Pharmacodynamics as applied to animal models

- how to uncover the PK/PD properties of antibiotics
- how to classify the main antibiotics

This part uses material from presentations of W.A. Craig (Madison, WI.) made at the 2000 and 2002 ISAP Educational Workshops
Pharmacodynamics

Something you can relate to dosing and that you can measure

- Cmax / MIC
- 24h AUC / MIC
- Time above MIC

Dosage

Therapeutic effects

Toxic effects
### A reminder ...

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Influenced by</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;:</td>
<td>+ dose * – clearance – Vd</td>
</tr>
<tr>
<td>Half-life:</td>
<td>– clearance + Vd</td>
</tr>
<tr>
<td>AUC:</td>
<td>+ dose ** – clearance</td>
</tr>
</tbody>
</table>

* + directly proportional
  - – indirectly proportional
  * * unit dosis
  ** ** total dose for the period considered (usually 24h)

**this is what you do ...**
### Parameter influenced by

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- directly proportional
- indirectly proportional

* unit dose

** total dose for the period considered (usually 24h)

---

**those are drug properties**

**A reminder ...**
Why are animal models essentials

• Explore the conditions of success AND of failures

• Dissociate the pharmacokinetic co-variables
Why are animal models essentials

• Explore the conditions of success AND of failures

This is fairly obvious but often neglected by

• teachers (why bother ? …)
• regulators (viz. the design of clinical trials !!)
• and clinicians (who wants to fail too often ? … and to report it ????)

Why are animal models essentials?

- Explore the conditions of success AND failures.
- Dissociate the pharmacokinetic co-variables.

Ignoring this is one of the biggest mistakes made by:

- Regulators
- Clinicians who draw conclusions from clinical trials only!!
Dissociating the pharmacokinetic co-variables ...

Adapted from F. O. Ajayi, ISAP-FDA Workshop, 1999
C_{\text{max}} \text{ and } "\text{Time above a given value}" \text{ are co-variables because they are both directly related to the unit dose}

quite different \( C_{\text{max}} \text{ and } T > \text{MIC} \ldots !!! \)
24h AUC is only dependent of the total daily dose...

- Dose = 3 in one administration
- Different $C_{\text{max}}$ and $T > \text{MIC}$ but same 24h AUC!!!
- Dose = 3 DIVIDED in 3 administrations
Looking for PK/PD parameters predictive of $\beta$-lactam activity (cefuroxime) in a model of murine pneumonia (*Klebsiella pneumoniae*) in neutropenic mice (after W.A. Craig *)

![Graph showing log10 CFU per Lung at 24 Hours vs. Peak/MIC Ratio](image)

* 2d ISAP Educational Workshop, Stockholm, Sweden, 2000
Looking for PK/PD parameters predictive of β-lactam activity (cefuroxime) in a model of murine pneumonia (*Klebsiella pneumoniae*) in neutropenic mice (after W.A. Craig *)

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Looking for PK/PD parameters predictive of β-lactame activity (cefuroxime) in a model of murine pneumonia (*Klebsiella pneumoniae*) in neutropenic mice (after W.A. Craig *)
Available antibiotic can be divided in 3 groups

- time-dependent (T > MIC)
- AUC / MIC - dependent
- both AUC / MIC AND peak / MIC - dependent
1. **Antibiotics with time-dependent effects and no or little persistent effects**

<table>
<thead>
<tr>
<th>AB</th>
<th>PK/PD parameter</th>
<th>Goal</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lactams</td>
<td>Time above MIC</td>
<td>Maximize the exposure time</td>
</tr>
<tr>
<td>clindamycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>oxazolidinones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>flucytosine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*2d ISAP Educational Workshop, Stockholm, Sweden, 2000; revised accord. to Craig, et al. ICAAC 2002*
2. Antibiotics with **time-dependent effects**, with little or no influence of the concentration **BUT** with persistent effects

