A multi-organ MPS-PBPK workflow for profiling CYP2D6 population activity through dextromethorphan metabolism

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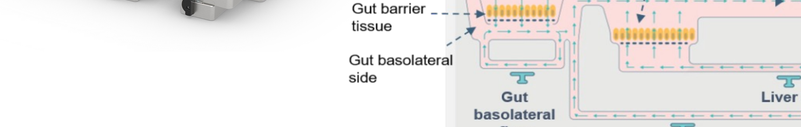
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Introduction

New approach methodologies (NAMs) are now delivering on their promise as practical alternatives that accelerate and improve drug discovery [1,2]. Conventional *in vitro* and *in vivo* models used to estimate absorption, clearance and oral bioavailability often lack translational reliability, resulting in poor prediction of clinical dosing for Investigational New Drug applications. While physiology based pharmacokinetic (PBPK) modeling can help bridge this gap by integrating preclinical data into a systems-level framework, its predictive performance is fundamentally limited by quality, mechanistic scaling and human relevance of the input parameters.

This work presents a microphysiological system (MPS)-to-PBPK workflow for clinical dose prediction from MPS-derived time course data. As an example, the enzyme Cytochrome P450 2D6 (CYP2D6) was chosen as a highly polymorphic enzyme exhibiting substantial interindividual variability in metabolic activity. Consequently, drugs metabolized by CYP2D6 frequently require dose adjustment based on patient-specific activity scores. Dextromethorphan was utilized as a probe substrate for the CYP2D6 enzyme.

In this study, a primary human Gut/Liver MPS was utilized to generate human-relevant parameters. This system utilizes primary cells using RepliGut® Planar jejunal epithelial monolayers and liver microtissue derived from human hepatocyte donors. Experimental concentration-time profiles from the MPS were used to construct an *in silico* compartmental model, enabling estimation of key parameters including intestinal absorption, hepatic intrinsic clearance and first-pass extraction. These experimentally derived parameters were subsequently scaled and incorporated into a PBPK model for prediction of clinical pharmacokinetics.



Methods

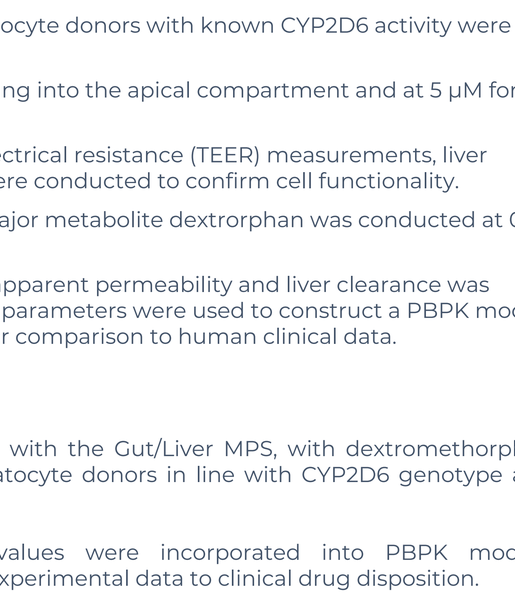
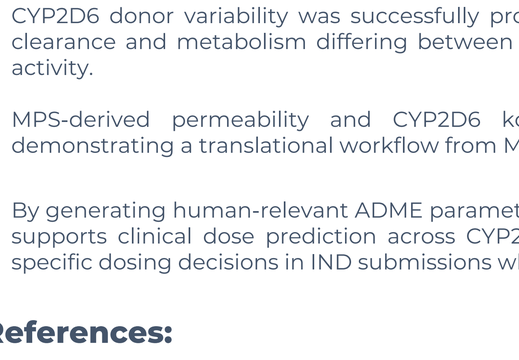
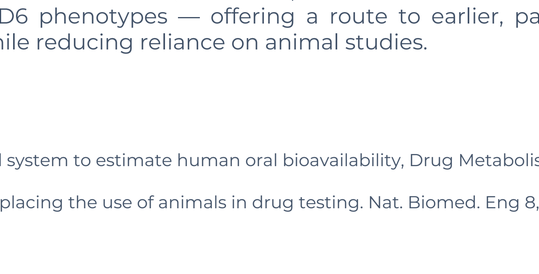


Figure 1: The human-relevant PhysioMimix® Bioavailability assay.

A. The PhysioMimix® Core microphysiological system



C. Dual-organ plate schematic and directions of flow



- Liver microtissues from three primary human hepatocyte donors with known CYP2D6 activity were tested in the PhysioMimix® Bioavailability Assay.
- Dextromethorphan was dosed at 50 µM for oral dosing into the apical compartment and at 5 µM for IV dosing into the liver compartment.
- Cell functionality assays including transepithelial electrical resistance (TEER) measurements, liver CYP3A4 activity, albumin and LDH quantification were conducted to confirm cell functionality.
- Quantification of both dextromethorphan and its major metabolite dextrorphan was conducted at 0, 2, 6, 24, 48, and 72 hours with LC-MS/MS.
- Extraction of relevant ADME parameters including apparent permeability and liver clearance was conducted with *in silico* modeling. Extracted ADME parameters were used to construct a PBPK model with PK-SIM® from Open Systems Pharmacology for comparison to human clinical data.

