Educational Workshop

EW02: Pharmacokinetics and pharmacodynamics of anti-infective agents

arranged with ISAP

(International Society of Anti-Infective Pharmacology)

Convenor: Johan W. Mouton, Nijmegen, NL

Faculty: Hartmut Derendorf, Gainesville, US
Johan W. Mouton, Nijmegen, NL
Ursula Theuretzbacher, Wien, AT
Inga Odenholt, Malmö, SE
William A. Craig, Madison, US
The General Concepts of Pharmacokinetics and Pharmacodynamics

Prof. Hartmut Derendorf
University of Florida

PHARMACOKINETICS
what the body does to the drug

PHARMACODYNAMICS
what the drug does to the body

Pharmacokinetics
conc. vs time

Pharmacodynamics
conn. vs effect

PK/PD
effect vs time
Pharmacokinetics

the time course of drug and metabolite concentrations in the body

Pharmacokinetics helps to optimize drug therapy:

- dose
- dosage regimen
- dosage form

What happens to a drug after its administration?

("Fate of drug")

- Liberation
- Absorption
- Distribution
- Metabolism
- Excretion
Pharmacokinetic Parameters

- Clearance
- Volume of distribution
- Half-life
- Bioavailability
- Protein Binding

Clearance

- quantifies **ELIMINATION**
- is the volume of body fluid cleared per time unit (L/h, mL/min)
- is usually constant

\[ CL = Q \cdot E \]

- \( Q \) Blood Flow
- \( E \) Extraction Ratio
Clearance

\[ E = \frac{C_o - C_i}{C_i} \]

\[ CL = Q \cdot E \]

\[ CL = \frac{Q \cdot f_u \cdot CL_{\text{int}}}{Q + f_u \cdot CL_{\text{int}}} \]

Parameters: Blood Flow, intrinsic clearance, protein binding
Good prediction of changes in clearance
Steady state

---

High-extraction drugs

\[ CL = \frac{Q \cdot f_u \cdot CL_{\text{ex}}}{Q + f_u \cdot CL_{\text{ex}}} \]

\( Q \ll f_u \cdot CL_{\text{ex}} \) → \( CL = Q \)

Low-extraction drugs

\[ CL = \frac{Q \cdot f_u \cdot CL_{\text{int}}}{Q + f_u \cdot CL_{\text{int}}} \]

\( Q \gg f_u \cdot CL_{\text{int}} \) → \( CL = f_u \cdot CL_{\text{int}} \)

---

Clearance can be calculated from

- Excretion rate / Concentration
  e.g. \( \text{mg/h} \) / \( \text{mg/L} \) = \( \text{L/h} \)
- Dose / Area under the curve (AUC)
  e.g. \( \text{mg} \) / \( \text{mg-h/L} \) = \( \text{L/h} \)
Clearance

Total body clearance is the sum of the individual organ clearances

\[ CL = CL_{\text{ren}} + CL_{\text{hep}} + CL_{\text{other}} \]

Volume of Distribution

\[ Vd = \frac{X}{Cp} \]

- quantifies DISTRIBUTION
- relates drug concentration (Cp) to amount of drug in the body (X)
- gives information on the amount of drug distributed into the tissues

Apparent Volume of Distribution

\[ C_1 = \frac{X}{V} \]
\[ V = \frac{X}{C_1} \]
\[ C_2 = \frac{X}{Vd} \]
\[ Vd = \frac{X}{C_2} \]
Hartmut Derendorf
Introduction to PK/PD

Volume of Distribution

<table>
<thead>
<tr>
<th>Drug</th>
<th>Volume (L/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicloxacillin</td>
<td>0.1</td>
</tr>
<tr>
<td>Gentamicin (ECF)</td>
<td>0.25</td>
</tr>
<tr>
<td>Antipyrine (TBW)</td>
<td>0.60</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1.8</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>8</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>31</td>
</tr>
</tbody>
</table>

Half-Life

Half-life is the time it takes for the concentration to fall to half of its previous value.

Half-life is a secondary pharmacokinetic parameter and depends on clearance and volume of distribution.

\[
t_{1/2} = \frac{0.693 \cdot Vd}{CL}
\]

\[
t_{1/2} = \frac{\ln 2}{k} = \frac{0.693}{k}
\]

\[CL = k \cdot Vd\]
Bioavailability

\[ F = \frac{AUC_{po}}{AUC_{iv}} \]

- quantifies **ABSORPTION**

\( f \) is the fraction of the administered dose that reaches the systemic circulation

**Bioavailability**

Rate and Extent of Absorption

**Protein Binding**

- reversible vs. irreversible
- linear vs. nonlinear
- rapid equilibrium

The free (unbound) concentration of the drug at the receptor site should be used in PK/PD correlations to make predictions for pharmacological activity.
Hartmut Derendorf
Introduction to PK/PD

