How to integrate in vitro PK/PD information for toxicity prediction

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An integrated modelling approach for *in vitro* to *in vivo* extrapolations

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Summary

Predicting *in vivo* drug toxicity from *in vitro* testing requires modelling those processes that are not reproduced *in vitro*. The most obvious difference between the two settings is the lack of integrated absorption, distribution, metabolism, and excretion (ADME) that govern target tissue exposure *in vivo*. For equal input doses, the concentrations to which *in vitro* systems are exposed may not correspond to those found *in vivo*. The partners of the European project PREDICT-IV in charge of modelling develop prediction models for ADME processes and integrate them into generic physiologically based pharmacokinetic (PBPK) models. Examples of simulations of concentration-time profiles for diazepam and ciclosporin A, in various human or rat organs and tissues, are presented here. *In vivo* drugs' toxicity will be forecasted by coupling the predicted target tissue concentration profiles to dose-response relationships observed *in vitro*. Human variability will be evaluated for each component of the approach.

1 Introduction

It is now recognised that replacing animals by *in vitro* systems for assessment of medium to long-term toxicity of drugs or general chemicals requires an integration of pharmacokinetics (Bouvier d'Yvoire, et al., 2007). Predicting *in vivo* drug toxicity from *in vitro* testing requires modelling the absorption, distribution, metabolism, and excretion (ADME) processes that are not reproduced *in vitro*. For equal input doses, the concentrations to which *in vitro* systems are exposed may not correspond to the target tissue exposure experienced *in vivo*. INERIS, Simcyp Limited, the German Cancer Research Centre, Emergentec Biodevelopment GmbH, and ECVAM, which constitute the modelling team of the European project PREDICT-IV, will integrate prediction models for ADME processes into global generic physiologically based pharmacokinetic (PBPK) models able to simulate concentration-time profiles in human or rat blood and tissues. *In vivo* drugs' toxicity will be predicted by coupling the predicted target tissue concentration profiles to dose-response relationships observed *in vitro*. Human variability will be evaluated for each component of the approach.

While the establishment of predictive dose-response relationships for neurotoxicity, liver and kidney toxicity is a very important part of the project, this article focuses on the progress made so far on PBPK modelling by PREDICT-IV.

PBPK models are increasingly used in drug development and regulatory toxicology to predict the kinetics and metabolism of substances in the body (Barton, et al., 2007, Bouvier d'Yvoire, et al., 2007, Loizou, et al., 2008). In these models, the body is represented by a set compartments corresponding specific organs or tissues, and the transfers or transports of drugs are dictated by various

physiological flows (blood, bile, pulmonary ventilation, *etc*) (Bois and Paxman, 1992). A system of differential equations can be written, with parameters representing blood flow rates, organ volumes *etc*., for which information is available in the published scientific literature or may be obtainable *in vitro* (Woodruff and Bois, 1993, Parrott, et al., 2005). Numerical integration of that differential system computes the quantity and concentration of the drug considered in each compartment, as a function of time and exposure dose. Indeed, such a description of the body is approximate, if not rough, but a balance has to be found between precision (and therefore complexity) and simplicity (ease of use).

We will briefly describe the global approach taken by PREDICT-IV to integrate experimental evidence for a predictive approach to toxicology. We will then describe Simcyp's generic PBPK model and the specific parameter setting adopted for ciclosporin A and diazepam in rats and humans. Modelling results for those two substances are presented and discussed.

2 Methods

2.1 General modelling framework

Fig. 1 presents graphically the integrated prediction and validation framework of PREDICT-IV. *In vitro* studies will provide the necessary data for the development of a set of ADME QSARs and multivariate dose-response models for cellular and organ-level effects. Those models will be coupled to a generic PBPK model and will provide an *in silico* pharmacokinetic-pharmacodynamic (PK/PD) tool predictive of medium- to long-term toxicity. In order to validate that tool, its predictions for plasma and tissue drug levels will be confronted to pharmacokinetic data obtained in humans or rats. We do not expect to get enough

data relating plasma to pathological effects in humans. Therefore the pharmacodynamic component will be validated on the basis of already published rat data. We do not expect perfect predictions of pharmacokinetics or toxicity data and meta-analysis models will be developed to analyse and correct for the discrepancies.

