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PUBLISHABLE SUMMARY

We here describe physiologically-based pharmacokinetic (PBPK)-based in vivo contextualization of in vitro toxicity data (PICD), which quantitatively predicts in vivo drug response over time by integrating multiple levels of biological organization. Explicitly, in vitro toxicity data at the cellular level were integrated into whole-body PBPK models at the organism level by coupling in vitro drug exposure with in vivo drug concentration-time profiles simulated in the extracellular environment of the organ. The predictive accuracy of PICD was assessed by comparing in vivo drug response predicted for rats with observed in vivo measurements. PICD provides a generic platform to investigate drug-induced toxicity at a patient level and thus may facilitate risk assessment during drug development in the future.

OBJECTIVES

Changes at different biological levels can nowadays be measured by omics technologies to describe cellular alterations in response to toxic drug concentrations. However, a systematic consideration of in vitro toxicity data within an in vivo context to describe drug-induced cellular changes in vivo still remains challenging. In this regard, the consideration of experimental measurements within a whole-body context would allow the characterization of adverse outcome pathways at patient level.

INTRODUCTION

Physiologically-based pharmacokinetic (PBPK) models are nowadays routinely used in pharmaceutical development. In general, PBPK modelling aims for a mechanistic understanding of physiological processes governing drug pharmacokinetics (PK) within the human body. Relevant tissues and organs are explicitly represented in PBPK models and are connected through convective blood flow (Figure 1). Organs are further subdivided into plasma, red blood cells, interstitial and intracellular space. On the one hand, PBPK are based on large collections of physiological and anatomical information to quantify for example organ volumes or perfusion rates. On the other hand, physicochemical information of a specific drug is used to calculate partition organ-tissue coefficients as well as tissue permeabilities. Since PBPK models include a large amount of mechanistic information, these models are well-suited for extrapolation of various treatment scenarios such as dose extrapolations, pediatric scaling or cross-species extrapolations.

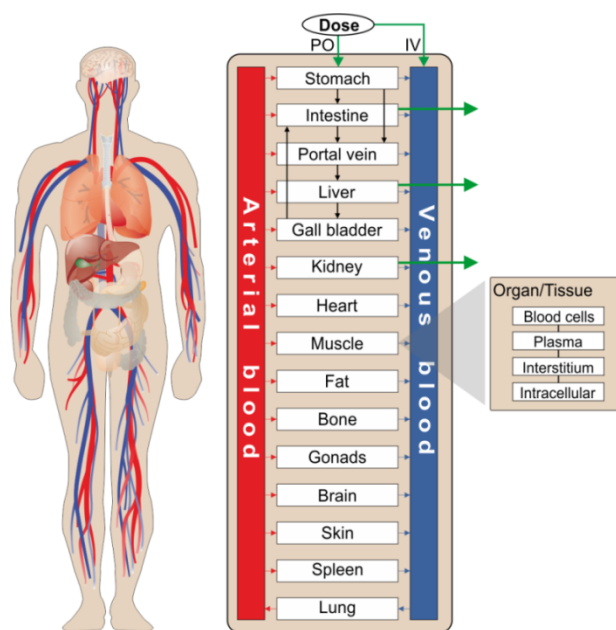


Figure 1: Physiologically-based pharmacokinetic (PBPK) modelling

Notably, PBPK models allow the simulation of time-concentration profiles in specific tissues. Moreover they enable quantification of plasma protein binding in the vascular space. The fraction unbound hence corresponds to the medium drug concentration of many *in vitro* assays. Matching simulated interstitial tissue concentrations to *in vitro* media composition ensures a direct comparability of *in vitro* to *in vivo* drug exposure. At the cellular scale, network models describe molecular responses in the face of external stimuli. This extracellular concentration corresponds in turn to the unbound fraction of a drug in a specific tissue. We here use PBPK to investigate molecular networks within a whole-body context.