<table>
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<tr>
<th>AB</th>
<th>PK/PD parameter</th>
<th>Goal</th>
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<tbody>
<tr>
<td>glycopeptides</td>
<td></td>
<td>Optimize the quantity of AB administered</td>
</tr>
<tr>
<td>tétracyclines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>macrolides</td>
<td>24h AUC / MIC</td>
<td></td>
</tr>
<tr>
<td>streptogramines</td>
<td>ratio</td>
<td></td>
</tr>
<tr>
<td>fluconazole</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* 2d ISAP Educational Workshop, Stockholm, Sweden, 2000; revised accord. to Craig et al., ICAAC 2002
Antibiotics Group # 2
(after W.A. Craig, 2000; revised 2002)

3. Antibiotics with concentration-dependent activity and with persistent effects (PAE)

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<thead>
<tr>
<th>AB</th>
<th>PK/PD parameter</th>
<th>Goal</th>
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<tr>
<td>aminoglycosides</td>
<td>$C_{\text{max}} / \text{MIC}$ and</td>
<td>Optimize both the peak and the quantity of drug</td>
</tr>
<tr>
<td>fluoroquinolones</td>
<td>24h AUC / MIC ratios</td>
<td></td>
</tr>
<tr>
<td>daptomycin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ketolides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>amphotericin</td>
<td></td>
<td></td>
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Are PK/PD Parameters universals?

- Is the magnitude of the parameter required for efficacy the same in different animal species?
- Does the magnitude of the parameter vary markedly with:
  1. the dosing regimen?
  2. different drugs within the same class?
  3. different organisms?
  4. different sites of infection (e.g. blood, lung, peritoneum, soft tissue)?

From WA. Craig: 7th ISAP Educational Workshop, San Diego, CA, 2002
The 'saga' of the “Static Dose” ...

- Determine cfu/thing in untreated controls and mice treated with 4-5 different total doses
- Use nonlinear regression and modified Hill equation to estimate $E_{\text{max}}$ (difference from untreated control), $P_{50}$ (dose giving 50% of $E_{\text{max}}$) and slope (N) of dose-response relationship

$$\Delta \text{CFU} = (E_{\text{max}}) \frac{\text{Dose}^N}{\text{Dose}^N + P_{50}^N}$$

- Calculate the “static dose”

From WA. Craig: 7th ISAP Educational Workshop, San Diego, CA, 2002
Relationship Between 6-Hour Dose and Number of Klebsiella pneumoniae in Thighs of Neutropenic Mice

- **Dose (mg/kg/6 hrs)**: 10, 30, 100, 300
- **Log10 CFU per Thigh at 24 Hrs**: 5, 6, 7, 8, 9

From WA. Craig: 7th ISAP Educational Workshop, San Diego, CA, 2002
### Time Above MIC Required for a Static Effect After 24-hours of Therapy with Four Cephalosporins

**Time Above MIC (Percent of Dosing Interval)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Enterobacteriaceae</th>
<th>S. pneumoniae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone (Total)</td>
<td>72 (66-79)</td>
<td>74 (69-78)</td>
</tr>
<tr>
<td>Ceftriaxone (Free)</td>
<td>38 (34-42)</td>
<td>39 (37-41)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>38 (36-40)</td>
<td>38 (36-40)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>36 (27-42)</td>
<td>39 (35-42)</td>
</tr>
<tr>
<td>Cefpirome</td>
<td>35 (29-40)</td>
<td>37 (33-39)</td>
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Data is mean (95 % CI)

*From WA. Craig: 7th ISAP Educational Workshop, San Diego, CA, 2002*
Relationship Between T>MIC and Efficacy for Amoxicillin against *Streptococcus pneumoniae* in Rat Pneumonia and Murine Thigh-Infection Models

![Graph showing relationship between T>MIC and efficacy for Amoxicillin against Streptococcus pneumoniae in rat pneumonia and murine thigh-infection models.](image)

From WA. Craig: 7th ISAP Educational Workshop, San Diego, CA, 2002
Why have we sometimes hesitated?

