Computational Tools and Models to Facilitate In Vitro to In Vivo Exposure Extrapolation: Lessons from the Drug Development World

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Outline

• Quantitative technologies in pharmaceutical R&D

• What are we measuring? Exposure-response relationships vs. safety thresholds

• Data is not knowledge: Requirements for PK and PKD modelling

• Applications of inferential, integrative methods in toxicology

• Conclusions
What is not a poison? All things are poison and nothing is without poison. Only the dose makes a thing not to be poison (Paracelsus).

The impact of empirical protocols in quantitative pharmacology and toxicology.
Quantitative technologies currently used to enhance pharmaceutical R&D
(Bio)analytical Technologies

Dried blood spot – (DBS)
Full PK profiles from a single animal

Blood-Brain Barrier Transport in a Kainate model of epilepsy
In Vivo Study Using $^{11}$C-Flumazenil and PET

From Sadayappan et al. Bioanalysis. 4(9) 2012

(Bio)analytical Technologies

Key Terms

Antisense oligonucleotides: Short fragments (typically 0–20 bp) of single-stranded deoxynucleotides that could be used to modulate target mRNA expression through specific hybridization to the complementary mRNA sequences via Watson–Crick base pairing.

Radioisotope probe assay: An analytical tool to quantify the target molecule by detecting the emission of a radioisotope tracer.

Figure 2. Comparison of mean kidney, liver and plasma concentrations (ng/ml) of a 14-mer antisense oligonucleotide following single subcutaneous doses in Ob/Ob mice.

Figure 3. Equivalent concentration (µg eq./g) of 125I-labeled protein conjugate in ocular tissues (vitreous humor, retina, choroid-retinal pigmented epithelium, iris-ciliary body and aqueous humor) as well as plasma after intravitreal injection at a dose of 0.25 mg per eye in male Dutch Belted rabbits over 28 days.

Mechanisms contributing to drug distribution/disposition

Mechanisms contributing to drug distribution/disposition

Summary of methodologies available to estimate intracellular fraction of unbound drug in cells

<table>
<thead>
<tr>
<th>Method</th>
<th>Predictive equations developed for $f_{u,cell}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Empirical prediction of $f_u$ for nontransporter substrates</td>
<td>$\log\left(\frac{1}{f_u}\right) = 0.40\log D(P) - 1.38$</td>
</tr>
<tr>
<td>2. Empirical prediction of $f_{u,cell}$ for nontransporter substrates</td>
<td>$f_{u,cell} = \frac{1}{1 + (125 \times VR \times 10^{0.072\log P(D)^2 + 0.67\log P(D) - 1.126})}$</td>
</tr>
<tr>
<td>3. Indirect estimation from $K_{puu}$ and $K_p$ data for transporter substrates</td>
<td>$K_{puu} = \frac{CL_{act,uptake} + CL_{diff}}{CL_{diff}}, f_{u,cell} = \frac{K_{puu}}{K_p}$</td>
</tr>
<tr>
<td>4. Mechanistic compartmental uptake model</td>
<td>$\frac{dC_{cell}}{dt} = \frac{V_{max} \times C_{med,u}}{K_{m,u} + C_{med,u}} + CL_{diff} \times C_{med,u} - CL_{diff} \times C_{cell} \times f_{u,cell}$</td>
</tr>
</tbody>
</table>

Integration of *in vitro* and *in vivo* data as input for *in silico* models

*From Lowe et al, Xenobiotica 37: 1331-1354, 2007*
Variability

- e.g., Genotype
- Age
- Disease
- Environmental Factors

Intra-individual variability

Inter-individual variability

Inter-occasion variability
Components of a population PK model

- **Fixed effects**
- **Structural model**
  - E.g. how many compartments, how to model elimination?
- **Covariate model**
  - E.g. Weight effect on clearance and volume of distribution?
- **Statistical model**
  - How to model between-subject, between-occasion, residual variability?
- **Mostly random effects**
Dose-exposure-response paradigm for toxic effects, relating observed response as consequence of perturbations of the normal control processes in the cell.