- **Plasma**: Protein binding
- **Blood cell binding**: Diffusion into blood cells, binding to intracellular biological material
- **Extracellular biological material**: Binding to extracellular biological material
- **Interstitial**: Cell binding, diffusion into tissue cells, binding to intracellular biological material

Effect of Protein Binding on Antimicrobial Activity
MICS of Staphylococcus aureus (Data from Kanon et al. 1973)
Microdialysis

Pharmacokinetic profile of cefpodoxime
(400 mg oral dose, n = 6)

Pharmacokinetic profile of cefixime
(400 mg oral dose, n = 6)
Pharmacokinetics

<table>
<thead>
<tr>
<th></th>
<th>Cefpodoxime</th>
<th>Cefixime</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_P [\text{mg} \cdot \text{h}/\text{L}]$</td>
<td>22.4 (8.7)</td>
<td>25.7 (8.4)</td>
</tr>
<tr>
<td>$AUC_T [\text{mg} \cdot \text{h}/\text{L}]$</td>
<td>15.4 (5.2)</td>
<td>7.4 (2.1)</td>
</tr>
<tr>
<td>$C_{\text{max},P} [\text{mg}/\text{L}]$</td>
<td>3.9 (1.2)</td>
<td>3.4 (1.1)</td>
</tr>
<tr>
<td>$C_{\text{max},T} [\text{mg}/\text{L}]$</td>
<td>2.1 (1.0)</td>
<td>0.9 (0.3)</td>
</tr>
</tbody>
</table>

Two-compartment model

Dose
$X_c$: Drug in the central compartment
$X_p$: Drug in the peripheral compartment
Drug eliminated

Two-compartment model
Short-term infusion

\[ C_p (\mu g/ml) \]

\[ t [h] \]

Cp\textsuperscript{max}, Cp\textsuperscript{min}

Three-compartment model

D Dose
E Drug eliminated

\( X_p \) Drug in the central compartment
\( X_{ps} \) Drug in the shallow peripheral compartment
\( X_{pd} \) Drug in the deep peripheral compartment

\[ C = a \cdot e^{-\alpha t} + b \cdot e^{-\beta t} + c \cdot e^{-\gamma t} \]
Biomarker vs. Surrogate Endpoint

Biomarker
Drug- or disease-induced measurable physiological, pathophysiological or biochemical change

Surrogate Endpoint
Biomarker that has predictive value for therapeutic outcome
Sigmoid $E_{\text{max}}$ - model

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

- $E$ = intensity of effect
- $E_{\text{max}}$ = maximum effect
- $C$ = concentration
- $EC_{50}$ = concentration at 0.5 $E_{\text{max}}$
- $n$ = shape (slope) factor, Hill factor

Pharmacodynamics of Anti-infective Agents

- in vitro studies
  - steady state
dilution models
diffusion models
- animal studies
- clinical studies
Hartmut Derendorf
Introduction to PK/PD

![Graphs showing various PK/PD metrics such as Time above MIC, C_max/MIC, and AUC_24/MIC.]

**Kill Curves of Ceftriaxone**

*S. pneumoniae ATCC6303*  
MIC: 20 ng/mL  

*H. influenzae ATCC10211*  
MIC: 5 ng/mL

---

---

---
Hartmut Derendorf  
Introduction to PK/PD

**Pharmacokinetics**  
conc. vs time

**Pharmacodynamics**  
conc. vs effect

**PK/PD**  
effect vs time

---

**Concentration-dependent vs. Time-dependent**

![Graph showing concentration-dependent vs. time-dependent](image)

---

**Kill Curves**

- reservoir
- flask
- tubing
- connector
- waste
- pump
  - Auto-dilution system
PK-PD Model

\[
dN \frac{dt}{dt} = \left( k - \frac{k_{\text{max}} \cdot C_f}{EC_{50} + C_f} \right) \cdot N
\]

Maximum Growth Rate Constant \( k \)
Maximum Killing Rate Constant \( k-k_{\text{max}} \)

Initially, bacteria are in log growth phase

PK-PD Model

In animals

Bacterial survival fraction of \( P. \) aeruginosa in a neutropenic mouse model at different doses (mg/kg) of piperacillin (Zhi et al., 1988)

Single Dose
Piperacillin vs. \( E. \) coli
Providing Clinical Evidence of Effectiveness for Human Drug and Biological Products

New Dosage Form of a Previously Studied Drug
In some cases, modified release dosage forms may be approved on the basis of pharmacokinetic data linking the new dosage form from a previously studied immediate-release dosage form. Because the pharmacokinetic patterns of controlled-release and immediate release dosage forms are not identical, it is generally important to have some understanding of the relationship of blood concentration to response to extrapolate to the new dosage form.