2.2 Simcyp's generic PBPK models

The structure of the PBPK model developed by Simcyp for rats and humans is shown on Fig. 2. A set of ADME sub-models, able to predict substance-specific parameter values for a large range of molecules, is already coupled to this model (Howgate, et al., 2006, Jamei, et al., 2009a, Jamei, et al., 2009b, Jamei, et al., 2009c). Obviously, while the model structure remains the same, parameter values and corresponding absorption, distribution, metabolism and excretion (ADME) sub-models differ between species. Dosing can be by intra-venous injection (*i.v.*) or *per os* (*p.o.*) (the dermal and inhalation routes are also developed for humans). The liver and gut are sites of metabolism. Renal excretion takes place in the kidney. Solving numerically the corresponding set of differential equations yields the time-course of the quantity or concentration of a given drug in any of the organs or tissues included in the model.

2.3 Specific parameters for diazepam in rats

Simcyp also provides default parameter values for diazepam with its rat model. A total *in vivo* clearance of 20.4 mL/min is reported (Klotz, et al., 1976, Igari, et al., 1983). To model the extra-hepatic metabolism of diazepam in rats, hepatic clearance was set equal to three-quarters of total clearance ($CL_{iv} = 0.75 \times CL_{tot} =$ 15.3 mL/min; $CL_r = 0.25 \times CL_{tot} = 5.1$ mL/min).

The intravenous injection of 1.2 mg/kg used by Igari et al. (1983) in their experiments on Wistar was simulated.

2.4 Specific parameters for diazepam in humans

Diazepam is not part of the default substances provided by Simcyp's human model. We used the physico-chemical characteristics specified by Simcyp's rat model (molecular weight: 284.7 g/mol; log octanol to water partition coefficient: 2.9; monoprotic base with a *pKa* of 3.4). Blood binding parameters where obtained from the literature (fraction unbound in plasma, fu_p: 0.022; blood to plasma ratio: 0.58) (Klotz, et al., 1975, Klotz, 1985, Jones and Larsson, 2004). Tissue-to-plasma concentration ratios (K_p) were automatically estimated by the corresponding ADME model. For *in vivo* clearance, CL_{iv}, the published value of 1.596 L/h was used (Klotz, et al., 1975).

To simulate Klotz et al. (1975) data, a two-minute intravenous infusion of 0.1 mg/kg diazepam was simulated for a representative healthy volunteer, followed up for 72 hr.

2.5 Specific parameters for ciclosporin A in rats

Ciclosporin A was not one of the drugs predefined for in Simcyp's rat model. We used the physico-chemical properties provided by the Simcyp's human model (molecular weight: 1202 g/mol; log octanol to water partition coefficient: 4.3; neutral compound). Blood binding parameters where obtained from the literature (fraction unbound in plasma, fu_p: 0.062; fraction unbound in blood, fu_b: 0.05 for intra-venous doses up to 6 mg/kg and 0.094 for doses above) (Kawai, et al., 1998, Tanaka, et al., 2000). The tissue-to-plasma concentration ratios (or partition

coefficients, K_p) (Tab. 1) were derived by multiplying published tissue-to-blood ratios (Tanaka, et al., 2000) by fu_p / fu_b.

In vivo plasma clearance values were derived from Tanaka et al., (2000) by multiplying the blood clearance by fu_p / fu_b: (CL_{iv}: 0.82 mL/min for an intra-venous dose of 1.2 mg/kg; 0.94 mL/min for 6 mg/kg; 0.35 mL/min for 30 mg/kg). Three experiments on Sprague-Dawley rats with 2-minute intra-venous infusions of 1.2 mg/kg, 6 mg/kg and 30 mg/kg ciclosporin A, respectively, were simulated (Tanaka, et al., 2000).

2.6 Specific parameters for ciclosporin A in humans

Simcyp provides default parameter values for ciclosporin A pharmacokinetics in humans. We used the predefined "Healthy volunteers" population. To match the characteristics of the population studied by Gupta et al. (1990), the reference body mass of the subjects was set to an average of 64 kg, with age ranging from 24 to 34 years, with 50% males. The duration of the simulated study was 24 hr after an oral administration of 10mg/kg of ciclosporin A. A first-order oral absorption model was used, with rate constant 0.91 h^{-1} (Kawai, et al., 1998).

For extrapolation to a larger population the age range was set from 20 to 70 years. To study variability, Monte Carlo simulations (Bois, et al., 1990, Bois, et al., 1991, Bois and Paxman, 1992, Rostami-Hodjegan and Tucker, 2007) of 100 subjects were performed.

3 Results

3.1 Diazepam in rats and humans

A set of simulations was performed to compare the model predictions to actual data published on rat and human diazepam pharmacokinetics. Only minimal adjustments to the parameter values, has described in the Methods section, were made. Fig. 3 shows the results obtained for the rat. Only plasma, brain, liver and kidney data (Igari, et al., 1983) and model simulations are shown because these are the compartments of primary interest in PREDICT-IV. Similar results are obtained in the other tissues.