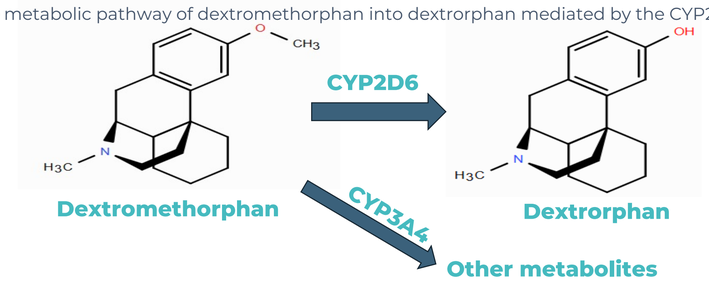
Conclusions

- CYP2D6 donor variability was successfully profiled with the Gut/Liver MPS, with dextromethorphan clearance and metabolism differing between hepatocyte donors in line with CYP2D6 genotype and activity.
- MPS-derived permeability and CYP2D6 kcat values were incorporated into PBPK models, demonstrating a translational workflow from MPS experimental data to clinical drug disposition.
- By generating human-relevant ADME parameters from the Gut/Liver MPS, the MPS-to-PBPK workflow supports clinical dose prediction across CYP2D6 phenotypes — offering a route to earlier, patient-specific dosing decisions in IND submissions while reducing reliance on animal studies.

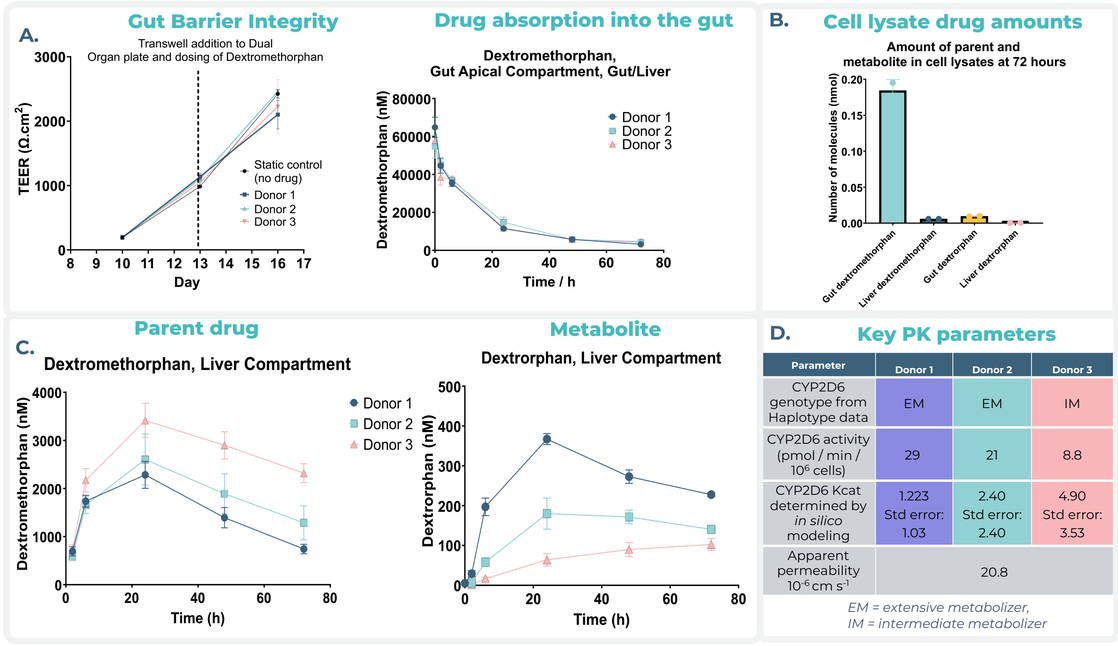
References:

[1] Abbas, Y, *et al.* ,A primary human Gut/Liver microphysiological system to estimate human oral bioavailability, Drug Metabolism and Disposition, 53, 9, (2025).
[2] Stresser, D.M., *et al.* Towards in vitro models for reducing or replacing the use of animals in drug testing. Nat. Biomed. Eng 8, 930–935 (2024).

Figure 2: The metabolic pathway of dextromethorphan into dextrorphan mediated by the CYP2D6 enzyme.



Results



Parameter	Donor 1	Donor 2	Donor 3
CYP2D6 genotype from Haplotype data	EM	EM	IM
CYP2D6 activity (pmol / min / 10^6 cells)	29	21	8.8
CYP2D6 Kcat determined by *in silico* modeling	1.223 Std error: 1.03	2.40 Std error: 2.40	4.90 Std error: 3.53
Apparent permeability 10^{-6} cm s^{-1}	20.8		

EM = extensive metabolizer, IM = intermediate metabolizer

Figure 3: Dextromethorphan profiling in the Gut/Liver MPS aligns with liver donor CYP2D6 activity . **(A)** Gut cells maintain a tight barrier throughout the dosing period. **(B)** Enterocyte and hepatocyte lysate quantities of dextromethorphan and dextrorphan concentration, potentially indicative of lysosomal trapping of dextromethorphan **(C)** Clear distinctions in both parent and metabolite concentrations can be observed between hepatocyte donors. Faster clearance of dextromethorphan in the liver compartment corresponds with greater production of dextrorphan. **(D)** Extraction of PK parameters from time-course data can be conducted using an *in silico* model.