Plasma and free tissue levels
500 mg IR

n = 12 (means +/- S.D.)
- total plasma concentrations
- free tissue concentrations
Plasma and free tissue levels

500 mg MR

750 mg MR

n = 12 (means +/- S.D.)
- total plasma concentrations
- free tissue concentrations

750 mg MR bid vs 500 mg IR tid

Streptococcus pneumoniae

Summary

- A simple comparison of serum concentration and MIC is usually not sufficient to evaluate the PK/PD-relationships of anti-infective agents.
- Protein binding and tissue distribution are important pharmacokinetic parameters that need to be considered. Microdialysis can provide information on local exposure.
- PK-PD analysis based on MIC alone can be misleading.
- Microbiological kill curves provide more detailed information about the PK/PD-relationships than simple MIC values.
Conclusions

• PK/PD can help to streamline rational clinical dose selection
• The final dose needs to be confirmed in a clinical trial

PK/PD in Drug Development

Streamlining
Rational Approach

Cost Saving
Time Saving
Dosing should be such that the level of antimicrobial activity is associated with a high likelihood of therapeutic success.

**Dose Finding - The Past**
How can PK/PD help here?

Efficacy of the drug

- Potency of a drug (MIC)
- Exposure to the bug \textit{In vivo} (PK)

Lowest concentration with no visible growth after 18 hour incubation

- MIC

PK

- X-acin 500 mg

\begin{align*}
\text{MIC} &= 2 \text{ mg/L} \\
\end{align*}

Pharmacokinetic Parameter (and Dose)

- Thus, we have to:
  - Establish a relationship between the MIC \textit{in vitro} and concentrations \textit{in vivo} (thus, dosing regimens)
  - Determine which dosing regimens are optimal for \textit{Treatment} in relation to the MIC
Any idea where we are today?

No idea... may be a mouse?

Might be a human, though...

---

PK/PD

- Neutropenic mouse thigh model
- Various doses and dosing regimens (q1 to q24)
- Outcome parameter: cfu counts after 24 h
- Plot PD parameter (AUC, Peak T>MIC) to effect

---

K. pneumoniae, imipenem

Based on data from Craig
For *K. pneumoniae*, there is no clear relation between total daily dose of imipenem and efficacy in an in vivo model of infection.

For beta-lactams, there is a direct relation between Time > MIC and efficacy.
Relationship between T>MIC, Peak, AUC and effect of levofloxacin for S. pneumoniae in mice. Each dot represents one mouse/dosing regimen.

Based on data from Scaglione & Mouton, 2001, 2003

PK/PD relationship is Class Dependent

Relationship PkPd and Effect

<table>
<thead>
<tr>
<th>T&gt;MIC</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillins</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Fluoroquinolones</td>
</tr>
<tr>
<td>Carbapenems</td>
<td>Metronidazole</td>
</tr>
<tr>
<td>Monobactams</td>
<td>Lipopeptides</td>
</tr>
<tr>
<td>Tribactams</td>
<td>Ketolides</td>
</tr>
<tr>
<td></td>
<td>Macrolides</td>
</tr>
<tr>
<td></td>
<td>Clindamycin</td>
</tr>
<tr>
<td></td>
<td>Streptogramins</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td>Glycylcyclines</td>
</tr>
<tr>
<td></td>
<td>Oxazolidinones</td>
</tr>
<tr>
<td></td>
<td>Tetracyclines</td>
</tr>
<tr>
<td></td>
<td>Azoles</td>
</tr>
</tbody>
</table>
Relationship AUC and effect

- What has the MIC to do with this?

Fluconazole efficacy in mice

Dose vs MIC

Pharmacokinetic parameters:

Measures of Exposure

AUC and Peak are usually linearly related to Dose
Fluconazole Pharmacodynamics Against Isogenic Strain Pairs of Susceptible and Resistant C. albicans

Andes et al. ISHAM 2003
Johan W. Mouton
PK/PD indices

Thus, 2 factors influence the value of the pk/pd index:
MIC and its Errors/variation
Pharmacokinetics and its variation

Why is the term pk/pd index used instead of pk/pd parameter?
-a ratio (e.g.) of two independent parameters, not a parameter by itself
The MIC

The MIC is a result of:

• kill over time (kill rate) by the antibiotic
• growth over time (growth rate)
• for a certain number of micro-organisms (the inoculum)

AT STATIC CONCENTRATIONS

Kahlmeter et al, JAC 2003
Johan W. Mouton
PK/PD indices

- Growth and/or kill rate dependent:
  - strain, species
  - medium composition, brand
  - MH, supplements, ISO
  - number of bacteria
  - inoculum
    - $5 \times 10^5$ (CLSI) vs $10^5$ (BSAC)
  - temperature ($35^\circ$ vs $37^\circ$)
  - growth phase
  - CO$_2$
  - etc.

