

Toxicokinetic modelling of hepatotoxic pyrrolizidine alkaloids: a combined *in silico*, *in vitro*, and *in vivo* approach

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Introduction and objective

- **Pyrrolizidine alkaloids (PAs)** are a class of secondary metabolites in plants of which some are highly **hepatotoxic, genotoxic, and carcinogenic** [1].
- **High concentrations of PAs** are found in contaminated food and especially in herbal supplements [2-4].
- To date, there are only few attempts to model PA kinetics and none of them include a model of hepatic PA metabolism [5]. However, the **combination of PAs with CYP-inducing compounds**, e.g. the drug phenobarbital, has been shown to **dramatically increase PA toxicity** [6].

Our aim Develop a physiologically-based toxicokinetic (PBTK) model for PAs that is tailored to **predict hepatic CYP interactions**.

Methods

- In contrast to most pharmaceutical compounds, **PAs are not well characterized** in terms of their physico-chemical/biochemical properties.
- **Kinetic data is sparse** due to general efforts towards reduction of animal testing in chemical risk assessment.

Our approach

A *in silico*

- Modelling framework in R (v3.2.4) including species database based on [7]
- *RxODE* (v0.7.2-1)
- Parameter estimation via Maximum Likelihood Estimation and DRAM-MCMC [8]
- L1 Regularization (LASSO regression)
- Prediction of ionization (SPARC v2018), tissue to plasma partition coefficients, plasma protein binding (GastroPlus v9.5)

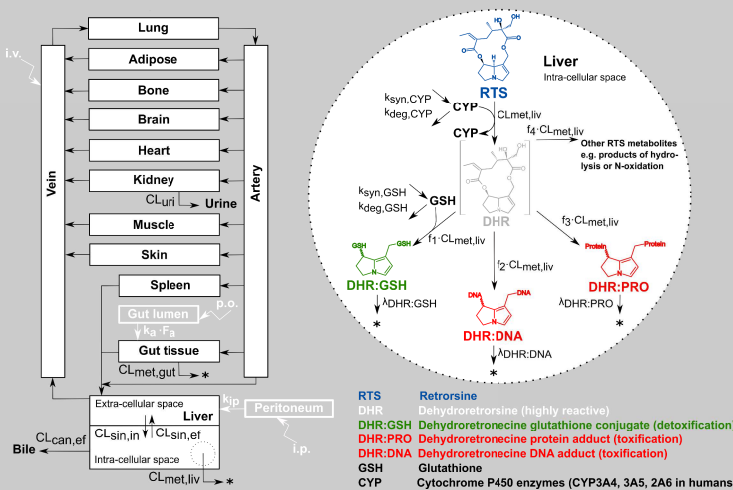
B *in vitro*

- Metabolic stability in microsomes
- Physico-chemical properties (e.g. log P)
- Caco-2 permeability
- Blood to plasma concentration ratio

C *in vivo*

- *In vivo* data of the PA retrorsine and selected metabolites in rat and mouse [9-12]
- *In vitro* to *in vivo* extrapolation (IVIVE) of metabolic clearance (CL_{met,liv})

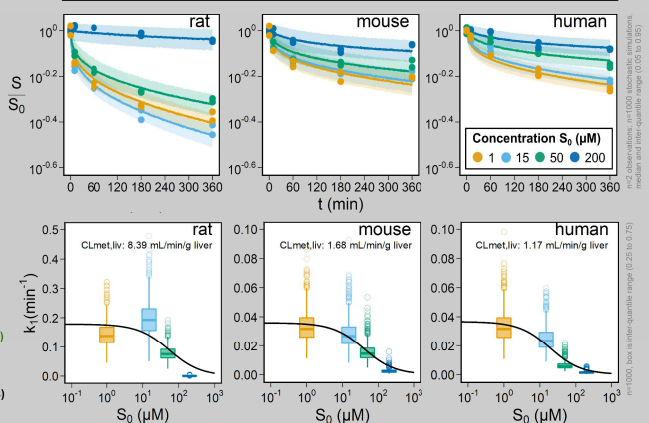
A PBTK model of retrorsine (RTS)



B *In vitro* metabolic stability in microsomes

End-product inhibition model:

$$S \xrightarrow{k} P \text{ with } k(P) = k_1 \cdot \left(1 - \frac{P(t)}{P(t) + EC_{50}}\right); \quad k_1(S_0) = \frac{V_{max}}{K_M} \cdot \left(1 - \frac{S_0}{S_0 + K_M}\right)$$



C Fitting of the PBTK model to *in vivo* data of retrorsine (RTS)

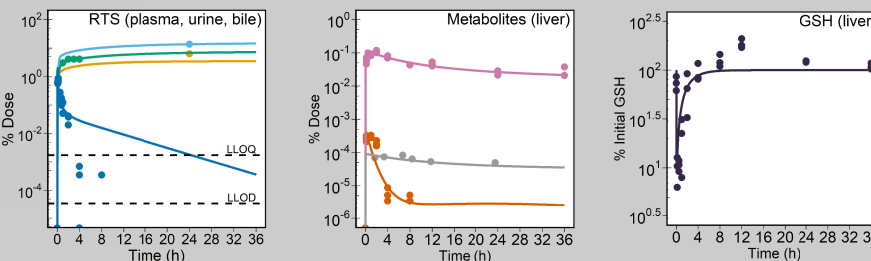


Figure 1: A: PBTK model of the hepatotoxic PA retrorsine (RTS) including a simplified model of RTS metabolism. B: Metabolic stability of RTS in rat, mouse, and human liver microsomes described by end-product inhibition model. CL_{met,liv} (linear PK) was obtained by IVIVE. C: *In vivo* kinetic data of RTS and metabolites in mouse and rat together with PBTK model simulations. Unknown PBTK model parameters were estimated via Maximum Likelihood Estimation.

Results

- For PBTK model development, we used the **extended clearance model of the liver** [13] and adapted it via a basic representation of **PA toxification and detoxification pathways** (Fig. 1 A).
- Metabolic clearance of retrorsine determined from liver microsomes revealed inter-species differences:** CL_{met,liv} (mL/min/g liver) was 5- or 7-fold higher in rat compared to mouse or human (Fig. 1 B).
- Our developed **PBTK model appropriately describes *in vivo* kinetic data of retrorsine** in mouse and rat (i.p./i.v. administration) (Fig. 1 C).

Conclusions

- With our approach, we demonstrate that **translational toxicology is an efficient tool** for the development of PBTK models, especially in **sparse data situations**.
- We suggest to test whether **EC₅₀ of the end-product inhibition model** is a suitable **potency factor** for predicting the differences in the liver toxicity of PAs.
- Next steps include the **simulation of real-life consumer exposure scenarios in combination with CYP inducing drugs**, e.g. rifampicin. Thereby, we want to address highly relevant interactions between medical and foodborne compounds that should be considered in both drug dosing and food risk assessment.

References

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