days.

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Zaccard et al. AAC 2011; 55: 1606.  
An approach to Combating Resistance

• Thus, for single drug regimens in order to combat resistance we need to:
  – “Hit the bacteria hard and hit them fast”
  – Use higher dosages for a shorter duration to rapidly reduce the total (susceptible and less-susceptible) population to below $10^5$ CFU/g of tissue, which is predicted to restore granulocyte function. The restored function of the granulocytes can eliminate the residual bacteria without additional antibiotics.
  – Drug tolerability may be a limiting factor for some drugs.

• Although not discussed today, combination antibiotic regimens is very effective for combating resistance.
  – For example: beta-lactam plus aminoglycoside.
Conclusions

• Drug exposures for the killing of the Abx-susceptible microbial population is a monotonic function.
• The killing of the less-susceptible population is characterized by an “inverted U.”
  – Doses of drugs that yield a stasis effect and 1-log reductions of the parent strain also amplify the less-susceptible subpopulations.
• The intensity of drug exposures needed to suppress resistance may increase with the duration of therapy.
• The PD indices for the killing of the parent strain and for resistance prevention are not the same.
• The immune system has a role in controlling infections provided the bacterial concentrations are < 10⁵ CFU/mL.
• To prevent resistance: higher dosages for shorter durations.
Acknowledgements

George Drusano, M.D.

The laboratory crew