A reference MIC method has been described by ISO/CEN
Accepted by memberstates, pending final vote
ALL METHODS USED UN THE FUTURE SHOULD BE VALIDATED AGAINST THIS METHOD

The method complies with CLSI and EUCAST

Several Posters at This ECCMID
The reproducibility of the MIC is within 2 2-fold dilutions. The variation introduced in the AUC/MIC and Peak/MIC values by the MIC is there for at least 0.5 to 2 x the pk/pd index value!

• SC = The concentration of antimicrobial at which growth equals kill, i.e. no net growth or kill at a certain point in time
• = NOT equal to MIC (which includes time)
• Distinguish between MIC in vitro and in vivo.

Mouton & Vinks Clin Pharmacokinet 2005 44:201-10

MISCONCEPTION:
'Drug is active for a prolonged period of time, and remains above the MIC long enough to…'

The SC may be lower or higher than the MIC, depending on its kill kinetics

In general the SC is lower, especially for concentration dependent drugs

Mouton & Vinks Clin Pharmacokinet 2005 44:201-10
**AUC**

**Definition:** The Area under the Concentration-time curve over 24 hours.

**Note:** It should be stated how the AUC is determined: based on (log) linear trapezoidal rule, based on clearance, or based on microconstants.

**Dimensions:** concentration x time e.g. mg.h/L or µg.h/mL


AUC 0-24 = 3033
AUC inf = 5100
AUC 0-24 sd = 1361
AUC inf sd = 1700

Mg.h/L

---

**PHARMACOKINETIC parameters**
WHICH AUC?

- AUC\_\_0-24h or AUC\_\_∞
- Steady State?
- (log) trapezoidal rule?
- Derived? (A/α + B/β or other)

AUC/MIC

Definition: The area under the concentration-time curve over 24 hours in steady state divided by the MIC.

Note: For unbound fraction of the drug, use FAUC/MIC.

Dimensions: no dimensions

AUIC

Definition: The area under the inhibitory curve over 24 hours.

Note: the AUIC should be reserved for those cases where actual inhibitory titers have been measured and used in the calculations. The AUIC is not equal to the AUC/MIC. See also Flaherty et al, AAC 1988;32(12):1825-29; Hyott JM et al AAC 1994;38(12):2730-7; Occhipinti DJ et al, AAC 1997;41(11):2511-7.

Dimensions: none
**Peak/MIC**

*Definition:* the peak level divided by the MIC.

*Dimensions:* no dimensions.

---

**WHICH PEAKLEVEL?**

- After the 1st, 2nd or later dose?
- If more than one compartment, the peak level in compartment 1, 2 or even 3?

---

**fig 1**

Scaglione et al, AAC 2003
**Johan W. Mouton**

**PK/PD indices**

---

**Time > MIC**

**Definition**: the % of time above the MIC over a period of 24 hours.  
**Note**: if the period is other than 24 h, this should be stated explicitly.  
**Dimensions**: %.

---

**Variation in methods, definitions**  
Variation in *estimation*  
Variation in *population*

---

**For all indices**:  
how are they determined  
how are they calculated  
what is the error?  

Only when these questions have been answered do we know the true impact and value of the index.
### Protein binding

<table>
<thead>
<tr>
<th>Protein binding (%)</th>
<th>Antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;90%</td>
<td>Oxacillin, ceftriaxone, ertapenem, teicoplanin, daptomycin, dalbavancin, televancin, fusidic acid, rifampicin, isapaprin</td>
</tr>
<tr>
<td>&gt;70%</td>
<td>Cefazolin, ertapenic, oritavancin, tigecycline</td>
</tr>
<tr>
<td>&gt;30%</td>
<td>Penicillin G, cefotaxime, ceftriaxone, metoxolacacain, erythromycin, clarithromycin, azithromycin, telithromycin, vancomycin, linezolid</td>
</tr>
<tr>
<td>&lt;10%</td>
<td>Meropenem, doripenem, aminoglycosides, tetracyclins</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>Amoxicillin, piperacillin, cephapirin, cefuroxim, imipenem, meropenem, levofloxacin, gatifloxacin, metronidazol</td>
</tr>
</tbody>
</table>

### Activity

The achievable active drug concentration at the site of infection are high enough to inhibit the bacteria.