RESULTS

We have developed a generic approach called PICD (PBPK-based *in vivo* contextualization of *in vitro* toxicity data) for the vertical integration of *in vitro* omics data and molecular networks at the cellular scale into PBPK models at the whole body level [1]. PICD integrates *in vitro* toxicity data into drug-specific PBPK models to translate drug-induced *in vitro* findings to an actual *in vivo* situation thereby predicting drug-specific response profiles induced by different dose levels administered in patients. At the cellular level, *in vitro* toxicity data are coupled with equivalent PBPK-simulated concentration-time profiles at the organism level to allow a quantitative description of time-resolved *in vivo* drug response in key cellular processes and biological pathways (Figure 2).

In an initial step, drug-specific PBPK models are developed to identify *in vivo* doses that are directly related to *in vitro* drug exposure. We used the Open TG Gates library [2] as an exemplary *in vitro* assay. Notably, the *in vitro* setup is explicitly represented in the PBPK models by specifically adjusting *in vivo* drug plasma protein binding in the PBPK model correspondent to the *in vitro* concentrations. PK profiles simulated in the interstitial space of the liver are then coupled with *in vitro* toxicity data to *predict in vivo* drug response at the cellular level following *in vivo* drug administration at the organism level (Figure 2). To couple interstitial concentration-time profiles with *in vitro* toxicity data from the Open TG Gates library [2] with whole-body PK models, *in vivo* doses are identified by PBPK simulations for intravenous drug administration such that the *in vitro* drug exposure equals the interstitial area under the curve in the liver

or the heart at each experimental time point. Dose-response curves are then generated for all time points by mapping *in vitro* toxicity data to the identified *in vivo* doses (Figure 2).

The identified *in vivo* doses are next averaged horizontally to three doses (d_{low} , d_{middle} , and d_{high}), which thus represent the *in vivo* equivalents to *in vitro* concentrations (low, middle, and high). Drug response values are next calculated and assigned to doses d_{low} , d_{middle} , and d_{high} by linearly interpolating dose-response curves (Figure 2) to predict *in vivo* drug response in relevant Gene Ontology terms, as well as in human pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and in toxicity-related pathways (TOX) (SABiosciences). The use of PICD enables thus a time-resolved description of drug-induced *in vivo* response at the cellular level by the integration of several levels of biological organization.