Fig. 4 presents similar results in human plasma. The experimental data of Klotz et al. (1975) for two healthy volunteers are slightly overestimated, but we have not attempted here to account for the heterogeneity of the human population.

3.2 Ciclosporin A in rats and humans

Fig. 5 shows the data obtained by Tanaka et al. (2000) in Sprague-Dawley rats after intra-venous administrations of 1.2, 6 or 30 mg/kg of ciclosporin. The model-predicted time-courses are shown on the same Figure. Here again, no specific adjustment was made to "fit" the data, beyond the obvious adaptations mentioned in the Methods section.

For humans, Fig. 6 shows the data of Gupta et al. (1990), obtained after oral administration of 10 mg/kg of ciclosporin A to a healthy volunteer, in low fat or high fat diet conditions. Monte Carlo simulation of 100 similar subjects (diet not considered) were performed, and the average, 5^{th} and 95^{th} percentiles of the simulated values are plotted.

Fig. 7 illustrates the very purpose of all this modelling exercise: predicting the concentration time-course of a substance (here ciclosporin A) in the brain, liver and kidney for a varied human population. We simulated 100 healthy subjects, aged from 20 to 70 years, taking a daily dose of 10 mg/kg of ciclosporin A for 60 days. Note that during the first week of treatment, liver exposure is the highest of the three, but brain exposure after 60 days is higher than liver or kidney exposure.

Discussion

Predicting organ toxicity beyond acute effects is a significant scientific challenge. The team of PREDICT-IV is taking an inclusive approach to the problem, coupling *in vitro* testing, *in silico* ADME, PBPK and dose-response models in an integrated predictive tool. An important aspect of the work will be the validation, or benchmarking, of the model predictions with a set of about 30 reference drugs, known for their neurotoxicity, hepatotoxicity or kidney toxicity. To that effect, Simcyp Ltd has already extended its human pharmacokinetic model and developed a rat model. The results presented here are indeed limited to only two compounds, diazepam and ciclosporin, but give an idea of the quality of the predictions obtained with the current model. We will test the approach with whole set of reference drugs, and we even expect improvements when additional, more precise, ADME properties prediction models will be included.

We have performed here Monte Carlo simulations to describe inter-individual variability only to a limited extent, and only for ciclosporin A. This type of simulations will be performed for more diverse populations, potentially more susceptible to organ toxicity. Accessing this type of results is difficult for *in vitro*

testing alone, hence the synergy with mathematical modelling, which is one of the goals of PREDICT-IV.

The results presented here go only as far as the prediction of organ exposures after medium term repeated administration of a drug. We could have extended our simulations to longer times or different doses, but stable values were obtained for ciclosporin A after 60 days of dosing and we would have gained no more insight with additional follow-up. Obviously this may not be true for other compounds and will have to be assessed on a case by case basis. In any case, long term target organ exposure is the relevant measure of dose for the prediction of organ toxicity after sustained exposure. We have still to couple our PBPK model to dose-response models for a complete PK/PD approach. The dose-response component will also add a time dimension of its own, but the work on correlated multi-dimensional dose-response models in PREDICT-IV has just begun.

We are focusing on local organ toxicity, for which the target tissue concentration can be considered as the relevant measure of dose. Systemic toxicity would be much more of a challenge, combining effects of the dysfunction of several organs. However, we are only a step away from being able to describe the toxic effects of co-administered substances, since our models have the capability to account for at least pharmacokinetic and metabolic interactions (Rostami-Hodjegan and Tucker, 2004, Bois, submitted). We are therefore confident in our ability to make significant contributions to the 3Rs agenda and to predictive clinical and environmental toxicology in the near future.

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Tables

Tab. 1: Tissue-to-blood partition coefficients used for ciclosporin A in rats.

Tissue	Dose (mg/kg)		
	1.2	**6**	**30**
Adipose	8.36	7.77	4.94
Bone	2.81	3.76	1.08
Brain	0.01 [a]	0.30	0.53
Gut	6.39	6.49	2.55
Heart	7.29	5.74	2.52
Kidneys	9.54	12.90	4.42
Liver	14.63	13.64	5.67
Lungs	8.85	6.32	5.79
Muscle	1.22	1.46	0.71
Skin	5.17	3.04	1.12
Spleen	9.15	8.64	3.61

[(a)] The brain concentration was below the limit of detection in Tanaka et al., (2000).

Figure legends

Fig. 1: Graphical representation of the integrated prediction and validation framework of PREDICT-IV.

