Pharmacokinetics as applied to \textit{in vitro} and animal models

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Topics

- In vitro pharmacodynamic models
  - Post-antibiotic effects (PAE) – effects that continue after antibiotic removal or when antibiotic concentration is subinhibitory
  - Chemostat models – antimicrobial effect in the presence of a varying drug concentration – drug variation simulates variation over time of drug concentration in blood or site of infection

- Value of animal models
  - Differences between virulence of pathogens in humans vs animals
  - Difference in pharmacokinetics between humans and animals, and how these can be modified in animals

- Extrapolation of results of in vitro PD models and animal models to human infections
Chemostat PK/PD models

- In a one-compartment model the antimicrobial agent is added to a central compartment containing medium and antibiotic.
- Medium is displaced by pumping in fresh medium at a fixed rate.
- This simulates first order pharmacokinetic clearance and half-life and results in an exponential decrease in drug concentration.
- Disadvantage of this system is that bacteria are eliminated from the central compartment as well – can be prevented using membrane filter or compensated for mathematically.

**Figure 1** One-compartment model. The bacterial inoculum and antibiotic are introduced into the central compartment. \( C_A = \) concentration of antibiotic A, \( CC = \) central compartment, \( Cl_A = \) clearance of antibiotic A, \( FM = \) fresh medium, \( P = \) peristaltic pump, \( SP = \) sampling and injection port, \( V_c = \) volume of distribution of antibiotic A, \( SB = \) magnetic stir bar, \( WB = \) water bath (37.5°C), \( WM = \) waste medium.

Ryback et al: Ch 3 in Antimicrobial Pharmacodynamics in Theory and Clinical Practice
Chemostat model – drug concentrations achieved

Mouton, 1995, 97
To simulate QD dosing in humans, an initial bolus of cefdinir was injected into the chemostat at time zero (achieving a peak concentration of 3 mg/liter), whereas for BID dosing, boluses were instilled at time zero and at h 12 (achieving a peak concentration of 1.6 mg/liter).

Targeted concentrations were derived from reported data on human cefdinir pharmacokinetics. Although 60 to 70% of cefdinir is protein bound, we chose to simulate total serum concentrations in the model, as the significance of protein-binding values below 85 to 90% and the effect on tissue penetration and clinical impact are unclear.

By pumping of antibiotic-free medium into the system at a rate of 1.7 ml/min with a peristaltic pump, an equal volume of antibiotic-containing medium was displaced.

This resulted in the simulation of a monoexponential pharmacokinetic process that was adjusted to attain the desired cefdinir half-life of 2 h.

Ross et al. AAC 2001, 45:2936-8
Comparison of Once-Daily versus Twice-Daily Administration of Cefdinir – concentrations achieved in chemostat

Standard dosing – 600 mg/d or 14 mg/kg/d

Prediction:
For organisms with MICS of 0.25 mg/L, cefdinir should work BID but not QD

Adapted from Ross et al. AAC 2001, 45:2936-8
Activity of cefdinir in chemostat at dose of 600 mg QD (●) and 300 mg BID (●)
Growth controls are represented by the symbol ▲

HF 1746 (beta-lactamase-producing *H. influenzae*)
Cefdinir MIC 0.25 mg/L

HF 2019 (beta-lactamase negative *H. influenzae*)
Cefdinir MIC 0.25 mg/L

SP30 (penicillin-sensitive *S. pneumoniae*) penicillin
MIC <0.06 mg/liter (C).
Cefdinir MIC 0.25 mg/L

S-53 (penicillin-intermediate *S. pneumoniae*) penicillin
MIC 0.25 mg/liter (D).
Cefdinir MIC 0.5 mg/L
Comparison of Once-Daily versus Twice-Daily Administration of Cefdinir – concentrations achieved in chemostat

Standard dosing – 600 mg/d or 14 mg/kg/d

Prediction:
For organisms with MICS of 0.25 mg/L, cefdinir should work BID but not QID

Findings:
Cefdinir was bactericidal for 3 of 4 isolates BID and for 0/4 isolates QD

Adapted from Ross et al. AAC 2001, 45:2936-8
Alexander Project USA 2000: S. pneumoniae (n=1362), H. influenzae (n=634), M. catarrhalis 2000 (n=206)
Cefdinir (Omnicef)
14 mg/kg/day (qd or bid)
300 mg bid or 600 mg qd

Conc. (ug/ml)

