PK-PD analysis and modelling
Why modelling? (*)

• to move from mere description to underlying phenomena…
  – nature can often be better explained in terms of equations than mere description
  – this has been essential in physics (think about gravity law, radioactive decay, study of electromagnetic field and optics, … up to the equivalence of mass and energy…)

• to allow predictions over and beyond what is immediately accessible by the experience…

• to generate rules that can be applied widely…

* CAUTION: modelling in UK English but modeling in US English …
In vitro studies
Response to an antimicrobial

an example with ceftobiprole and S. aureus (one strain)

![Graph showing the effect of different concentrations of ceftobiprole over time on the log10 CFU of S. aureus. The graph plots concentration (multiples of MIC) on the y-axis and time (h) on the x-axis, with the effect over time demonstrated by the lines representing different concentrations.](image-url)
Response to an antimicrobial

an example with ceftobiprole and S. aureus (2 strains)

Effect-over-time (2 strains)

concentration (multiples of MIC)

- 0.01
- 0.2
- 1
- 2
- 20
- 50
- 200

This where the phenomenon happens
Response to an antimicrobial: the model

an example with ceftobiprole and *S. aureus* (2 strains)
Response to an antimicrobial: the model
an example with ceftobiprole and S. aureus (multiple strains)
**Analyses**

Sigmoidal dose-response:  
\[ Y = \text{Bottom} + \frac{\text{Top-Bottom}}{1 + \left(10^{\text{LogEC}_{50} - X}\right)^{\text{HillSlope}}} \]

also called "4-parameters logistic equation", i.e.
- bottom (\(E_{min}\))
- Top (\(E_{max}\))
- \(EC_{50}\)
- Hill slope

**Equation for Prism**

Equation:Sigmoidal dose-response  
\[ Y=\text{Bottom} + \frac{\text{Top-Bottom}}{1+10^{(\text{LogEC}_{50} - X)}} \]

;\(X\) is the logarithm of concentration. \(Y\) is the response; \(Y\) starts at Bottom and goes to Top with a sigmoid shape
## Analyses

**Equation:**

\[
Y = \text{Bottom} + \frac{\text{Top} - \text{Bottom}}{1 + 10^{\left(\text{LogEC}50 - X\right)}}
\]

; \(X\) is the logarithm of concentration.

\(Y\) starts at \(\text{Bottom}\) and goes to \(\text{Top}\) with a sigmoid shape.

<table>
<thead>
<tr>
<th></th>
<th>MSSA</th>
<th>HA-MRSA</th>
<th>CA-MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Y</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Best-fit values</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOTTOM</td>
<td>-1.042</td>
<td>-0.9878</td>
<td>-1.006</td>
</tr>
<tr>
<td>TOP</td>
<td>3.063</td>
<td>2.596</td>
<td>2.741</td>
</tr>
<tr>
<td>LOGEC50</td>
<td>-0.6931</td>
<td>-0.5582</td>
<td>-0.4805</td>
</tr>
<tr>
<td>EC50</td>
<td>0.2027</td>
<td>0.2766</td>
<td>0.3307</td>
</tr>
<tr>
<td><strong>Std. Error</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOTTOM</td>
<td>0.1109</td>
<td>0.1087</td>
<td>0.1346</td>
</tr>
<tr>
<td>TOP</td>
<td>0.2756</td>
<td>0.2025</td>
<td>0.2325</td>
</tr>
<tr>
<td>LOGEC50</td>
<td>0.1134</td>
<td>0.1069</td>
<td>0.1148</td>
</tr>
<tr>
<td><strong>95% Confidence Intervals</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOTTOM</td>
<td>-1.273 to -0.8117</td>
<td>-1.207 to -0.7684</td>
<td>-1.278 to -0.7347</td>
</tr>
<tr>
<td>TOP</td>
<td>2.490 to 3.637</td>
<td>2.187 to 3.005</td>
<td>2.271 to 3.210</td>
</tr>
<tr>
<td>LOGEC50</td>
<td>-0.9291 to -0.4572</td>
<td>-0.7739 to -0.3425</td>
<td>-0.7122 to -0.2489</td>
</tr>
<tr>
<td>EC50</td>
<td>0.1177 to 0.3490</td>
<td>0.1683 to 0.4544</td>
<td>0.1940 to 0.5637</td>
</tr>
</tbody>
</table>