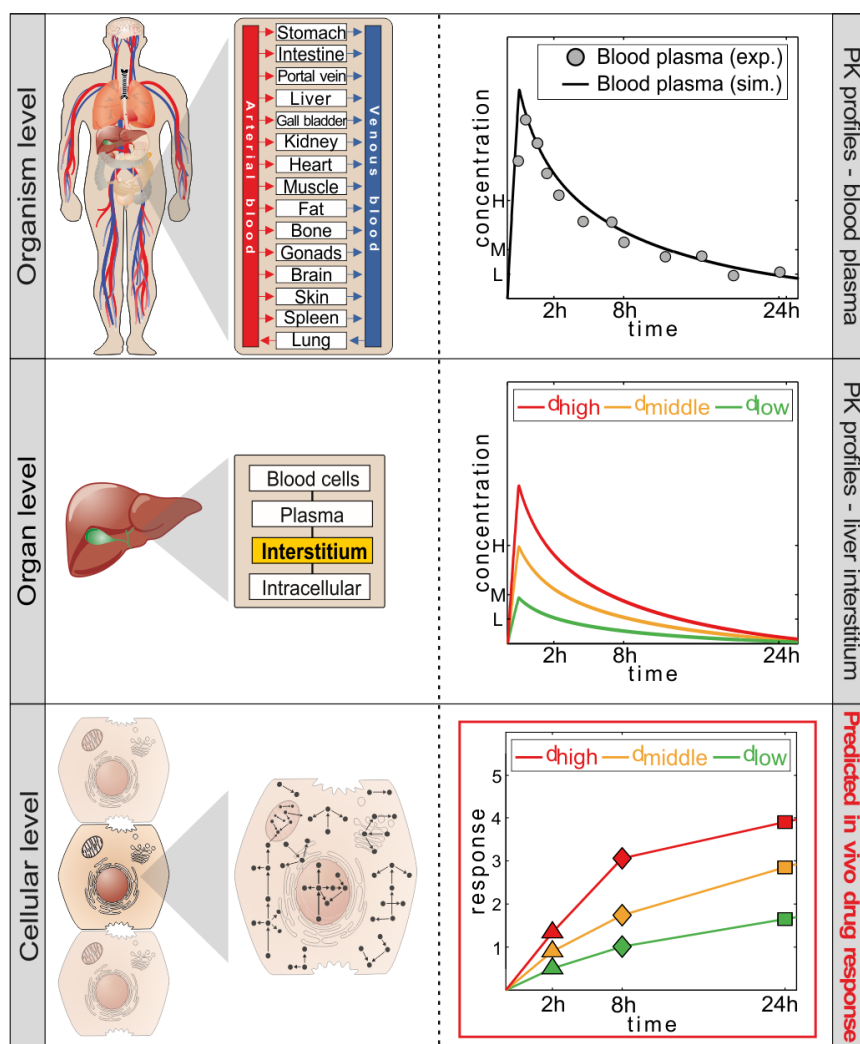


Figure 2: PBPK-based *in vivo* contextualization of *in vitro* toxicity data (PICD). Integration of *in vitro* toxicity data into whole-body PBPK models allows to quantitatively predict *in vivo* drug responses in molecular networks in human patients.

So far we have applied PICD in the analyses of 15 hepatotoxicants: acetaminophen (APAP), amiodarone (AD), azathioprine (AZA), cyclophosphamide (CPA), cyclosporine A (CSA), diclofenac (DFN), erythromycin (ERY), flutamide (FT), haloperidol (HPL), isoniazid (INH), phenobarbital (PB), phenytoin (PHE), rifampicin (RIF), simvastatin (SST), valproic acid (VPA) (3) (Figure 3,4). The drugs were selected from the HeCaTos list of compounds based on pharmaceutical and chemical diversity, physicochemical properties, availability of *in vitro* toxicity data and experimental drug concentration-time profiles as well as concern for drug-

induced liver injury (DILI). PBPK models were developed for each of the compounds and carefully evaluated against clinical PK data from the literature (Figure 3, 4).

To assess the predictive accuracy of PICD, *in vivo* toxicity data measured in rat livers [2] were used. Rat PBPK models of the various hepatotoxicants together with *in vitro* toxicity data obtained in rat hepatocytes [2] were translated with PICD to predict *in vivo* drug response in rats. Cellular processes and biological pathways were then predicted for all three doses orally administered in rats and were subsequently correlated with corresponding *in vivo* observations. To check whether the application of PICD actually improved predictions *in vivo* compared to the *in vitro* situation, temporal *in vitro* patterns and predicted *in vivo* drug responses were both correlated to respective *in vivo* observations. *In vitro* drug response profiles of perturbed biological pathways and biological processes showed almost no relevance for the *in vivo* situation (Pearson's $r = -0.04 - 0.36$, $p > 0.05$). In contrast, applying PICD obviously increased the concordance with *in vivo* measurements. Observed *in vivo* responses of affected molecular functions were in agreement with the predicted *in vivo* drug responses (Pearson's $r = 0.81$, $p = 1.7E-05$) (Figure 5). The comparison of *in vivo* rat expression data [3] with corresponding profiles with PICD hence shows the general improvement achieved through the vertical integration of compound specific molecular networks into PBPK models.

To conclude, the application of PICD provides a generic platform for a systematic analysis of drug-induced hepatotoxicity by coupling *in vitro* omics data [2] with *in vivo* pharmacokinetics simulated for therapeutic and toxic doses using PBPK modeling. Drug-induced hepatotoxicity could be thus analyzed at the patient level to facilitate the understanding of differences in drug response following oral administration of therapeutic and toxic doses in humans and to identify drug specific adverse outcome pathways. The vertical integration of compound specific molecular networks into PBPK models might help to improve the clinical risk assessment in the future [4].