0 0.1 1 10 64

0 12 24 hr

PenR: MIC$_{90}$= 8 ug/ml
PenI: MIC$_{90}$= 2 ug/ml
PK/PD bkpt. 0.5 ug/ml
H infl and M cat: MIC$_{90}$=0.5 ug/ml
PenS: MIC$_{90}$= 0.12 ug/ml

Peak 3.2-4.4 ug/ml QD

Adapted from Craig et al. Pediatr Infect Dis J 1996;15:944–948
Animal PK/PD models
<table>
<thead>
<tr>
<th>Drug</th>
<th>Half-life in Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>Mice: 5, Humans: 30</td>
</tr>
<tr>
<td>Imipenem</td>
<td>Mice: 8, Humans: 60</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>Mice: 15, Humans: 108</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Mice: 18, Humans: 150</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Mice: 32, Humans: 240</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Mice: 35, Humans: 180</td>
</tr>
<tr>
<td>Minocycline</td>
<td>Mice: 120, Humans: 1080</td>
</tr>
</tbody>
</table>
## Pharmacokinetics of Ciprofloxacin in Animals

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Cmax</th>
<th>T1/2</th>
<th>AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>5</td>
<td>1.5</td>
<td>0.52</td>
<td>1.8</td>
</tr>
<tr>
<td>Rat</td>
<td>5</td>
<td>1.2</td>
<td>1.2</td>
<td>2.2</td>
</tr>
<tr>
<td>Dog</td>
<td>5</td>
<td>1.5</td>
<td>3.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Man</td>
<td>7</td>
<td>2.7</td>
<td>4.4</td>
<td>11</td>
</tr>
</tbody>
</table>

WA Craig, 2002
Ways to Reduce Clearance and Prolong Half-life

• Probenecid - reduces tubular secretion of beta-lactam antibiotics

• Renal impairment - can be induced in mice and rats by administering uranyl nitrate. Slows elimination of renally excreted drugs

• Increase protein binding of drugs eliminated primarily by glomerular filtration

WA Craig, 2002
## Serum Protein Binding of Antimicrobials in Animals

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mice</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefonacid</td>
<td>78%</td>
<td>97%</td>
</tr>
<tr>
<td>Ceftiazone</td>
<td>76%</td>
<td>95%</td>
</tr>
<tr>
<td>Cefditoren</td>
<td>87%</td>
<td>88%</td>
</tr>
<tr>
<td>Telithromycin</td>
<td>88%</td>
<td>60%</td>
</tr>
</tbody>
</table>

WA Craig, 2002
Other Factors to Consider with Pharmacokinetics in Animal Models

- Infection can significantly alter pharmacokinetics in animals. Usually get higher concentrations and larger AUCs.
- Penetration of antimicrobials into fibrin can vary remarkably.
- Drug concentrations is extracellular fluid of tissues related to ratio of the surface area for diffusion and the volume of fluid.
- Good correlation in interstitial fluids with those in serum. Lower peak levels and higher trough levels in fluid collections.

WA Craig, 2002
Correlation of Pharmacodynamic Parameters with Efficacy

- Use neutropenic murine thigh-and lung-infection models
- Evaluate 20-30 different dosing regimens (5 different total doses given at 4-6 different dosing intervals)
- Measure efficacy from change in $\log_{10}$ CFU per thigh or lung at the end of 24 hours of therapy
- Correlate efficacy with various pharmacodynamic parameters (Time above MIC, peak/MIC, 24-Hr AUC/MIC)
Animal Models for Susceptibility Breakpoint Determinations

- Simulate human pharmacokinetics in animals (induce renal impairment with uranyl nitrate)
- Infect groups of animals with organisms with varying MICs
- Treat the animals for at least 24 hours with dosage regimen used to treat human infections
- Find the MIC value that separates bacterial killing from bacterial growth
PK/PD Parameters

- Is the magnitude of the parameter required for efficacy the same in different animal species?
  
  YES

- Does the magnitude of the parameter vary with:
  1. the dosing regimen? NO
  2. different drugs within the same class? NO
  3. different organisms? Minimal
  4. different sites of infection (e.g. blood, lung, peritoneum, soft tissue)? NO
Conclusions

- Serum clearance of most antimicrobials is faster in animals than in man
- Serum protein binding is usually less in animals than in man
- The higher doses required for studies in animal models may result in non-linear kinetics
- Sensitive drug assays should be used to identify deep tissue compartments that could prolong activity against very susceptible organisms

WA Craig, 2002