### Goodness of Fit

- **Degrees of Freedom:**
  - MSSA: 21
  - HA-MRSA: 43
  - CA-MRSA: 43

- **\(R^2\):**
  - MSSA: 0.9296
  - HA-MRSA: 0.8795
  - CA-MRSA: 0.8499

- **Absolute Sum of Squares:**
  - MSSA: 3.232
  - HA-MRSA: 10.99
  - CA-MRSA: 15.35

- **Sy.x:**
  - MSSA: 0.3923
  - HA-MRSA: 0.5056
  - CA-MRSA: 0.5974

### Data

- **Number of X values:**
  - MSSA: 32
  - HA-MRSA: 98
  - CA-MRSA: 164

- **Number of Y replicates:**
  - MSSA: 1
  - HA-MRSA: 1
  - CA-MRSA: 1

- **Total number of values:**
  - MSSA: 24
  - HA-MRSA: 46
  - CA-MRSA: 46

- **Number of missing values:**
  - MSSA: 8
  - HA-MRSA: 52
  - CA-MRSA: 118
Type of functions

Fitting Models to Biological Data using Linear and Nonlinear Regression

A practical guide to curve fitting

Harvey Motulsky & Arthur Christopoulos

Do not forget to use the appropriate axes!

how would you fit those data
Type of functions

This would be a good model
Run statistics
Run tests
Two examples
Impact of MIC on the response of intracellular bacteria to moxifloxacin

The extent and rate of killing of *P. aeruginosa* by colistin were markedly decreased at high CFUo compared to those at low CFUo. Bulita et al. Antimicrob. Agents Chemother. (2010) 54:2051-2062
In search of models with Prism
In search of models (including your own)
In search of models (including your own)

\[ Y = (\text{Bottom1} + (\text{Top1} - \text{Bottom1})/(1 + 10^{(\text{LogEC501} - X)\text{HillSlope1}})) + (\text{Bottom2} + (\text{Top2} - \text{Bottom2})/(1 + 10^{(\text{LogEC502} - X)\text{HillSlope2}})) + 0.5 \]

\( X \) is the logarithm of concentration. \( Y \) is the response.

\( Y \) starts at Bottom and goes to Top with a sigmoid shape.

This is identical to the "four parameter logistic equation"
And here you are …

**Azithromycin**
- Cmax: 2 mg/L
- MIC: 0.01-0.03 mg/L
- Cs: 3 mg/L

**Clarithromycin**
- Cmax: 1 mg/L
- MIC: 0.008 mg/L
- Cs: 0.06 mg/L

**Telithromycin**
- Cmax: 1 mg/L
- MIC: 0.008 mg/L
- Cs: 0.04 mg/L

**Ciprofloxacin**
- Cmax: 4 mg/L
- MIC: 0.01 mg/L
- Cs: 0.002 mg/L

**Moxifloxacin**
- Cmax: 4 mg/L
- MIC: 0.01 mg/L
- Cs: 0.001 mg/L

**Finafloxacin**
- Cmax: 12 mg/L
- MIC: 0.01 mg/L
- Cs: 0.05 mg/L
In vivo pharmacokinetics
What is PK analysis and modeling?

• **Noncompartmental analysis**
  Noncompartmental PK analysis examines total drug exposure and looks for function(s) fitting the change of concentration over time without reference to where the drug may distribute.

  Analysis is simple and does not imply anything concerning the actual fate of the drug.

  The results are purely descriptive and non-predictive unless the function selected is linked to physical phenomena (e.g. 1\textsuperscript{st} order kinetics).
What is PK analysis and modeling?

• **Compartmental analysis**
  Describes and predicts the concentration-time curve based on the movements of the drug between compartments (kinetic or physiological model)

Once the model is identified, it can be used to predict the concentration at any time.

The model may be (very) difficult to develop

The simplest PK compartmental model is the one-compartmental PK model with IV bolus administration and first-order elimination.

The most complex PK models rely on the use of physiological information to ease development and validation.
What is PK analysis and modeling?