• Not enough experimental data
• Not enough separation of the covariables
• Specificities of the animal models
More experimental data with ertapenem ...

Activity against *S. aureus* in a Murine Thigh Infection Model

- **Peak/MIC**: $R^2=24\%$
- **24 Hour AUC/MIC**: $R^2=46\%$
- **Time Above MIC (%)**: $R^2=81\%$
More experimental data with ertapenem ...

T > MIC ~ 6 % : bacteriostatic

T > MIC ~ 60 % : max. bactericidal

*S. pneumoniae* in a murine neutropenic thigh infection model

Xuan *et al*, AAC 1998, 46: 2990-2995
More experimental data with penicillins, cephalosporins and carbapenems ...

Fig. 7. Relationship between the change in log_{10} CFU per thig or lung for various pathogens following 24 h of therapy with different doses of penicillins (△), cephalosporins (○), and carbapenems (□).

- different pathogens
- same shape of dose response
- diff. In T > MIC for a static effect (penicill. > carbap.)
- diff $E_{\text{max}}$ (penicill. < carbap.)

Why have we sometimes hesitated?

• Not enough experimental data

• Not enough separation of the covariables

• Specificities of the animal models
Lack of separation of the PK co-variables ...

• As already stated, this is a common problem in all clinical trials
  – which are not specifically designed to test for one PK variable independetly from the other
  – have not enough PK-related failures to unambiguously assess success or failures to one PK variable...
The saga of the AUC / MIC vs $C_{\text{max}} / \text{MIC}$ ratio for fluoroquinolones ...
24h-AUC/MIC : actual data ...

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<td>&lt;0.125</td>
<td>28</td>
<td>82</td>
<td>79</td>
</tr>
<tr>
<td>0.125-0.25</td>
<td>13</td>
<td>75</td>
<td>69</td>
</tr>
<tr>
<td>0.5</td>
<td>14</td>
<td>54</td>
<td>79</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>33</td>
<td>44</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0</td>
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Forrest et al., AAC, 1993
## 24h-AUC/MIC: actual data ...

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- **success**
- **failure**

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<td>0-125</td>
<td>19</td>
<td>32</td>
<td>42</td>
</tr>
<tr>
<td>125-250</td>
<td>16</td>
<td>81</td>
<td>88</td>
</tr>
<tr>
<td>250-1000</td>
<td>14</td>
<td>79</td>
<td>71</td>
</tr>
<tr>
<td>1000-5541</td>
<td>15</td>
<td>87</td>
<td>80</td>
</tr>
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- **failure**
- **success**

Forrest et al., AAC, 1993
Is 24h AUC/MIC the only parameter for fluoroquinolones...

- All patients in the Forrest et al's study received ciprofloxacin on a bid or tid schedule

  ➔ the study was, therefore, not powerful enough to assess $C_{\text{max}}$ independently from the total daily dose

  ➔ since success was linked mostly to a low MIC, and the only independent variable was the total daily dose, only 24h AUC / MIC could emerge as a predictive PK/PD parameter
Fq PK/PD: a study demonstrating the role of peak to MIC ratio in the clinic (1/2)


OBJECTIVE:
To prospectively quantitate the relationship between plasma levels of levofloxacin and successful clinical and/or microbiological outcomes and occurrence of adverse events in infected patients.

PATIENTS: 313 with clinical signs and symptoms of bacterial infections of the respiratory tract, skin, or urinary tract.

MAIN OUTCOME MEASURES: Clinical response and microbiological eradication of pathogenic organisms.
Fq PK/PD: a study demonstrating the role of peak to MIC ratio in the clinic (1/2)


RESULTS (as presented by the authors):

- 134 / 313 had both PK and MIC
- **clinical AND bacterial outcomes were related to peak/MIC** (logistic regression; p < 0.001)
- **results were favourable if peak / MIC > 12.2**
Is $C_{\text{max}}$ / MIC ratio truly the only parameter for predicting fluoroquinolones efficacy?