Translating Exposure into Effect

NOAEL, Study Design

Main Toxicology Group

Satellite Group

Adverse Event (AE) Data

Dose-Exposure Data

ex·po·sure  n.
An act of subjecting or an instance of being subjected to an action or an influence
Example of typical toxicology package

- 7.5mg/kg
  - 1 Week
  - 2 Weeks
  - 4 Weeks

- 15mg/kg
  - 1 Week
  - 2 Weeks
  - 4 Weeks

- 40mg/kg
  - 1 Week
  - 2 Weeks
  - 4 Weeks

Legend:
- No AEs
- AEs
- Satellite Group

NOAEL
Empirical Calculation of Exposure
Model-predicted exposure

Not limited to the profile on sampling days
Model-derived PK measures
Simulations: Beyond the experimental evidence

(Left panel): True maximum risk (over 6 months daily dosing) vs. exposure relationship for simulation model.
(Right panel): Estimated risk-exposure relationship according to: \( p(AE) = \logit(\theta_1 + \theta_2 \cdot \text{AUC}_{0-24h}) \).
The wider distribution appropriately reflects estimation uncertainty.
Biomarker-guided evaluation of drug exposure

For PGE2, differences in the IC80 values were statistically significant across species. No differences were observed for TXB2. Target related toxicity is therefore predicted to manifest closer to the minimum therapeutic concentration in humans.

A well-known topic: QTc prolongation
Translational Pharmacology – TIPharma Consortium
Translation to man

- Compounds which show no QTc prolonging effects
- Compounds with borderline activity
- Positive controls: moxifloxacin, sotalol, cisapride, methadone, dofetilide

In-Vitro Experiments
Predictive / Translational Model
In-Vivo Experiments
Predictive / Translational Model
Clinical Trials
Predictive / Translational Model
Real life Observations (QT, SCD)

Inter-species Translational Modelling

Dogs
Guinea pigs
Primates

FTIH, dose escalation, Phase II/III
Translational Modelling in R&D

Translational model development

- *In vitro* experiments
- *In vivo* pre-clinical species
- Humans (healthy subjects)

Drug development
In vitro-in vivo correlations

Relationship between hERG channel activity, action potential and ECG

Normal conditions  hERG block

hERG channel ($I_{Kr}$)

Action potential

ECG

QT interval  QT prolongation

Torsades de Pointes
Interspecies differences

A. Diagram of heart with labels:
- SA Nodal
- Atrial
- AV Nodal
- Purkinje Fiber
- Endocardial
- Midmyocardial
- Epicardial
- Septum
- RV
- LV

B. Diagram of ECG waveforms:
- P
- QRS
- T

C. ECG waveform with annotations:
- PR
- QRS
- QT
Model-based prediction of QT prolongation
Bayesian model

\[ QT = QT_0 \times RR^\alpha \cdot (1 + A \cdot \cos(2\pi/24 \cdot (\text{clocktime} - \phi))) + \text{slope} \cdot C \]

- \( QT \) = observed QT interval
- \( QT_0 \) = intercept of QT-RR relation
- \( RR \) = interval between successive R waves
- \( \alpha \) = individual heart rate correction factor
- \( A \) = amplitude of circadian rhythm
- \( \phi \) = phase
- slope = linear pharmacodynamic relation
- \( C \) = drug concentration
Modeling of QTc interval prolongation in humans

Translational Pharmacology
Translation to man PK moxifloxacin
Translational Pharmacology
Translation to man PKPD moxifloxacin
Probability of QT interval prolongation in dogs and humans

Cisapride
- dog: 0.0045 ms/conc
- man: 0.09 ms/conc

Sotalol
- dog: 0.002 ms/conc
- man: 0.021 ms/conc

Moxifloxacin
- dog: 0.00056 ms/conc
- man: 0.0039 ms/conc

From: Chain ASY, Dubois VFS et al. submitted for publication
Animal to human extrapolation
moxifloxacin in dog, monkey and man

Dubois et al., (2014) Submitted for publication
Establishing in vitro in vivo correlations
Establishing *pre-clinical to clinical* correlations

<table>
<thead>
<tr>
<th></th>
<th>Moxifloxacin</th>
<th>Cisapride</th>
<th>Sotalol</th>
<th>NCE03</th>
<th>NCE04</th>
<th>GSK945237</th>
<th>SB237376</th>
<th>Carbersat</th>
<th>GSK618334</th>
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<td><img src="2.1-0.6" alt="0.98" /></td>
<td><img src="0.01-0.03" alt="0.0098" /></td>
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<td><img src="0.17" alt="0.17" /></td>
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<td>4005</td>
<td>33.2</td>
<td>&gt;9000</td>
<td>1.3</td>
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</tbody>
</table>
Summary

• Highlighted issues with current experimental design, which hamper characterisation of exposure-response relationships, both from a mechanistic and statistical perspective

• Data is not knowledge: example of how PK and PKD modelling can be used to disentangle species and drug specific differences

• Bayesian (population) hierarchical modelling enables accurate estimation of risk or exposure-relatedness, especially for delayed, unfrequent events

• Inferential, integrative methods must be integrated into mainstream data analysis in toxicology
Conclusion:
What is not a poison? All things are poison and nothing is without poison. Only the dose makes a thing not to be poison (Paracelsus)

The impact of empirical protocols in quantitative pharmacology and toxicology.