Tra in Vitro e in Vivo

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Introduction: a Dantesque vision of toxicology

"Tra fletro e feltro" really meant "Between in vitro and in vivo"

The first real toxicologist is not Paracelsus but Dante.

(in fact the “Divine Comedy” should be called “Toxicology Testing in the 14th Century”)

DANTE IN VITRO

MODELING PURGATORIUM

EXTRAPOLATION PARADISE

DANTE'S JOURNEY IN VITRO
Outline

- QIVIVE general scheme
- Our software tools
- *In vitro* PK
- *In vitro* effects
- *In vivo* PK
- *In vivo* effects
- Perspectives

Is this all about literature?
Quantitative *in vitro* to *in vivo* extrapolation

General scheme

![Diagram of in vitro to in vivo extrapolation process](image)
To recapitulate, we need data and models on:

- **PK in vitro**: getting at cellular concentration (increasingly done)
- **Dose-response in vitro**: always done
- **PK in vivo**: getting at cellular concentration (PBPK models, increasingly done)
- **Dose-response in vivo**: adapting *in vitro* dose-response to *in vivo* (rarely done)

We also need good software.
GNU MCSim is a modeling package, written in C, which allows you to:
- Design and run your own statistical or simulation models (algebraic or dynamic, via differential equations)
- Do parametric simulations (for example for sensitivity analysis)
- Perform Monte Carlo (stochastic) simulations
- Do Bayesian inference for hierarchical models through Markov Chain Monte Carlo simulations (Metropolis-Hasting by component or y vector; tempered MCMC too)
- Compute optimal experimental designs

It runs under Unix/Linux, Windows, Mac OS
The code is compiled for speed, model and input files have their own grammar (SBML can also be used for model definition)
In vitro PK

Data and problems

Cyclosporine A in kidney cells

- Non-linearities
- Missing data
- Variability
- Censored data
- Complex kinetics

...in primary hepatocytes

Cisplatin in kidney cells (total Pt)
In vitro PK

Structural models

We adapted the model to each case, and there are many variants.

In vitro PK model used for cyclosporine A, amiodarone, chlorpromazine, ibuprofen.

In vitro PK model used for cisplatin and adefovir dipivoxil.
We typically use Bayesian inference:

- *a priori* knowledge (expertise or historical data)
- Experimental data
- *a posteriori* knowledge after updating
We use hierarchical modeling when variability needs to be disentangled from uncertainty

**Cell type level**

**Experiment or cell donor level**
**In vitro PK**

Results for cyclosporine A in kidney cells

- The intra-cellular kinetic profile alone explains the shift in metabolic perturbations
- Interpolations and extrapolations can be performed correctly
- Identification of meaningful parameters which can be transposed *in vivo*

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**Application of integrated transcriptomic, proteomic and metabolomic profiling for the delineation of mechanisms of drug induced cell stress**

Anja Wiltmes¹, Alice Limonciel², Lydia Aschauer³, Konrad Moenkoh⁴, Chris Bielow⁵, Martin O. Leonard⁶, Jeremy Hamori⁷, Donatella Carpi⁸, Silke Ruzek⁹, Andreas Handler⁹, Olga Schmal¹, Karin Herrger¹, Patricia Bellw¹, Christof Burek¹, Germaine L. Trussi-m¹, Philipp Hewitt¹, Emma Di Consiglio¹, Emanuela Testa¹, Bas J. Blauhofer¹, Claude Guillou¹, Christian G. Huber¹, Arno Lukas¹, Walter Pfatter¹, Stefan O. Mueller¹, Frederic Y. Boie¹, Wolfgang Dekant¹, Paul Jenninges¹

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Bois – SOT – Sept 2017
In vitro PK

Results for cyclosporine A in liver cells

- The data finally make sense
- You can start correlating the underlying parameters (e.g. metabolic clearance) to cell genotype for example
- A challenge is the temporal evolution of the cellular processes (non-stationarity): you see toxicity (or adaptation) induced pharmacokinetic changes
In vitro PK

Results for cisplatin in kidney cells

- Not much variability between experiments
- But kinetics are complex and poorly identified (tempered MCMC was needed)
- Cisplatin equilibration between compartments is rapid (minutes), but metabolites formed accumulate slowly in cells (hours)
Predicting *in vitro* effects