- Most patients in the Preston et al.’s study received levofloxacin at a fairly large dose (for the MIC of *S. pneumoniae* at that time...; 500 mg) and with only one administration scheme (once-a-day)...
- This very design caused most patients to have a $C_{\text{max}}$/MIC ratio > 10...
- There were very few failures...
Is Cmax / MIC ratio truly the only parameter for predicting fluoroquinolones efficacy?

- the data, actually, showed that both $C_{\text{max}}$/MIC and 24h AUC/MIC were linked to clinical success

"Peak/MIC ratio, AUC/MIC ratio, and Time>MIC were virtually indistinguishable in their ability to alter the probability of a successful outcome (Table 2). This is understandable as, when examined, Peak/MIC and AUC/MIC ratios were highly correlated, with an r value of 0.942 (Spearman rank correlation)."

The authors decided to select $C_{\text{max}}$/MIC as the critical parameter… but could have use 24h AUC/MIC even time > MIC since these three parameters are true co-variables when only one schedule is used…
Fluoroquinolones: the role of the peak for efficacy: demonstrations in animal models

- peak/MIC ratio becomes predictive at ratios > 10
  AUC / MIC is more predictive at peak/MIC < 10
  no influence of time > MIC when tested specifically

- Dose-dependency ( = AUC) in vivo
  Dalhoff, J Antimicrob Chemother 1999 May;43 Suppl B:51-9)

- Penetration in inflammatory fluids and interstitial fluids, and rapid equilibration between compartments ( = AUC)
  • Wise et al., Antimicrob Agents Chemother 1999 Jun;43(6):1508-10)
  • Muller et al., Antimicrob Agents Chemother 1999 Oct;43(10):2345-9)

FQ are dependent of both the peak /MIC and the AUC/ MIC ratios …
Why have we sometimes hesitated?

- Not enough experimental data
- Not enough separation of the covariables
- Specificities of the animal models
  - non-neutropenic vs. neutropenic animals
  - differences in PK parameters between rodents and man
  - timing of the infection / treatment schedule
Difficulties of animal models

- Most models need to use neutropenic animals as many pathogens for man (incl. S. pneumoniae and H. influenzae) are relatively avirulent to small rodents.
- Small rodents need also to be made renally-impaired in order to obtain drug pharmacokinetic parameters similar to those observed in humans.
- Most studies start treatment quite soon after the infection, which is not what one does in humans ...
Why do we (often) use neutropenic animals?

In order to be able to **kill** them **rapidly** …

- if animals are not rapidly killed, you start having problems …
  - lack of clear-cut endpoint
  - building up of humoral and tissular means of defense …
  - cost, nursing, etc…

- Many interesting pathogens for man (incl. *S. pneumoniae* and *H. influenzae*) are relatively innocuous to small rodents and animals eventually never die ..
But using neutropenic animals may modify the dose / effect response ...

Relationship Between 24 Hr AUC/MIC and Mortality for Fluoroquinolones against *S. pneumoniae* in Immunocompetent vs. Immunocompromised animal Models

Adapted from W.A. Craig : 7th ISAP Educational Workshop, San Diego, CA, 2002
But beyond the difficulties, there is now a consensus for ...

- C_{max} / MIC
- AUC / MIC
- time > MIC

to be predictive of the activity of

- fluoroquinolones
- aminoglycosides
- macrolides
- tetracyclines
- β-lactams
- oxazolidinones
- vancomycin

The difficulty remains to determine which is the minimal acceptable value for these parameters.
So, please, remember pharmacodynamics...

- Emax
- steepness
- point of initial response
And now, decide ...

- Emax
- steepness
- point of initial response

Which effect would you like to obtain?
Dosage

The black box is ... 

- $C_{\text{max}} / \text{MIC}$
- 24h $\text{AUC} / \text{MIC}$
- Time above MIC

Therapeutic effects

Toxic effects

This is where we are now ...