Left panel: Predictive component of project *In vitro* studies will provide the necessary data for the development of a set of ADME QSARs and multivariate dose-response models for cellular and organ-level effects. Those models will be coupled to a generic PBPK model for humans and rats and will provide an *in silico* pharmacokinetic-pharmacodynamic (PK/PD) tool predictive of medium- to long-term toxicity. Right panel: In order to validate that tool, its predictions for plasma and tissue drug levels will be confronted to pharmacokinetic data obtained in humans or rats. To relate plasma to pathological effects, the pharmacodynamic component will be validated on the basis of published animal (rat) data.

Fig. 2: Graphical representation of the physiologically based pharmaco-kinetic (PBPK) model of the human or rat body.

The model structure is the same for rats and humans, but parameter values and corresponding absorption, distribution, metabolism and excretion (ADME) sub-models differ between species. Dosing can be by intra-venous injection (*i.v.*) or *per os* (p.o.) (the dermal and inhalation routes are being developed for humans). The liver and gut are sites of metabolism. Renal excretion takes place in the kidney. Solving numerically the corresponding set of differential equations yields the time-course of the quantity or concentration of a given drug in any of the organs or tissues included in the model.

Fig. 3: Model predictions and published data on diazepam pharmacokinetics in the rat.

The rat PBPK model was run to simulate the intra-venous administration of 1.25 mg/kg of diazepam to Wistar rats (Igari, et al., 1983). The solid lines represent the predicted time-courses. The dots figure the corresponding data (average concentrations measured in 3 to 5 rats).

Fig. 4: Model predictions and published data on diazepam time-course in human plasma.

The human PBPK model (solid line) was run to simulate the intra-venous administration of 0.1 mg/kg of diazepam to human healthy volunteers. The data correspond to those obtained in two subjects (circles and squares, respectively) by (Klotz, et al., 1975).

Fig. 5: Model predictions and published data on ciclosporin A pharmaco-kinetics in the rat.

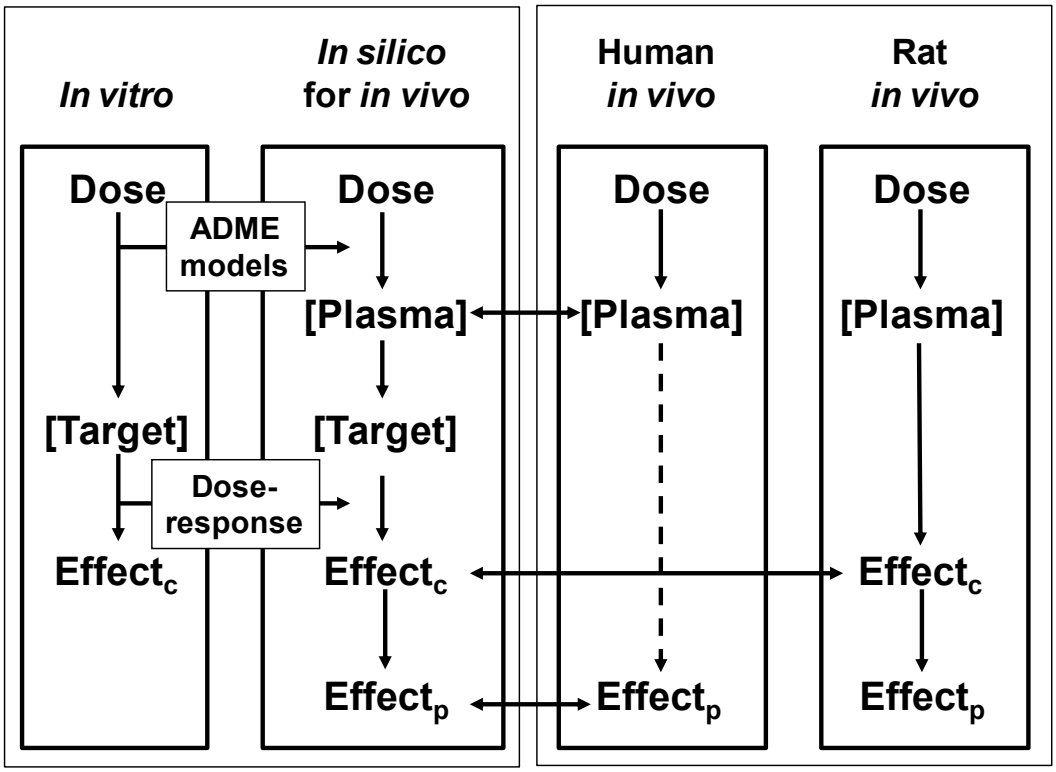
The rat PBPK model was run to simulate the intra-venous administration of 1.2, 6 or 30 mg/kg of ciclosporin to Sprague-Dawley rats (Tanaka, et al., 2000). The solid lines represent the predicted time-courses. The dots (circles: 30 mg/kg dose; squares: 6 mg/kg dose; diamonds: 1.2 mg/kg dose) figure the corresponding data (average concentrations measured in 3 rats).