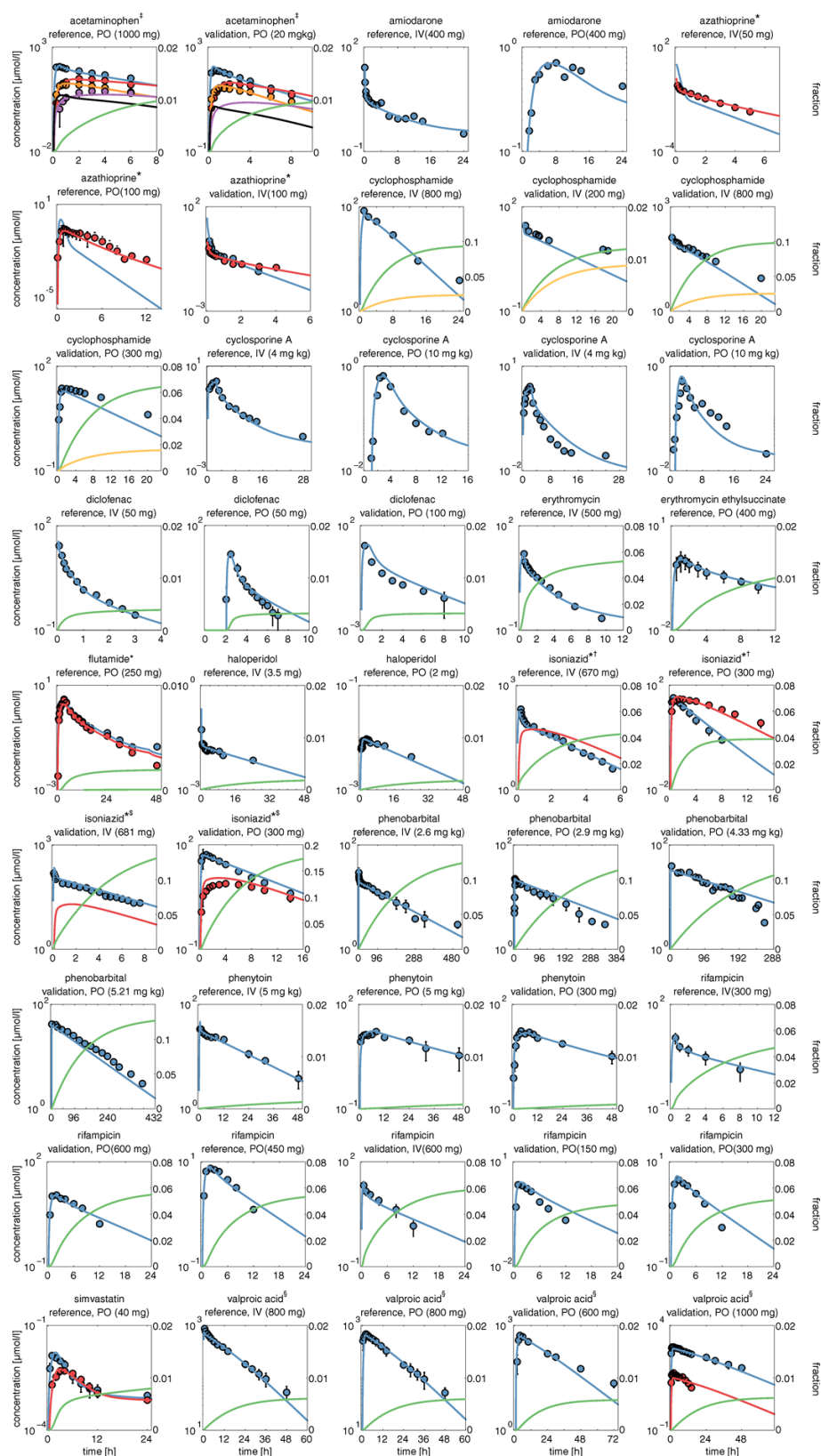


Figure 3: Simulated concentration-time curves (lines) for parent drugs (blue) were assessed with experimental PK profiles (circles). Renal (green) and biliary (dark yellow) excretion rates were simulated to match experimental measurements.