1. **Protein binding**
2. **Penetration**
3. **Activity**
4. **Pathogen**
5. **Antibiotic**

**microbiologic**

Tissue concentrations
Protein binding/tissue distribution

**Protein binding**

- small reservoirs
- large reservoirs

**Protein Binding - Examples**

**Activity!**

- Ertapenem
  - Relationships between EC50 and % human serum for *E. cloacae* (○) and *S. aureus* (●)
  - Mean total and free conc. of telithromycin in plasma, muscle, subcutis (800 mg p.o.)

**Distribution!**

- Telithromycin
  - Protein binding: ∼90%
  - Total conc.

**Protein Binding: Activity**

- High protein binding raises MICs (tested with protein)
  - Ertapenem (94%)
  - Imipenem (10%)
  - Meropenem (2%)

© Ursula Theuretzbacher
**Protein Binding: Activity**

Kill curves of moxifloxacin and trovafloxacin in media containing or lacking albumin

---

**Protein binding:**

Effect on Penetration of β-Lactams into Rabbit Peripheral Lymph

---

**Protein Binding: Cefotaxime - Ceftriaxone**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Serum: Total</th>
<th>Serum: Free</th>
<th>Pleural Fluid: Total</th>
<th>Pleural Fluid: Free</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>35%</td>
<td>95%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

---

© Ursula Theuretzbacher

© Ursula Theuretzbacher

© Ursula Theuretzbacher
Location - Pathogen

- Blood capillary
- Extracellular fluid
- Cells
- Chlamydia, Rickettsia, Ehrlichia
- Legionella, mycobacteria

Location - Antibiotic

- Blood capillary
- Intravascular
- Extra-, intracellular
- Bound + free fraction
- Homogenates, biopsies
- Antibiotics: macrolides, fluoroquinolones, ß-lactams, aminoglycosides

Tissue concentrations (biopsies)

- Ratio tissue concentration/serum
- Mouton et al. JAC 2008
Tissue concentration - Lung

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Method</th>
<th>C&lt;sub&gt;24h&lt;/sub&gt; Lung [µg/ml]</th>
<th>C&lt;sub&gt;0&lt;/sub&gt; Plasma [µg/ml]</th>
<th>No. Sampling</th>
<th>Time Period</th>
<th>No. Patients</th>
<th>Sex</th>
<th>Group no.</th>
<th>Pool Days</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steady</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftazidime</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long period</td>
<td>3.57-8.2</td>
<td>1 (SD) 30</td>
<td>3 3</td>
<td>1</td>
<td>Zeitlinger et al. 2005&lt;sup&gt;41&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6.6-15</td>
<td>1 (SD) 30</td>
<td>3 3</td>
<td>1</td>
<td>Zeitlinger et al. 2005&lt;sup&gt;41&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifaximin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long period</td>
<td>2.7-4.9</td>
<td>1 (SD) 25.9</td>
<td>6 6</td>
<td>1</td>
<td>Albarran et al. 2006&lt;sup&gt;44&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.4-14</td>
<td>1 (SD) 59.8</td>
<td>3 3</td>
<td>1</td>
<td>Bexiguer-Benito et al. 2007&lt;sup&gt;45&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rifaximin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long period</td>
<td>4.105</td>
<td>4 (SD) 126</td>
<td>12 12</td>
<td>1</td>
<td>Zeitlinger et al. 2005&lt;sup&gt;41&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.728</td>
<td>4 (SD) 186</td>
<td>1 1</td>
<td>1</td>
<td>Matia et al. 2007&lt;sup&gt;41&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Clarithromycin: 2x 500mg, 4 days
Azithromycin: 1x 500mg, 1x 250mg, 4 days

Tissue concentration - Lung

Clarithromycin: 2x 500mg, 4 days
Azithromycin: 1x 500mg, 1x 250mg, 4 days

Surrogate Blood Concentrations

<table>
<thead>
<tr>
<th>Infection site</th>
<th>Appropriate specimen</th>
<th>Surrogate free blood concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchopneumonia</td>
<td>ELF without inflammatory cells</td>
<td>β-lactamides, ketolides, linezolide, lincosamide and quinolones, streptogramins, monobactam, piperacillin, carbapenem, ceftazidime, etapenem, imipenem, meropenem, ciprofloxacin, levofloxacin</td>
</tr>
<tr>
<td>Otitis media</td>
<td>Middle ear fluid without inflammatory cells</td>
<td>β-lactamides, beta-lactams</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>Sinus fluid without inflammatory cells</td>
<td>Not enough information available</td>
</tr>
<tr>
<td>SST</td>
<td></td>
<td>β-lactamides, quinolones, linezolide</td>
</tr>
</tbody>
</table>


© Ursula Theuretzbacher
**Tissue concentration – Lung – Cardiac Surgery**

- Levofloxacin 500 mg
- Microdialysis in lung
  - Concentration in plasma
  - Concentration in pulmonary interstitial fluid

- PK/PD
  - unbound AUC\textsubscript{tissue}/MIC ratio → 30-40
  - Lung: unbound AUC\textsubscript{tissue}/MIC ratio → 1-4 for pseudomonas

**Interstitial Fluid Concentrations**

- Clarithromycin conc. in plasma and IF of soft tissues after multiple doses of 500 mg b.i.d.