- **Compartmental analysis**

  The simplest PK compartmental model is the one-compartmental PK model with IV bolus administration and first-order kinetic elimination.

  This can be developed with simple software accessible to lay users such as Prism (with some sophistication sometimes).

More complex PK models rely on the use of physiological information to ease development and validation.

This requires "high capacity" software that is often impossible to use without serious introduction.
Simple compartmental models

\[ \frac{dC_{\text{plasma}}}{dt} = -\frac{\text{Clearance}}{V_D} \cdot C_{\text{plasma}} \]

\[ \frac{dY}{dt} = -\frac{\text{Clearance}}{V_D} \cdot Y \]
Integrating a differential equation

Using calculus, you (or someone you delegate this job to) can integrate the equation to form a standard model that defines $Y$ as a function of $t$:

$$Y_t = Y_0 \cdot e^{-\frac{\text{Clearance}}{V_D} \cdot t} = Y_0 \cdot \exp\left(-\text{Clearance} \cdot \frac{t}{V_D}\right)$$

At time zero, the concentration of drug ($Y_0$) equals the dose you injected (D in mg) divided by the volume of distribution ($V_o$ in mL). So the equation can be rewritten like this:

$$Y_t = \frac{D}{V_D} \cdot e^{-\frac{C}{V_D} \cdot t}$$
From model to data and finding "best parameters" with a computer (curve fitting)

• choose (or enter) your equation
• enter your data
• enter initial parameter values
  (best estimate; optional but useful)
• the computer will then
  – compare equation-based curve to actual data
  – modify parameters by successive iterations
  until a "best" fit is obtained …
  – the limit is the number of iterations
From data to model with a computer (no calculus)
Example of monocapartmental analysis … (*)

Exponential-decay (1 compartment)

**Theoretical curve**

\[ C_0 = 142 \text{ mg/L} - 2 \text{ g} - 70 \text{ kg} - V_d = 0.2 \text{ L/kg} \]
\[ k_e = 0.185 \text{ (t1/2 = 2h)} \]
\[ \text{plateau} = 0 \]

**Equation:** 
\[ C = C_0 \cdot e^{-kt} \]

*This analysis and the following ones concern ceftazidime IV*
Fitting to ideal population data (*)

Ceftazidime: ideal patients

* data from a few volunteers
Ideal population: tests for 95 % CI

Ceftazidime: ideal patients
Ideal population: residuals

why are they much larger here?
Real population (*)

ceftazidime: real population

n = 27 patients
$C_0 = 98$ mg/L (2 g - 71.5 ± 10.9 kg)
$ke = 0.1865$ ($t_{1/2} = 3.716$ h)
plateau = - 8.2

* data from several patients
Real population: 95 % CI

ceftazidime: real population
Real population: residuals

![Real population: residuals graph]

Ideal population: Residuals

![Ideal population: Residuals graph]
More complex models: accumulation / decay

Bateman function
(applied to ceftazidime)

theoretical curve

D = 2 g / 70 kg (28.57 mg/kg) - V_d = 0.2 L/kg
ka = 8
ke = 0.3465 (t_1/2 = 2h)
plateau = 0

equation: C = D/Vd x ka/(ka-ke) [e^{-ket} – e^{-kat}]

<table>
<thead>
<tr>
<th>time (h)</th>
<th>0.0</th>
<th>2.5</th>
<th>5.0</th>
<th>7.5</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>concentration (mg/L)</td>
<td>150</td>
<td>125</td>
<td>100</td>
<td>75</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>20</td>
<td>15</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>
In search of more complex models with Prism
Accumulation / decay with Prism … (*)

Ceftazidime with Bateman function

\[ C = \frac{D}{Vd} \times \frac{ka}{(ka-ke)} \left[ e^{-ke} - e^{-kat} \right] \]

real data

\[ R^2 = 0.57 \]
Examples d'analyse monocompartimentale … (*)

Ceftazidime with Bateman

\[
equation: C = \frac{D}{V_d} \times \frac{ka}{(ka-ke)} \left[ e^{-ket} - e^{-kat} \right]
\]
When the data become really too complex…
The Mixed non-lin approaches

• A **mixed model** is a statistical model containing both fixed effects and random effects.