Figure 4: Simulated concentration-time profiles were compared to experimental PK data. Observed vs. predicted plots including the RMSD value and the coefficient of determination (R^2) were generated for all reference and validated PBPK models.

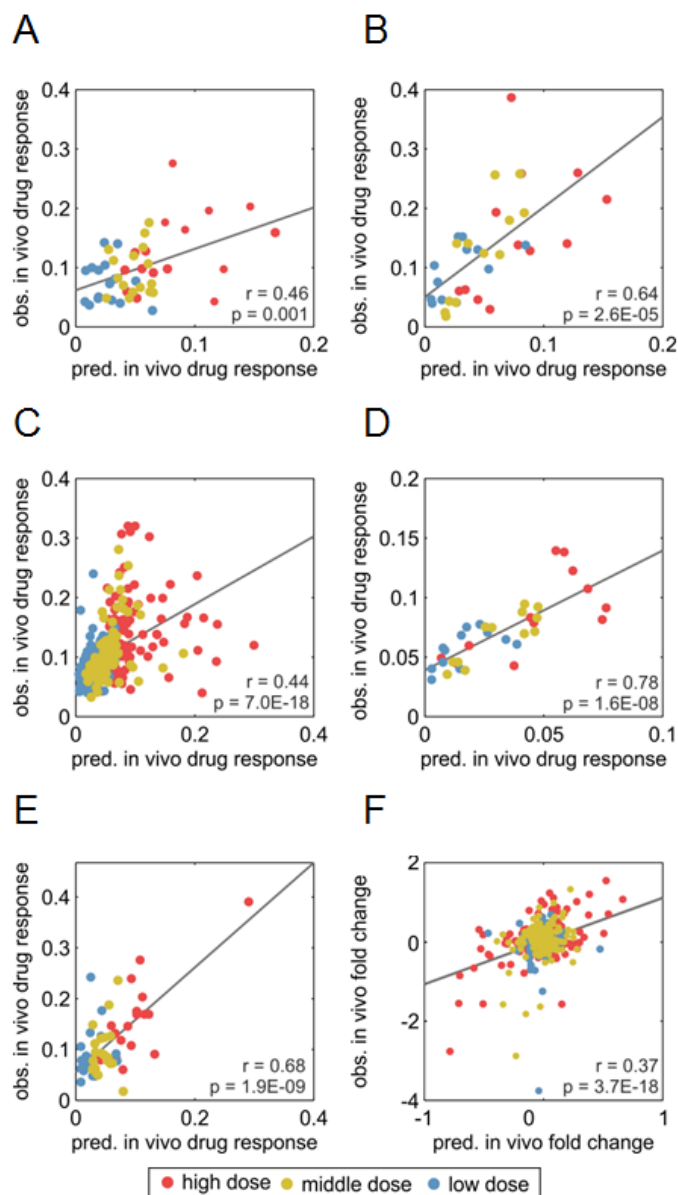


Figure 5: Correlation of predicted profiles with in vivo measurements in rats. Correlation between predicted (pred.) in vivo profiles of drug response and gene expression with observed (obs.) profiles measured in vivo following oral administration of the three doses used in the rat study (low dose = yellow, middle dose = blue, high dose = red). All cellular processes or biological pathways that were significantly regulated in at least one treatment and all genes analyzed in the case studies were considered for the correlation of drug response and gene expression, respectively. Correlation analyses were performed by calculating Pearson's correlation coefficient r and the corresponding p-value p . (A) Correlation of affected KEGG pathways. (B) Correlation of affected toxicity-related pathways. (C) Correlation of affected biological processes. (D) Correlation of affected cellular components. (E) Correlation of affected molecular targets.

DIFFICULTIES

Adequate necessary clinical patient data are required to further validate the approach.

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