Fig. 6: Modelled and observed ciclosporin A pharmacokinetics in human blood.

The data of Gupta et al. (1990), obtained after oral administration of 10 mg/kg of ciclosporin A to a healthy volunteer, are represented by either circles (low fat diet conditions) or square (high fat diet). The thick solid line represents the corresponding average model predictions for 100 similar subjects (diet not considered). The thin lines indicate the 5^{th} and 95^{th} percentiles of the 100 simulated values.

Fig. 7: Predictions of long-term ciclosporin A pharmacokinetics in humans.

Time-course of ciclosporin A concentrations predicted in the brain, liver and kidney of healthy subjects, aged from 20 to 70 years, and taking a daily dose of 10 mg/kg of ciclosporin A. The lines represent the average concentrations at any time, for 100 simulated subjects. The bars at the right indicate the range (from 5^{th} to 95^{th} percentiles) of concentrations found in the organs after 60 days of treatment. These are relevant values for the prediction of organ toxicity after sustained exposure.



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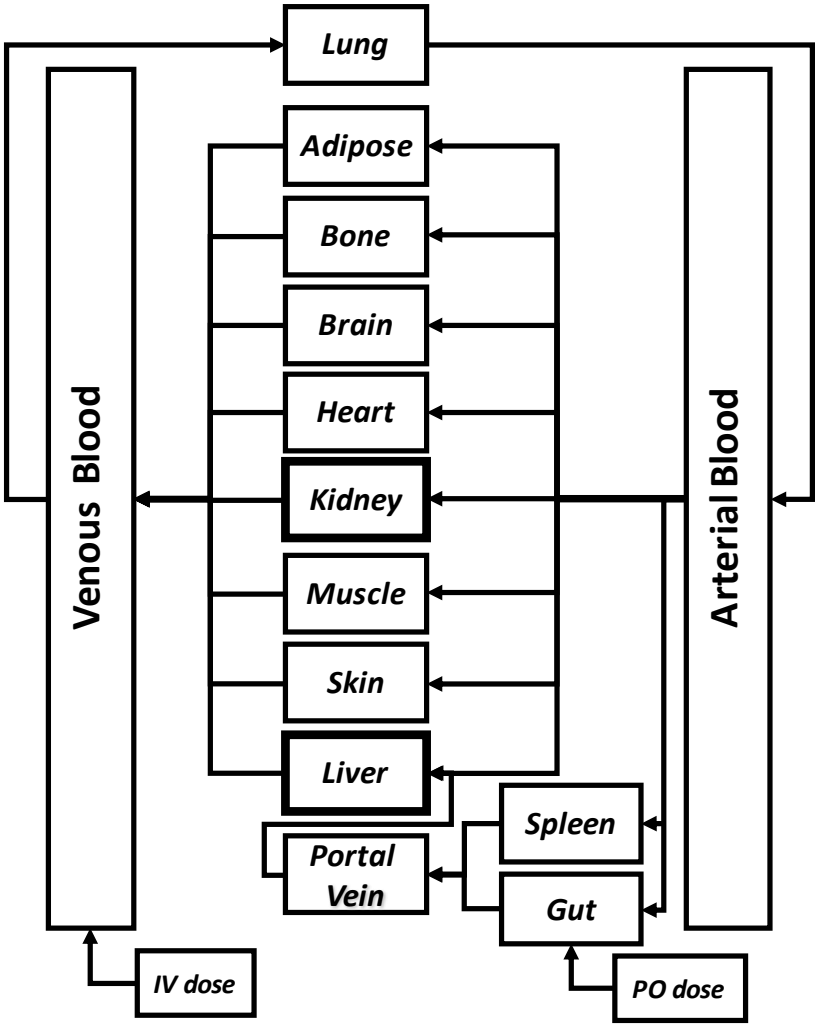