• These models are useful in a wide variety of disciplines in the physical, biological and social sciences.

• They are particularly useful in settings where repeated measurements are made on the same statistical units (longitudinal study), or where measurements are made on clusters of related statistical units.

• Because of their advantage in dealing with missing values, mixed effects models are often preferred over more traditional approaches such as repeated measures ANOVA.
The Mixed non-lin approaches

Different softwares, but all working by numerical integration based on pre-defined models

Noncompartmental

- Freeware: bear and PK for R
- Commercial: MLAB, EquivTest, Kinetica, MATLAB/SimBiology, Phoenix/WinNonlin, PK Solutions, RapidNCA

Compartment based

- Freeware: ADAPT, Boomer (GUI), SBPKPD.org (Systems Biology Driven Pharmacokinetics and Pharmacodynamics), WinSAAM, PKfit for R, PharmaCalc and PharmaCalcCL, Java applications.
- Commercial: Imalytics, Kinetica, MATLAB/SimBiology, Phoenix/WinNonlin, PK Solutions, PottersWheel, ProcessDB, SAAM II

Physiologically based

- Freeware: MCSim
- Commercial: acsIX, Cloe PK, GastroPlus, MATLAB/SimBiology, PK-Sim, ProcessDB, Simcyp, Entelos PhysioLab, Phoenix/WinNonlin, ADME Workbench

Population PK

- Freeware: WinBUGS, ADAPT, S-ADAPT / SADAPT-TRAN, Boomer, PKBugs, Pmetrics for R
- Commercial: Kinetica, MATLAB/SimBiology, Monolix, NONMEM, Phoenix/NLME, PopKinetics for SAAM II, USC*PACK, Navigator Workbench

Simulation

All model based software above.

- Freeware: COPASI, Berkeley Madonna, MEGen

Educational centres

Global centres with the highest profiles for providing in-depth training include the Universities of Buffalo, Florida, Gothenburg, Leiden, Otago, San Francisco, Beijing, Tokyo, Uppsala, Washington, Manchester, Monash University, and University of Sheffield. [1]
Exemples avec la témocilline
**Temocillin in a nutshell**

- Temocillin or \(6\alpha\text{-methoxy}\text{-ticarcillin}\)
- Registered in 1984 for the first time (Beecham)
- Maintained on the market since 1998 (Eumedica)
  - BE, LU, UK and now FR

- Narrow-spectrum antibiotic (Gram-negative oriented)
  - Enterobacteriaceae
  - *B. cepacia*
  - *Neisseria, Haemophilus, Pasteurella, Legionella*
  - Inactive against most strains of *P. aeruginosa, Acinetobacter, Stenotrophomonas*,
  - no useful activity against Gram-positive and anaerobes

- Stable to most \(\beta\)-lactamases
  - Class A (including ESBL, KPC), class C (AmpC), class D (OXA-1)
  - Hydrolysed by OXA-48-like (class D) and class B enzymes (metalloenzymes)
But what if you place the bulky group on the β-lactam ring?

(a) ticarcillin

(b) temocillin

Why me and temocillin?

doi:10.1093/jac/dkn511
Advance Access publication 18 December 2008

Temocillin revived

David M. Livermore\textsuperscript{1,*} and Paul M. Tulkens\textsuperscript{2}

\textsuperscript{1}Antibiotic Resistance Monitoring and Reference Laboratory, Health Protection Agency Centre for Infections, 61 Colindale Avenue, London NW9 5EQ, UK; \textsuperscript{2}Unité de Pharmacologie Cellulaire et Moléculaire & Centre de Pharmacie Clinique, Université Catholique de Louvain, Bruxelles, Belgium

Resistance in Gram-negative pathogens is an increasing concern, with carbapenems often appearing as the only acceptable treatment option in serious infections. Reviving older compounds that have fallen into disuse may help to alleviate this burden. Temocillin (6-\alpha-methoxy-ticarcillin) is resistant to most if not all classical and extended-spectrum \beta-lactamases and to AmpC enzymes. It is also chemically stable, allowing administration by continuous infusion. Pharmacokinetic/pharmacodynamic analysis, aided by Monte-Carlo simulations, suggests a breakpoint of 8 mg/L for the registered maximum dosage of 4 g daily. Temocillin’s weaknesses, explaining its limited previous use, are a lack of activity against Gram-positive organisms, anaerobes and \textit{Pseudomonas}. In settings where these are unlikely or are covered by other agents, temocillin may be useful, potentially sparing carbapenems and having little apparent potential to select for \textit{Clostridium difficile}. 
As a result …

