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APPLICATION NOTE

Improved prediction of oral bioavailability using a gut-liver microphysiological system

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Introduction

Central to the development of all new drugs is understanding the pharmacokinetic (PK) properties of a compound. One of the most important is bioavailability, defined as the fraction of a drug that reaches systematic circulation following absorption in the gut and first pass metabolism in the liver. For drugs with low bioavailability, it can be challenging to keep within a therapeutic window. A larger dose is often required to achieve the therapeutic effect, but by doing so risks inducing adverse safety effects. An estimation of bioavailability is required to guide the drug development process and therefore, its accuracy is linked to the success, or failure, of clinical trials.

Oral bioavailability is estimated using animals with dogs, rats, mice, and non-human primates among the most common. However, the overall correlation of these species with human bioavailability (R^2 = 0.34) is weak, as outlined in a seminal study by Musther *et al.,* who investigated the bioavailability of 184 compounds[1]. In addition, the large cost and ethical considerations associated with animal studies leads to the following question: is there a more human relevant *in vitro* alternative to obtain improved estimations of oral bioavailability?

Microphysiological systems (MPS) often referred to as organ-on-a-chip have emerged as promising tools to obtain relevant human data in pre-clinical drug ADME studies. They aim to recapitulate the structural and functional characteristics of human tissue by culturing cells in 3D scaffolds, and under perfusion to mimic blood flow. Thus far the development of MPS has largely focussed on single-organ tissue models, but more complex multi-organ systems are emerging to address the need for improved *in vitro* to *in vivo* PK predictions. For oral bioavailability, a combined gut-liver MPS is required to simulate drug absorption into the gut and subsequent first pass metabolism by the liver.

Aim

Here, we describe a gut-liver MPS to estimate human oral bioavailability using the PhysioMimix™ OOC MPS-TL6 consumable plate. This plate is compatible with CN Bio's PhysioMimix™ Multi-Organ lab-benchtop instrument and consists of six wells, each with two compartments, Transwell® (for gut) and liver. Media circulates within and between each compartment via an interconnecting channel. The gut MPS is formed from a mix of epithelial and goblet cell lines that form a barrier on the inside of the Transwell membrane. The liver MPS is comprised of primary human hepatocytes (PHH), which form 3D microtissues on a porous collagen-coated scaffold.

Oral bioavailability estimations were made by simulating an oral and intravenous (IV) dose administration using a single MPS-TL6 plate. A mathematical model was used to help design and optimise the experimental methodology.

Materials & Methods

Cryopreserved plateable PHH were obtained from ThermoFisher. 0.6 x 10^6 PHH were seeded into the liver compartment of each well of the PhysioMimix™ OOC MPS-TL6 plate (CN Bio Innovations Ltd) and cultured in hepatocyte maintenance media containing 500 nM hydrocortisone (Sigma). The gut Transwell® barrier, comprised of a mixture of Caco-2/HT-29 cells (ReadyCell), was pre-cultured in gut media containing serum for 21 days before being added into the MPS-TL6 plate at day 5 of PHH culture. An oral (100 µM in serum-free gut media) and IV (10 µM in hepatocyte maintenance media) dose of the following drugs were prepared: acebutolol, phenytoin, naproxen and methylprednisolone.

For each drug, one MPS-TL6 plate was used to estimate bioavailability. Three of the six wells were used to recreate an oral dosing regimen and the remaining three an IV dosing regimen. On day 5, hepatocyte maintenance media (without drug) was added to the liver and gut basolateral compartments of oral dose wells. For IV wells, drug was dosed into liver and gut basolateral compartments. Following a media change, the gut-MPS was then inserted into the MPS-TL6 plate. For oral dose wells, drug was added to the apical side of the Transwells. For IV wells, serum-free gut media (without drug) was added to the apical side of the Transwells.

Samples of media were taken at 0, 1, 4, 6, 24, and 48 hours, and analysed by LC-MS to determine the concentration of parent compounds in the liver compartment. An estimation of area under the curve (AUC) of both oral and IV concentration profiles were made using GraphPad Prism. An estimation of oral bioavailability was made thereafter using the following calculation:

$$\text{Oral bioavailability} = \frac{AUC_{oral} \cdot Dose_{IV}}{AUC_{IV} \cdot Dose_{oral}}$$

The metabolic potential of PHH was assessed using a P450-Glo™ CYP3A4 Assay (Promega) at day 4 and 7. Transepithelial electrical resistance (TEER) was measured to assess gut barrier stability before and after the drug dosing experiment. As a control, TEER was also assessed in gut Transwells cultured statically in a 24-well plate, in the same apical (serum-free gut media) and basolateral (hepatocyte maintenance media) media as gut-liver experiments.