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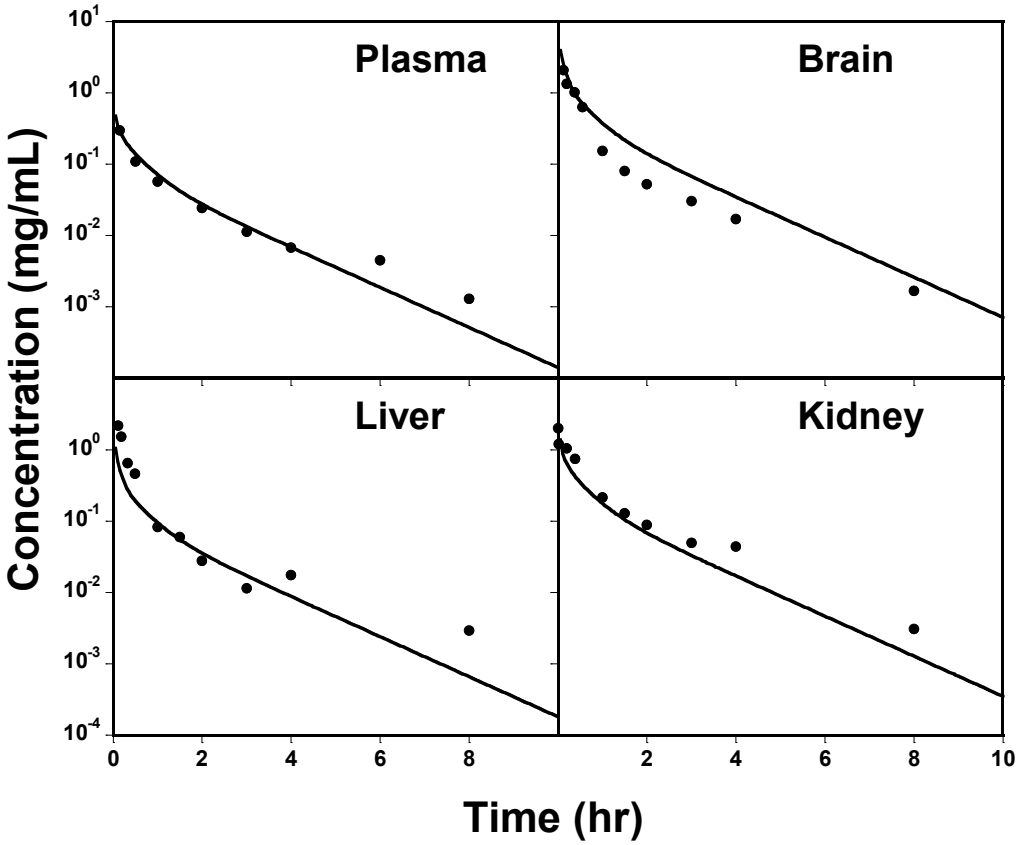

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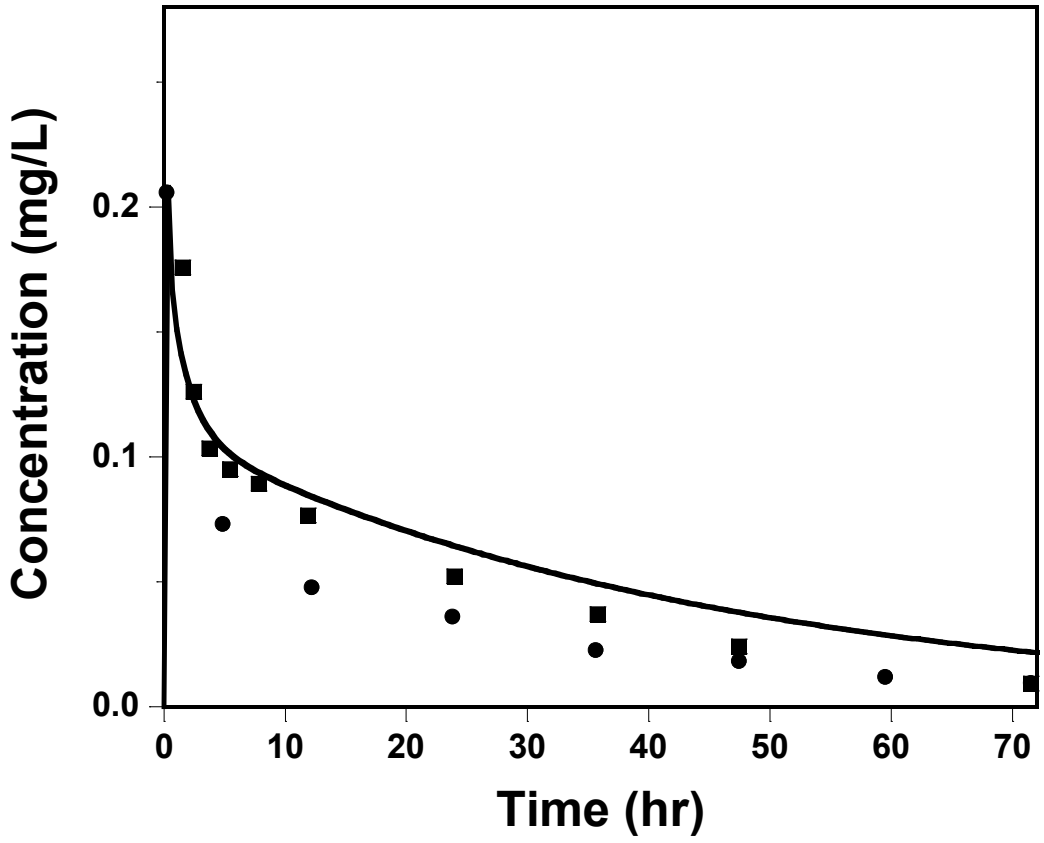