<table>
<thead>
<tr>
<th>Susceptible organisms</th>
<th>MIC &lt; 1 mg/L</th>
<th>1 mg/L &lt; MIC &lt; 10 mg/L</th>
<th>10 mg/L &lt; MIC &lt; 100 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moraxella catarrhalis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brucella abortus</td>
<td></td>
<td></td>
<td>Serratia marcescens</td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td></td>
<td></td>
<td>Enterobacter spp</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pasteurella multocida</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteus spp (indole +)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Providencia stuartii</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigella sonnei</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Intrinsically resistant organisms      |              |                        |                          |
| anaerobes                              |              |                        |                          |
| Gram(+) bacteria                       |              |                        |                          |
| *Acinetobacter spp*                    |              |                        |                          |
| *Pseudomonas aeruginosa*               |              |                        |                          |

Belgian SmPC, last revision 2012; Van Landuyt et al, AAC 1982; 22:535-40
Chemical stability of temocillin in concentrated solutions

**Table S1.** Stability of temocillin in concentrated aqueous solution (8.34% w/v; corresponding to a daily dose of 4 g in a 48 mL infusion syringe) at increasing temperatures maintained for 24 h.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Total (% of original amount)</th>
<th>R/S epimer ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>102.8±1.1&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.908±0.015&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>101.5±0.7&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.792±0.011&lt;sup&gt;B&lt;/sup&gt;</td>
</tr>
<tr>
<td>30</td>
<td>101.5±2.6&lt;sup&gt;A&lt;/sup&gt;</td>
<td>1.729±0.024&lt;sup&gt;C&lt;/sup&gt;</td>
</tr>
<tr>
<td>37</td>
<td>98.1±0.3&lt;sup&gt;B&lt;/sup&gt;</td>
<td>1.660±0.002&lt;sup&gt;D&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Samples were analysed by HPLC with differential detection of the R and S epimers. Data are means±SD (n=3).

Note that a drug loss upon storage ≤10% fulfills the requirements of the European Pharmacopeia [see Note for guidance on Manufacture of the Finished Dosage Form (CPMP/QWP/486/95), pp 1-6. The European Agency for the Evaluation of Medicinal Products (EMEA), London, UK].

## Comparative chemical stabilities of β-lactams upon storage of concentrated solutions at 25 and/or 37° C

<table>
<thead>
<tr>
<th>Conclusion</th>
<th>Molecule</th>
<th>Stability limit ¹</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>good</td>
<td>temocillin</td>
<td>&gt; 24 h at 37° C</td>
<td>De Jongh et al. JAC 2008</td>
</tr>
<tr>
<td></td>
<td>aztreonam</td>
<td>&gt; 30 h at 37° C</td>
<td>Chanteux et al. (abstract)</td>
</tr>
<tr>
<td></td>
<td>piperacillin</td>
<td>24 h at 37° C</td>
<td>Viaene et al. AAC 2002</td>
</tr>
<tr>
<td>weak</td>
<td>ceftazidime</td>
<td>24 h at 25° C / 8 h at 37° C</td>
<td>Servais et al. AAC 2001</td>
</tr>
<tr>
<td>problematic</td>
<td>cefepime</td>
<td>color appearance within 6 h</td>
<td>Baririan et al. JAC 2003</td>
</tr>
<tr>
<td>insufficient</td>
<td>imipenem</td>
<td>&lt; 5 h</td>
<td>Viaene et al. AAC 2002</td>
</tr>
<tr>
<td></td>
<td>meropenem</td>
<td>&lt; 5 h</td>
<td>Viaene et al. AAC 2002</td>
</tr>
<tr>
<td></td>
<td>doripenem</td>
<td>~ 6-10 h</td>
<td>Berthoin et al. JAC 2010</td>
</tr>
</tbody>
</table>