Drugs were selected for bioavailability estimation using the gut-liver MPS from a study comparing human and animal bioavailability by Musther *et al*[1]. Selection was narrowed down to those drugs with available reference data for hepatocyte clearance rate and permeability across a Caco-2

barrier on a Transwell[2,3]. These two input parameters were required for mathematical modelling, which we used to help guide and optimise the experimental design. Sixteen compounds, with a Biopharmaceutics Drug Disposition Classification System (BDDCS) class of 1 or 2, whose primary route for metabolism is the liver, were selected for mathematical modelling. Mathematical modelling was conducted using R programming language with ordinary differential equations (ODE) written to describe the concentration profiles over time in the MPS-TL6 plate. The package, RxODE was used to simulate the ODEs in R[4]. Out of the 16 drugs, those with LogD values of less than 3: acebutolol, phenytoin, naproxen and methylprednisolone, were selected for bioavailability estimation using the gut-liver MPS. LogD values greater than 3 can have adverse physiochemical drug properties, such as poor solubility and increased protein binding, making it more challenging to estimate PK properties.

The concentration of the 16 drugs in the small intestine was estimated using a model described by Maier *et al*[5]. Briefly, the model is based upon an *in situ* study for posaconazole, which delivered 40 mg (57 µmol) of drug to the stomach of volunteers in either an acidic or neutral solution[6]. The maximum concentration in the duodenum reached 26.3 ± 10.3 or 13.6 ± 5.8 µM respectively, which is equivalent to dissolving the drug in 300 mL of water and an absorption rate of 90%[6,7]. We used this relationship to estimate the drug concentration in the small intestine before and after absorption, taking the clinical recommended dose for the 16 drugs from the British National Formulary (BNF), WHO Anatomical Therapeutic Chemical (ATC) classification or Monthly Index of Medical Specialties (MIMS) data bases.

Results and Discussion

Using the PhysioMimix™ Multi-Organ System (Figure 1A), we demonstrated the use of a multi-organ gut-liver MPS to estimate oral bioavailability *in vitro*. In this study, the wells of each MPS-TL6 plate were divided into two dosing regimens to estimate drug bioavailability following an oral and IV dose (Figure 1B). For oral, a drug was added to the apical side of the gut MPS whereas for IV, drug was added to the media that circulates between the gut and liver compartments. Dosing occurred at day 5, following primary hepatocyte seeding and the insertion of gut cultures into the MPS-TL6 plate, with an assay window of 48 hours (Figure 1C).

A mathematical modelling approach, using equations that describe the concentration of a compound in each compartment of the MPS-TL6 plate (Figure 2A), was used to help guide the design of the bioavailability experiment. Sixteen compounds (R^2 = 0.32, Figure 2B) were selected for mathematical modelling from a study investigating the animal versus human bioavailability of 184 compounds (R^2 = 0.34), where a correlation was found to be weak[1]. Mathematical modelling was used to optimise several system parameters with the aim of designing an experiment that gets close to human bioavailability values. For example, a range of compartment volumes and flow rates in the MPS-TL6 were evaluated between the minimum and maximum allowed by the plate. The time and frequency of sample points was also investigated with an example shown for acebutolol (Figure 2C). The model found that restricting the study length to just six hours, or sampling too infrequently over the early time points, would result in a poor estimation of bioavailability.

For oral drug dosing, we aimed to use the same concentration for all compounds and for that to be relevant to the effective drug concentration in the small intestine. Relevant concentrations are important to avoid saturating the gut MPS with drugs that may induce toxicity and result in barrier loss. A methodology described by Maier *et al.,* was used to estimate gut concentrations of drugs, based on their clinical recommended dose and the resting volume of water in the small intestine[5]. We estimated the median drug concentration of the 16 drugs in the small intestine, before

and after absorption, to be 659.3 µM and 97.2 µM respectively (Figure 2D). A relevant oral drug concentration of 100 µM was chosen at the lower end of the estimations, for all bioavailability experiments, to reduce the possibility of drug-induced gut toxicity.