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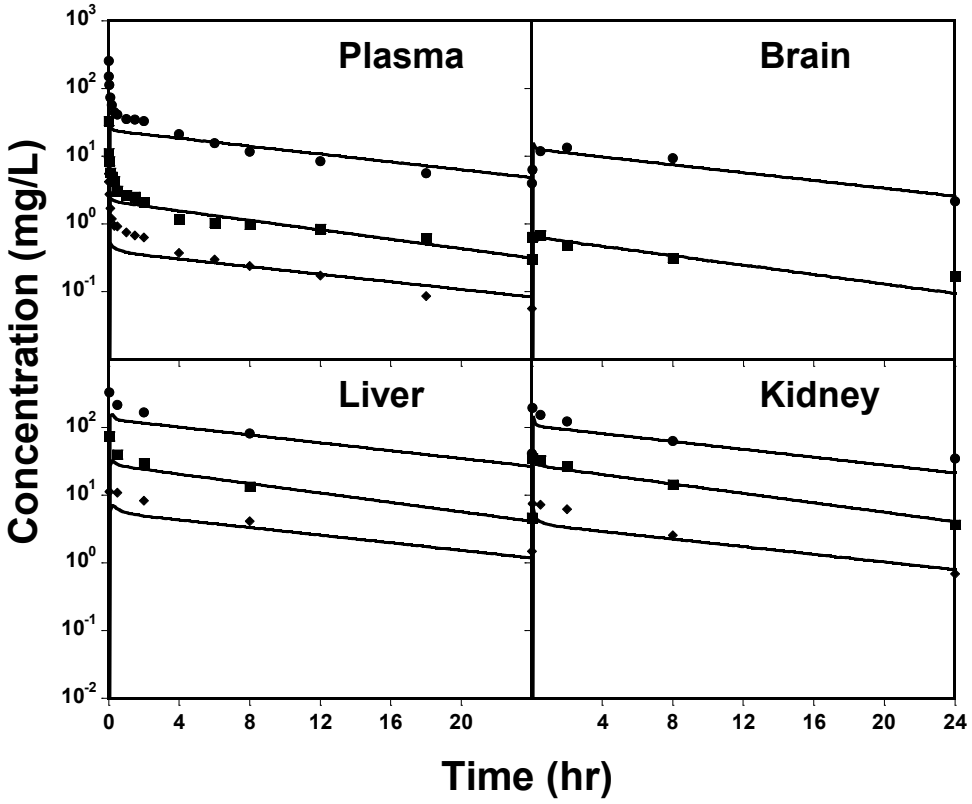

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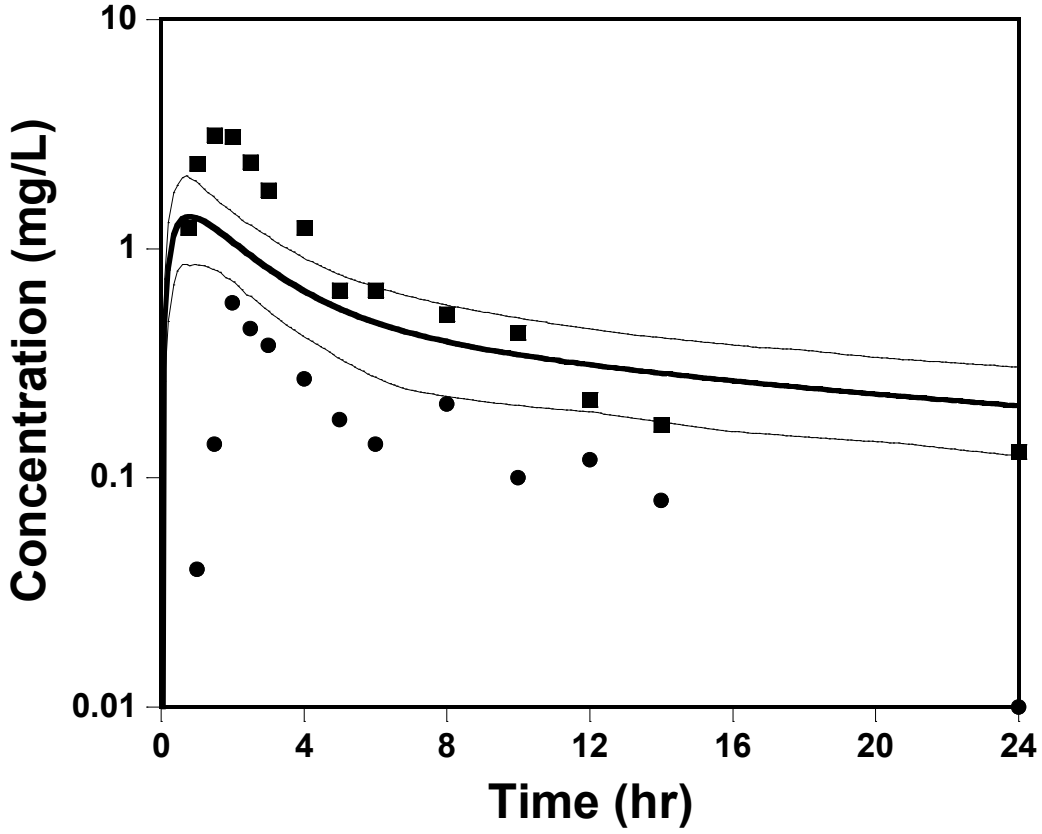