¹ > 90 % of original compound (European Pharmacopoiea)

² stable for 3 weeks at 4° C (for home medication) (Carryn et al., J Antimicrob Chemother 2010;65:2045-2046)
Temocillin pharmacodynamics: the lessons of β-lactams

• For β-lactams,
  – only the free fraction is (probably) active…
Exemple #1 (très court):
bolus et infusion continue
Application to clinical trials (ICU patients)

De Jongh et al, JAC 2008; 61:382-8
Exemple #2 (plus long):
patients de soins intensifs avec données manquantes
Population Pharmacokinetics of Temocillin in ICU patients and Monte Carlo Simulations to Evaluate Resistance Breakpoints

A.E. Muller¹, P.F. Laterre², T. Dugernier³, X. Wittebole³, N. Cauwenbergh³, P.M. Tulkens², S. Careyn¹, J.W. Mouton²,⁴

¹Erasmus Medical Centre Rotterdam, ²Hasbro Children’s Hospital, ³Katholieke Universiteit Leuven, ⁴Katholieke Universiteit Leuven, ²Université catholique de Louvain, Brussels, Belgium

Temocillin project (full)

Concentration (mg/L)

Time (h)
Outputs: individual curves
Total number of subjects: 4
Average number of doses per subject: 1
Total/Average/Min/Max numbers of observations: 15, 3.75, 3, 4

* not noodles!
Outputs: population curves
Outputs: population
Outputs: observations vs. predictions

Using the population parameters

Using the individual parameters
Outputs: residuals

- PWRES
- IWRES
- NPDE
Exemple #3 (long):
volontaires vs soins intensifs
et impact de la fraction libre

Cette partie est reprise du travail de Thèse en cours
de Mr Perrin Ngougni-Pokkem
There is growing evidence that standard antibiotic regimens may not provide adequate drug concentrations …

Critically-ill patients

- Hyperdynamic states
  - Increased cardiac output, and clearance
  - Decreased plasma concentrations

- Altered fluid balance / Altered protein binding
  - Increased volume of distribution
  - Decreased plasma concentrations

- Renal and hepatic impairment
  - Decreased clearance
  - Increased plasma concentrations

- Organ support (RRT/ECMO)
  - Increased volume of distribution / clearance
  - Increased/decreased plasma concentrations

Hosthoff et al, Swiss Med Wkly. 2016;146:w14368
A. Abdulla et al: University Medical Center Rotterdam; eposter 069; ECCMID 2017

RRT: renal replacement therapy
ECMO: extra corporeal membrane oxygenation
Consequences of PK alteration

Critically ill patients

Pharmacokinetic alteration

Variability in antibiotic concentration

underdosing

Therapeutic failure/antibiotic resistance

overdosing

Toxic effects

Therapeutic success

Hosthoff et al, Swiss Med Wkly. 2016;146:w14368
A. Abdulla et al: University Medical Center Rotterdam; eposter 069; ECCMID 2017
The main objectives

• Current literature data are based mainly on TOTAL temocillin concentrations
  • Only the free concentration is active!
  • Concentration in the infected tissue is important!

Part 1
Pilot study

Population Pharmacokinetic Analysis and Protein Binding Characteristics of Free and total Temocillin concentrations in Plasma of Healthy Volunteers and patients
Design of the in vitro study

- Comparing protein binding in spiked plasma of healthy donors (n=4) vs. plasma from patient donors hospitalized in intensive care unit (n=5) for temocillin concentrations ranging from 8 to 250mg/L.

- Free fraction of temocillin (%) = \( \frac{\text{free concentration} \times 100}{\text{total concentration}} \)

- Bound concentration of temocillin (mg/L) = \( \text{total concentration} - \text{free concentration} \)

- Study of the relationships between the free fraction of temocillin vs its total concentration.

- Bound concentration vs free concentration of temocillin in plasma

- Free Fraction at a given total concentration vs protein concentrations
Temocillin plasma protein binding In vitro study

Free fraction vs total concentration of temocillin in plasma for 4 healthy donors (D) compared with 5 patients donors (P) in vitro study

For the patient donors
✓ High free fraction up to 65%
✓ Free fraction which increases with the total concentration
✓ High variability between the patient donors.