**Tissue concentrations: Resistance**

- Selection of resistance with lower tissue concentrations
  - $C_{\text{max}} \downarrow$, $t_{\text{a}} \uparrow$
Ursula Theuretzbacher
Protein binding/tissue distribution

Simulated concentrations in ELF

Levofloxacin (500 mg OD) exposure in plasma and ELF

B Capitano et al. Chest 2004; 125: 965

Levofloxacin: S. pneumoniae

NR Florea et al. AAC 2004; 48: 1215

Take Home Message

The achievable active drug concentration at the site of infection are high enough to inhibit the bacteria.
Use of in vitro models to study the emergence of resistance

Professor Inga Odenholt
Department of Infectious Diseases
University hospital, Malmö
Sweden

Optimal dosing of antibiotics

Efficacy

Toxicity

Resistance

Costs
Inga Odenholt
Use of in vitro models to study emergence of resistance

How to study the emergence of resistance?

- In vitro with static concentrations (MPC concept)
- In vitro kinetic models with fluctuating concentrations
- In vitro foreign body kinetic model
- In animal models
- In human clinical trials

Different types of in vitro kinetic models

- Diffusion models
  - Two compartment models
- Dilution models,  
  - without filter-membranes  
  - with filter-membranes

The study of enoxacin to determine the Cmax/MIC ratio for bactericidal activity and emergence of resistance

Blaser et al.
AAC :31;1054-60, 1987
Inga Odenholt
Use of in vitro models to study emergence of resistance

Resistence was studied by population analysis and by inoculating the bacteria on antibiotic containing plates 0.33, 1 and 3 mg/L.

In conclusion: A Cmax/MIC > 8 was needed to prevent regrowth.
Inga Odenholt
Use of in vitro models to study emergence of resistance

The efficacy of enoxacin against P. aeruginosa with a MIC of 1 mg/l

Dose ranging and fractionation of ciprofloxacin against P. aeruginosa
Marchbanks et al.
AAC:37:1756-63, 1993

Hollow fiber cartridge

Diluent Reservoir
Central Reservoir
Elimination Reservoir
Inga Odenholt
Use of in vitro models to study emergence of resistance

Killing of P. aeruginosa at three different dosage regimens of ciprofloxacin

Initial MIC = 1 mg/L. Sampled at 24 h.
Inga Odenholt
Use of in vitro models to study emergence of resistance

Dilutions models

II. Dilution model with filter-membrane

Löwdin et al AAC, 40: 2478-2482, 1996

Silicon membrane for sampling

Magnet Filter Broth Pump Waste

Magnetic stirrer
Inga Odenholt
Use of in vitro models to study emergence of resistance

\[ C = C_0 e^{-kt} \]

- \( K \) = the rate of elimination
- \( C_0 \) = the concentration in the vessel at time 0
- \( K = \frac{F}{V} \)
- \( F = V \times 0.693/t_{1/2} \)

Pharmacodynamics of Penicillin G vs S. pneumoniae with different susceptibility for penicillin

Odenholt et al
AAC; 47:518,2003
Inga Odenholt
Use of in vitro models to study emergence of resistance

The concept of Mutant Selective Window

Emergence of resistant S. pneumoniae exposed to moxifloxacin inside and outside the MSW

Figure 4. Effect of AUC_{0-24}/MIC on the increase in frequency of recovery of resistant mutants. Agar plates used to detect mutants contained either 4 × MIC (squares) or 8 × MIC (diamonds). Equation (1): log_{10}N = 1.8 + 3.8 log_{10}(AUC_{0-24}/MIC). (Zinner et al. JAC, 2003:52)
Inga Odenholt
Use of in vitro models to study emergence of resistance

Selection of ciprofloxacin resistance in E. coli
Olofsson et al. JAC;57:1116-21, 2006.

Methods

• 2 quinolone susceptible strains (ATCC and Nu 14) and one gyr A mutant (nu 118)
• The MPC was 16xMIC for the susceptible strains and 4 x MIC for NU 118.