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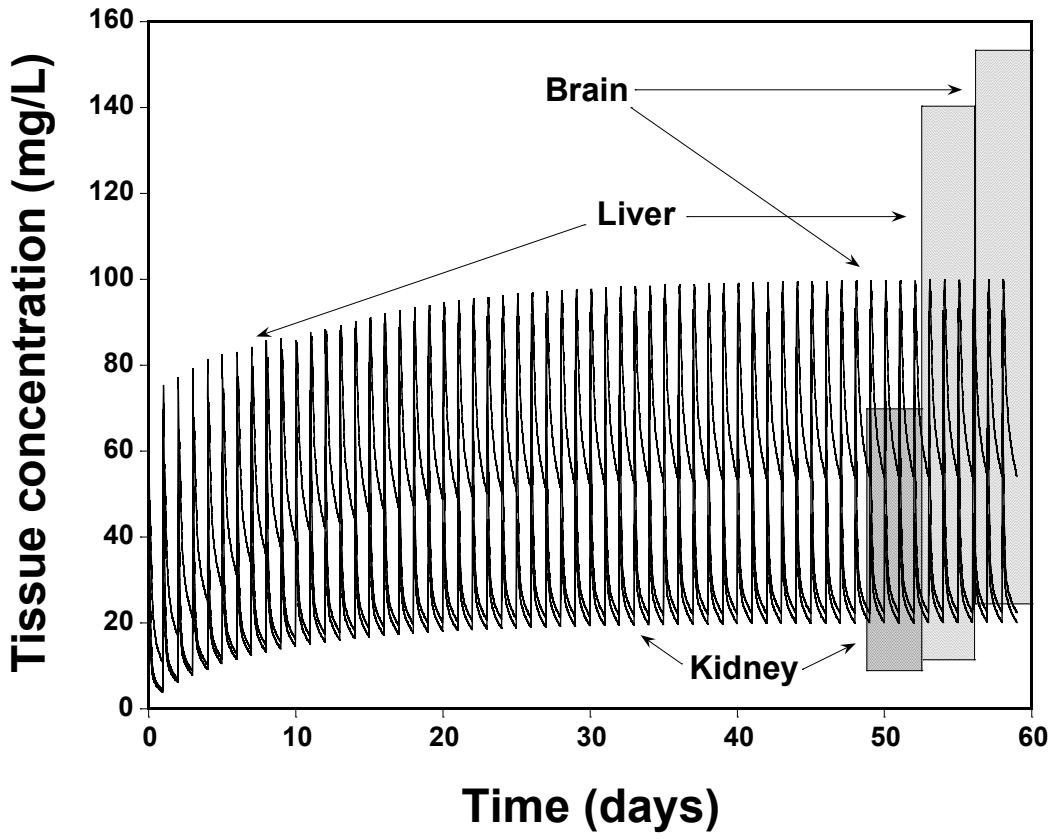

Fig. 7: Predictions of long-term ciclosporin A pharmacokinetics in humans.

Time-course of ciclosporin A concentrations predicted in the brain, liver and kidney of healthy subjects, aged from 20 to 70 years, and taking a daily dose of 10 mg/kg of ciclosporin A. The lines represent the average concentrations at any time, for 100 simulated subjects. The bars at the right indicate the range (from 5^{th} to 95^{th} percentiles) of concentrations found in the organs after 60 days of treatment. These are relevant values for the prediction of organ toxicity after sustained exposure.