For the healthy donors, except D4
✓ Low free fraction between 5 to 8%
✓ Free fraction which is not influenced by the total concentration
✓ Low variability between the healthy donors

Plasma total protein level (mg/L)
Reference range : 65-85g/L

P1: 52.89 g/L
P2: 48.34 g/L
P3: 61.17 g/L
P4: 55.31 g/L
P5: 55.53 g/L
D4: 57.03 g/L
D1: 71.75 g/L
D2: 84.91 g/L
D3: 70.55 g/L
Michaelis-Menten fitting of temocillin protein binding

\[ B = \frac{B_{\text{max}} \times C_{\text{free}}}{K_d + C_{\text{free}}} \]

Bound concentration vs free concentration of temocillin in plasma
In vitro study

✓ Plasma protein binding of temocillin is saturable
✓ Maximum binding is lower in patients
Design of the clinical study: « Phase1 »

- 8 healthy volunteers.
- Single dose of 2g TMO in 40 min infusion; IV administration.
- Blood sampling: 40min, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12h.
- Study of the relationship between free fraction of temocillin vs its total concentration.
- Study of the relationship between bound concentration of temocillin vs free concentration.

Principal Investigator according to Austrian drug law
Markus Zeitlinger, MD
Department of clinical Pharmacology,
Medical University of Vienna

Graph Pad 4 software
**Temocillin plasma protein binding**

Free fraction vs total concentration of TMO in plasma for 8 healthy volunteers (V) in vivo study compared with healthy donors (D) in vitro study

![Graph](Image)

- **Plasma total protein level**
  - Reference range: 65-85g/L
  - V1 = 73.58g/L
  - V2 = 77.45g/L
  - V3 = 66.16g/L
  - V4 = 74.08g/L
  - V5 = 72.45g/L
  - V6 = 64.50g/L
  - V7 = 65.25g/L
  - V8 = 69.45g/L

- **Free fraction (%)**
  - Mean total concentration (mg/L)

- ✓ Low free fraction (3-8%) for total concentrations below 150 mg/L, and increase in free fraction up to 20% for higher total concentrations
Michaelis-Menten fitting of temocillin protein binding

Bound concentration vs free concentration of temocillin in plasma

✓ Protein binding saturation observed
Michaelis-Menten fitting of temocillin protein binding

Comparison of plasma protein binding in healthy volunteers (V), healthy donors (D) and patient donors (P)

Bound concentration vs free concentration of temocillin in plasma

- V (in vivo study; n=8)
- D (in vitro study; n=3)
- P (in vitro study; n=6)

✓ Similar protein binding saturation observed
✓ Lower Bmax for patients!
PK modeling approaches

- "Data-rich" situation
- Simple to implement
  Individual analyzes in descriptive statistics

Adapted from I. Delattre. 2012
Plasma total and free concentration versus time

Pharmacokinetic profile of free and total concentration: individual data

- Free concentration decreases with the total concentration
- Important variability in the pharmacokinetic profiles between the volunteers
Comparison of pharmacokinetic parameters (free vs total)

Mean and IC95% of pharmacokinetic parameters of free and total TMO

The clearance of the free temocillin is very high compared to the total

The volume of distribution of the free temocillin is very high compared to the total

The half-life of the free temocillin has an important numerical effect; But not significant

\[ t_{1/2} = 0.693 \frac{V_d}{C_l} \]
1. Structural pharmacokinetic model

This PK profile suggests that the kinetics of the TMO is Bi-compartmental.
1. Goodness-of-fit plot

- **Mono compartmental model tested without covariate**

- **Bi-compartmental model tested without covariate**

- The correlation is better in this case and with less variability
1. Goodness-of-fit plot

- **Mono compartmental model tested without covariate**
  - WRE vs. $C_{\text{pred}}$ vs. Time
  - WRE vs. Time
  - Shapiro-Wilk. $P=0.002$

- **Bi-compartmental model tested without covariate**
  - WRE vs. $C_{\text{pred}}$ vs. Time
  - WRE vs. Time
  - Shapiro-Wilk. $P=0.214$