Back to the general scheme
What we really want is this: because now we just need to measure GSH inactivation

Frederic Y. Bois, Eurotox, 2017
**In vitro Effects**

**Models**

- We are using empirical statistical models and also systems biology-like mechanistic models, such as this one for oxidative stress control:
In vitro Effects
Software and statistical aspects

We are using R (for empirical models) or GNU MCSim (for mechanistic models)

For simpler models we can do reasonable statistical inference

For more complex model, the data is not sufficient to identify the model parameters (sensitivity analysis does not really help). So we used parameter values obtained from the literature and just use the model for exploring behavior and check our understanding of the data. Simple Monte Carlo simulations can be performed for checking the robustness of the conclusions.
In vitro Effects

Results of empirical modeling

KBrO₃ → GSH → ROX → Death

After parameterization:

Frederic Y. Bois, Eurotox, 2017
In vitro Effects

Results

• With the complex model we can start questioning the data more deeply:

Either KBrO₃ effect on GSH well modeled but baseline ROS production not so well.

Or the baseline ROS production well modeled but effect of KBrO₃ on GSH poorly fitted.

We probably do not understand well the exact interplay between KBrO₃, GSH, ROS and DCF inside cells. We should not be surprised if the empirical model is not robust.
Predicting *in vivo* PK

Back to the general scheme

- **Dose-response or Systems biology modeling**
- **PK in vitro**
  - *in vitro* PK data
  - *in vitro* PK model
  - *in vitro* PK predictions

- **Systems biology model**
- **Calibrated *in vitro* PK model**

- ***In vitro* PK data**

- **Calibrated *in vitro* PK model**

- **PKSB model**

- **In vitro effects data**

- **Calibrated PKSB model**

- **In vivo PK predictions**

- **PBPK model**

- **Physico-chemical data**

- **PBPK-SB model**

- **Integration**

- **In vivo effects predictions**

- **In vivo PK predictions**
The problem here is the lack of data (except ADME in vitro data, maybe). So we typically use PBPK models (expected to be robust to extrapolation because of their mechanistic basis)

INERIS’ lifetime model (woman)  INERIS’ pregnancy model
Chemical-specific parameters are the biggest problem. We are using QSPR models, *in vitro* measurements in improved *in vitro* systems (e.g. microfluidic systems, organoids *etc.*)

Monte Carlo simulation are important to characterize uncertainty and variability. The model can be calibrated if human data are available, but this gives an unrealistic sense of precision for purely predictive cases.
In vivo Effects

Results for a well know chemical, after calibration

- Predicted cyclosporin tissue concentrations after 10 mg/kg orally for 60 days
- Idem in kidney cells (A), plasma (B), and extracellular kidney water (C)
**In vivo PK**

Results for an “unknown” chemical, without calibration

Purely predictive simulations:

(in fact we have blood concentration data for this chemical, for checking)

Can be supplemented with sensitivity analyses to identify data needs
Predicting *in vivo* Effects

Back to the general scheme
In vivo Effects

Putting it all together (we don’t go up to here often, fortunately!)

After therapeutic CsA administration:

Loss of viability zone

Therapeutic zone
- For *in vitro* PK we are working on advanced partition models (in collaboration with Simcyp)
- We are also interested in moving toward high throughput (collaboration with the EPA-developed R package *httk*)
- We are working on improving *GNU MCSim* through a FDA grant (GUI, sensitivity analysis, parallelized computations)
- Looking at *in vitro* kinetics opens a whole Pandora’s box of either problems or potential discoveries
- The *in vitro* effect models are being discussed in the framework of quantitative AOPs
- In terms of *in vivo* PK modeling, the work is on specific organ refinements and mostly sources of parameter estimates
- An overall need for more case-studies and experimental “validation”
Acknowledgments

- C. Brochot
- R. Beaudouin
- E. Mombelli
- C. Tebby
- F. Zeman

- J. Deloffre
- W. Gao
- G. Gayraud
- N. Golbamaki
- A. Grech
- E. Leclerc
- N. Quignot
- W. Wiecek
- E. Zgheib