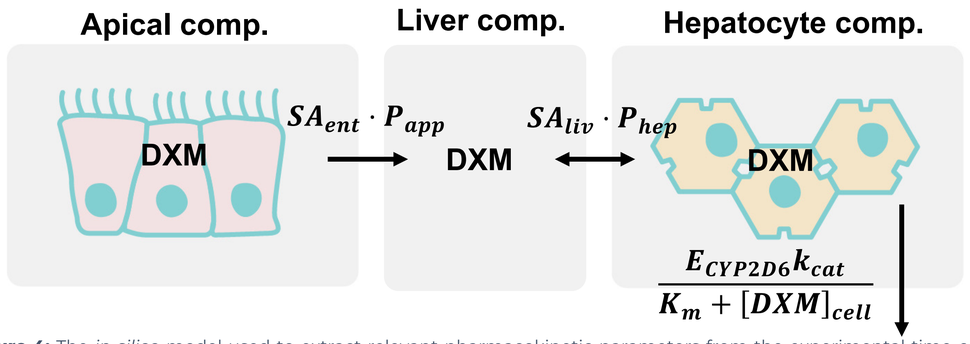


Figure 4: The *in silico* model used to extract relevant pharmacokinetic parameters from the experimental time-course data. DXM: dextromethorphan, SA_{ent}: Surface area of enterocytes, P_{app}: apparent permeability, SA_{liv}: surface area of hepatocytes, P_{hep}: hepatocyte permeability.

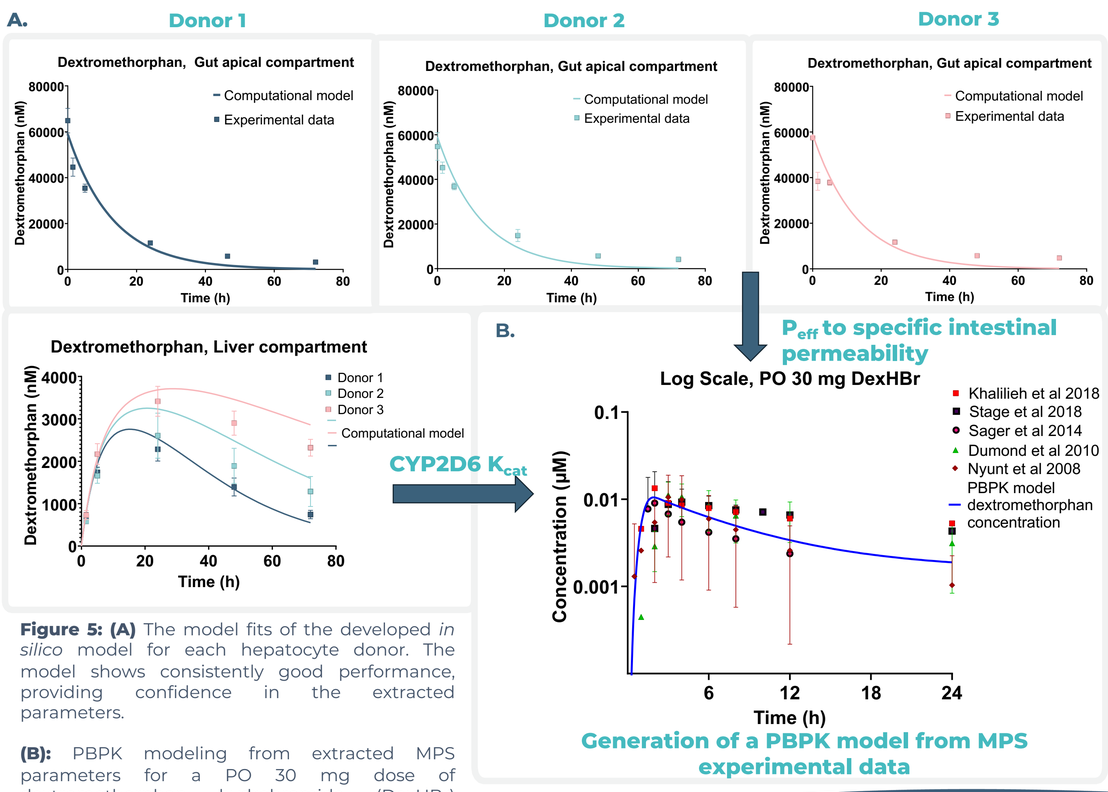


Figure 5: (A) The model fits of the developed *in silico* model for each hepatocyte donor. The model shows consistently good performance, providing confidence in the extracted parameters.

(B): PBPK modeling from extracted MPS parameters for a PO 30 mg dose of dextromethorphan hydrobromide (DexHBr) compared to extensive metabolizer clinical data.

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