Ciprofloxacin concentration fixed at MPC for different time points.
T>MPC for 18 h was needed to prevent growth of resistant bacteria.
Inga Odenholt
Use of in vitro models to study emergence of resistance

Results

• T>MPC of 18 h was sufficient to prevent selection of resistance
• A Cmax of 64xMIC was needed to prevent selection for the susceptible organisms (8 x MIC for Nu 118).
• The parameter best predictive of emergence of resistance was AUC/MPC
Regrowth was noted at 24 h when the strain was exposed to colistin alone at 1 and 4 x MIC
Even though the strain of S. maltophilia was resistant to rifampin, regrowth was prevented at 24 h.
Minocycline + colistin against imipenem-resistant A. baumannii

Yen Tan et al. JAC July, 2007

- Neither minocycline or colistin alone demonstrated bactericidal activity. Regrowth appeared at 6 hs.
- However, the combination was synergistic and bactericidal.
Inga Odenholt
Use of in vitro models to study emergence of resistance
Animal Models of Infection

William A. Craig, M.D.
University of Wisconsin
USA

Animal Models versus In Vitro Models

- Looks at infections in specific body sites
- Organisms grow more slowly in-vivo than in-vitro
- Some in-vitro phenomena are not observed in vivo (e.g. PAE with penicillins against pneumococci)
- Half-lives of drugs are shorter in small animals than in human-PK simulated in-vitro models
- Total inocula are smaller in animal models which makes study of the emergence of resistance more difficult than in in-vitro models

Animal Models for PK/PD Studies

- Murine thigh-infection model
- Pneumonia models in mice, rats and guinea pigs
- Peritonitis models in mice
- Septicemia models in mice
- Endocarditis in rats and rabbits
- Meningitis in rabbits
William A. Craig
From animal models to the patient

Neutropenic Mouse Thigh-Infection Model

1. Neutropenia induced by 2 injections of cyclophosphamide on days -4 and -1
2. Bacteria injected into thighs on day 0 ($10^6-7$)
3. Treatment (usually given SQ) started 2 hr after infection and continued for 1-5 days
4. Thighs removed, homogenized, serially diluted and plated for CFU determinations

Use of Animal Models in PK/PD Evaluation of Anti-Infective Agents

- Describing the time-course of antimicrobial activity at sites of infection
  - pattern of killing (concentration or time-dependent)
  - presence or absence of persistent effects

Time Course of Antibacterial Activity of Tobramycin and Ticarcillin Against Pseudomonas aeruginosa

Craig and Gudmundsson 1996
Use of Animal and In Vitro Models in PK/PD Evaluation of Anti-Infective Agents

- Identifying PK/PD indices correlating with efficacy (Cmax/MIC, AUC/MIC, Time>MIC)
  - dose-fractionation studies to reduce inter-dependence among the various indices

Relationship Between PK/PD Indices and Efficacy for Ceftazidime against *Klebsiella pneumoniae* in a Murine Pneumonia Model

PK-PD Relationships for Amikacin Against *Pseudomonas aeruginosa* and *Serratia marcescens* in Neutropenic Mice with Renal Impairment
William A. Craig
From animal models to the patient

Use of Animal and In Vitro Models in PK/PD Evaluation of Anti-Infective Agents

• Determining magnitudes of the PK/PD indice required for efficacy (and preventing emergence of resistance) and identifying factors that affect the magnitude
  - cfu changes (short durations of therapy) vs survival (longer courses)
  - predicting efficacy in humans

Mathematical Analysis of Dose-Response Data from Animal Models after 24 Hours of Therapy

Nonlinear regression and Hill equation to estimate Emax (difference from untreated control), P50 (dose giving 50% of Emax) and slope (N) of the dose-response relationship

\[ \Delta CFU = \frac{\text{Emax} \cdot \text{Dose}^N}{\text{Dose}^N + P_{50}^N} \]

PK/PD Magnitude Variables

• Animal
• Antibiotic Class
• Protein binding
• Organism/strain
• Presence of resistance mechanism(s)
• Immune status (normal vs neutropenic)
• Infection site
• Inoculum
• Location of organisms (intracellular vs extracellular)
• Kinetics and shape of concentration-time curve
• Duration of therapy
• Time survival is determined
Magnitude of PK/PD Indices

Does the magnitude of the indice vary with:

1. different dosing regimens? **NO,**
   providing drug elimination is not too fast

2. different drugs within the same class?
   **NO,** if free drug levels are used

3. different organisms, especially resistant strains?
   Less for staphylococci with β-lactams
   and pneumococci with fluoroquinolones;
   no difference with resistant organisms

---

24-Hr AUC/MIC with Total and Free Drug for
the Static Dose of Different Fluoroquinolones
with *S. pneumoniae* ATCC 10813