- Residues should be centered on 0
- 95% of the population residues should be between approximately -2 and 2
- Residue distribution should be normal

→ Bi-compartmental model + Proportional residual error model
2. Covariate model

- Relevant physiological, biological and demographic parameters that could change the pharmacokinetic parameters

- Make it possible to explain the inter and/or intra-individual variability

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean (sd)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>32.9 (12.1)</td>
<td>23.0-53.0</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>81.9 (10.9)</td>
<td>70.2-105.6</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.8 (0.1)</td>
<td>1.7-1.9</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>24.4 (2.9)</td>
<td>20.7-28.9</td>
</tr>
<tr>
<td>GFR (mL/min) (Cockcroft-Gault)</td>
<td>135.7 (16.1)</td>
<td>108.2-153.4</td>
</tr>
<tr>
<td>ASAT (U/L)</td>
<td>23.8 (4.5)</td>
<td>14.0-30.0</td>
</tr>
<tr>
<td>ALAT (U/L)</td>
<td>30.1 (9.2)</td>
<td>18.0-45.0</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>158.8 (28.4)</td>
<td>143.0-195.0</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>Not analyzed</td>
<td></td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>70.4 (4.4)</td>
<td>64.5-77.5</td>
</tr>
</tbody>
</table>

- Influence volume of distribution and clearance
- Renal excretion (80% found in the urine in 24h)
- Variable protein binding (-->93%)
Internal Validation: Monte Carlo simulations

- Simulated profiles (n=1000) compared to observed data.
- The observed concentrations should be distributed homogeneously around the median of the simulated concentrations.
- Less than 5% of observed concentrations must be outside the 5th and 95th percentiles of the simulated concentrations.

External Validation
Internal Validation: Visual Predictive Checks (VPC).

![Graph](image)

- Simulated free TMO profile
- Observed free concentration

Free concentration (mg/L) vs Time (h)

- Time (h): 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
- Free concentration: 0.05, 0.5, 0.95

Nov 2017

PK/PD and modelling
Temocillin pharmacodynamic targets

As every β-lactam, temocillin is

- bactericidal
- time-dependent
  (activity is driven by the time during which the drug plasma free concentration remains above the minimum inhibitory concentrations (MIC))

- 40% of time > MIC is enough for bacteriostatic activity
  → acceptable for non-immunocompromised patients

- 70% of time > MIC is recommended
  → for immunocompromised patients

- 100% of time > MIC is suggested
  → For critically-ill patients
  this could not only maximize efficacy but also minimize emergence of resistance

Delattre IK et al For submission to Expert Review on Antiinfective Therapy as Special Report
Probability of Target Attainment (PTA) of plasma free temocillin concentrations

For non-immunocompromised patients
Target: $f_{T} >$ BSAC breakpoint = 8 mg/L of 40% of the time, based on a mean free fraction of 6.0 ± 1.4% (mean of values observed for total concentration < 150mg/L),

- **Standard dosing (2g/12h)**: PTA = 0
- **newly proposed dosing (2g/8h)**: PTA = 0.5

[Graph showing PTA for different dosing regimens]
For non-immunocompromised patients

Target: $fT >$ BSAC breakpoint = 8 mg/L of 40% of the time, based on a mean free fraction of $13.0 \pm 4.0\%$ (mean of values observed for total concentration $> 150\text{mg/L}$),

**Probability of Target Attainment (PTA) of plasma free temocillin concentrations**

Standard dosing (2g/12h)  
PTA = 0.99

newly proposed dosing (2g/8h)  
PTA = 1
For critically-ill patients
Target: $f_T > \text{BSAC breakpoint} = 8 \text{ mg/L of 100\% of the time, based on a mean free fraction of 35.0 \pm 12.3\% (mean of values observed for patient),}$

![Graph showing Probability of Target Attainment (PTA) of plasma free temocillin concentrations.](image)

- **Standard dosing (2g/12h)**
  - PTA = 0.7

- **Newly proposed dosing (2g/8h)**
  - PTA = 1

Nov 2017
Which are the actual (and recent) observations?

MIC distributions of *E. coli*: ESBL/AmpC (n=1155) vs non-ESBL/AmpC (n=1473)

Source: Eumedica (data on file)