---

<table>
<thead>
<tr>
<th>Drug</th>
<th>GNB</th>
<th><em>S. pneumoniae</em></th>
<th>S.aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gati</td>
<td>40</td>
<td>80</td>
<td>120</td>
</tr>
<tr>
<td>Sita</td>
<td>80</td>
<td>120</td>
<td>160</td>
</tr>
<tr>
<td>Moxi</td>
<td>120</td>
<td>160</td>
<td>200</td>
</tr>
<tr>
<td>Gemi</td>
<td>160</td>
<td>200</td>
<td>240</td>
</tr>
<tr>
<td>Garen</td>
<td>200</td>
<td>240</td>
<td>280</td>
</tr>
<tr>
<td>Levo</td>
<td>280</td>
<td>280</td>
<td>320</td>
</tr>
<tr>
<td>Cipro</td>
<td>320</td>
<td>320</td>
<td>360</td>
</tr>
</tbody>
</table>

---

Time Above MIC Required for a Static Effect with 4 Cephalosporins

---

<table>
<thead>
<tr>
<th>Drug</th>
<th>GNB (%)</th>
<th><em>S. pneumoniae</em> (%)</th>
<th>S.aureus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftazidime</td>
<td>36 (27-42)</td>
<td>39 (35-42)</td>
<td>22 (19-24)</td>
</tr>
<tr>
<td>Cefpirome</td>
<td>35 (29-40)</td>
<td>37 (33-39)</td>
<td>22 (20-25)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>38 (36-40)</td>
<td>38 (36-40)</td>
<td>24 (20-28)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>38 (34-42)</td>
<td>39 (37-41)</td>
<td>24 (21-27)</td>
</tr>
</tbody>
</table>

---

Craig Diagn Microbiol Infect Dis 22:89, 1995
Magnitude of PK/PD Indice for Free Drug Required for Static Dose of Gemifloxacin Against S. pneumoniae in Thighs of Neutropenic Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC</th>
<th>Mean AUC/MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Susceptible</td>
<td>0.015</td>
<td>28.3</td>
</tr>
<tr>
<td>Gyrase, PAR C or E</td>
<td>0.03-0.5</td>
<td>31.3</td>
</tr>
</tbody>
</table>

Craig & Andes 2005 ECCMID

Magnitude of PK/PD Indices

Does the magnitude of the indice vary with:

4. different sites of infection? Generally NO, but penetration into ELF can vary for different drugs; some drugs more potent against intracellular bacteria

5. different inocula? Minimal for most drugs, but marked with vancomycin against S. aureus

6. presence of WBCs? Less reduction for gram-negative bacilli than for gram-positive cocci

Activity of Vancomycin against S. pneumoniae in Lungs and Thighs of Neutropenic Mice

![Graph showing the logarithm of CFU in lungs and thighs at different doses of therapy.](image)
Impact of Inocula on Static Dose against Staphylococci for Different Antibacterials

<table>
<thead>
<tr>
<th>Drug</th>
<th>Increase in Static Dose for Inocula from $10^5$ to $10^7$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>13- to 72-fold</td>
</tr>
<tr>
<td>Linezolid</td>
<td>2- to 8-fold</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>3- to 15-fold</td>
</tr>
<tr>
<td>Ceftobiprole</td>
<td>2- to 5-fold</td>
</tr>
</tbody>
</table>

Lee et al ICAAC 2007

In Vivo Activity of Moxifloxacin Against S. pneumoniae and K. pneumoniae in Thighs of Normal and Neutropenic Mice

Craig WA, Andes D. Unpublished data.

"PK-PD of antimicrobial therapy: It's not just for mice anymore"

<table>
<thead>
<tr>
<th>Disease</th>
<th>Drug</th>
<th>Human Value</th>
<th>Mice Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HAP</td>
<td>Quinolones</td>
<td>AUC/MIC 62-75</td>
<td>AUC/MIC 70-90</td>
</tr>
<tr>
<td>CAP</td>
<td>Quinolones</td>
<td>AUC/MIC 34</td>
<td>AUC/MIC 25-34</td>
</tr>
<tr>
<td></td>
<td>β-Lactams</td>
<td>T&gt;MIC 40%</td>
<td>T&gt;MIC 30-40%</td>
</tr>
<tr>
<td>SSTI</td>
<td>Linezolid</td>
<td>AUC/MIC 110</td>
<td>AUC/MIC 83</td>
</tr>
<tr>
<td></td>
<td>Tigecycline</td>
<td>AUC/MIC 18</td>
<td>AUC/MIC 15-20</td>
</tr>
</tbody>
</table>

Ambrose et al Clin Infect Dis 2007; 44:79
Conclusions

- Animal model studies have been very useful for determining the in-vivo PK/PD target for efficacy (PK/PD indice and appropriate magnitude required for bacteriologic cure and survival)
- Additional studies are identifying the PK/PD targets that enhance and suppress the emergence of resistance
- Despite the variety of techniques and models, there is marked consistency in the PK/PD data from animal model studies