To test the ability of our gut-liver MPS model to accurately predict oral bioavailability, 4 (of the 16) drugs with LogD values less than 3: acebutolol, phenytoin, naproxen, and methylprednisolone, were selected. QC metrics such as the metabolic capacity of PHH and the integrity of the gut barrier were all maintained during the bioavailability estimations (Figure 3). This provides confidence that bioavailability estimations were derived from a functionally stable *in vitro* multi-organ gut-liver MPS.

Oral and IV dosing were compared for each of the 4 drugs in the MPS-TL6 plate with permeability through the intestinal barrier and hepatocyte clearance, the main drivers of the concentration profiles (Figure 4). Importantly, the extent of drug clearance observed was in line with reported *in vitro* unbound intrinsic clearance rates[2] (CLint, u, Figure 4A). Human bioavailability predictions for acebutolol, phenytoin and methylprednisolone by the gut-liver MPS were markedly improved compared to those of animal models (Figure 4B). For naproxen, where animal models accurately predict bioavailability, performance by our gut-liver MPS was equivalent (Figure 4B).

Figures

Figure 1. Oral bioavailability estimation in MPS-TL6

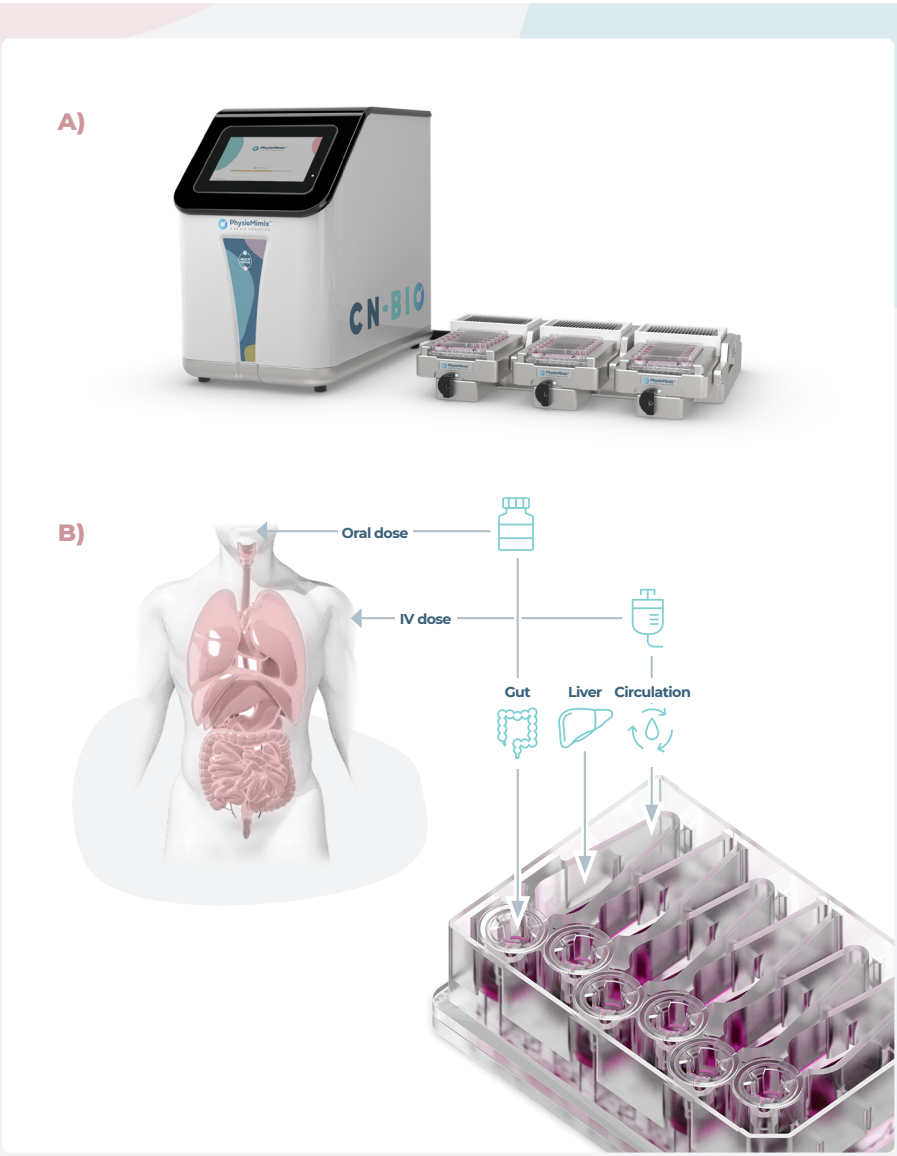
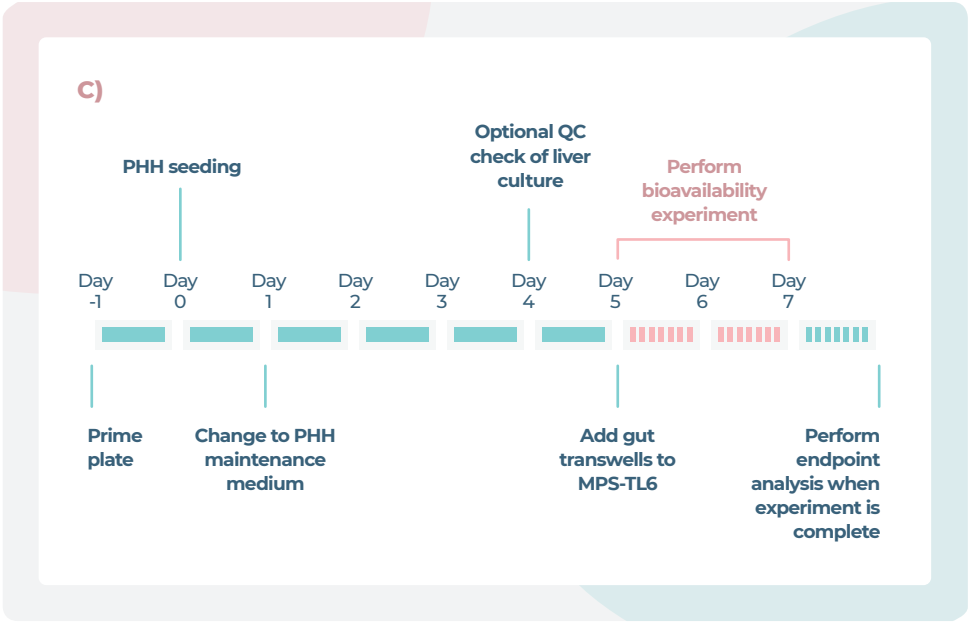
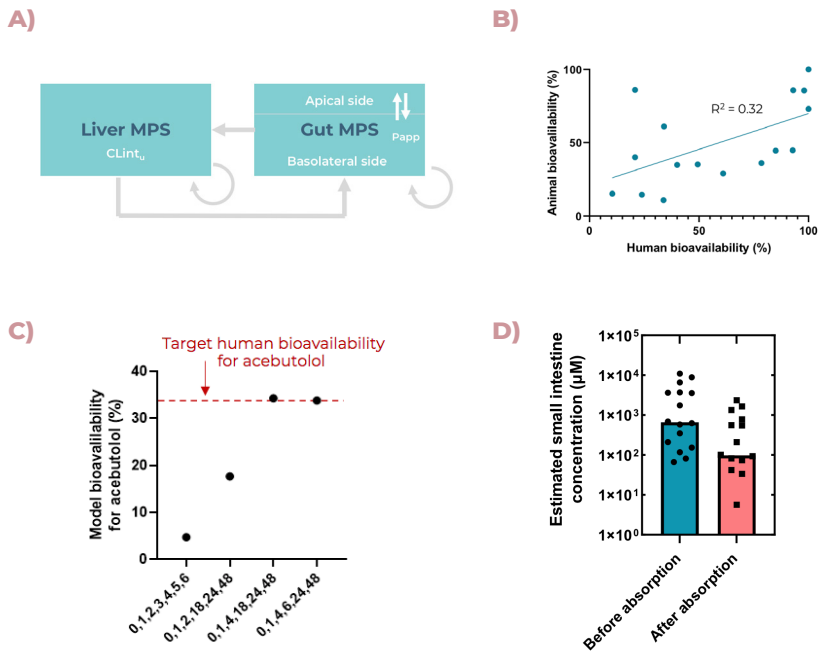


Figure 1. Continued...



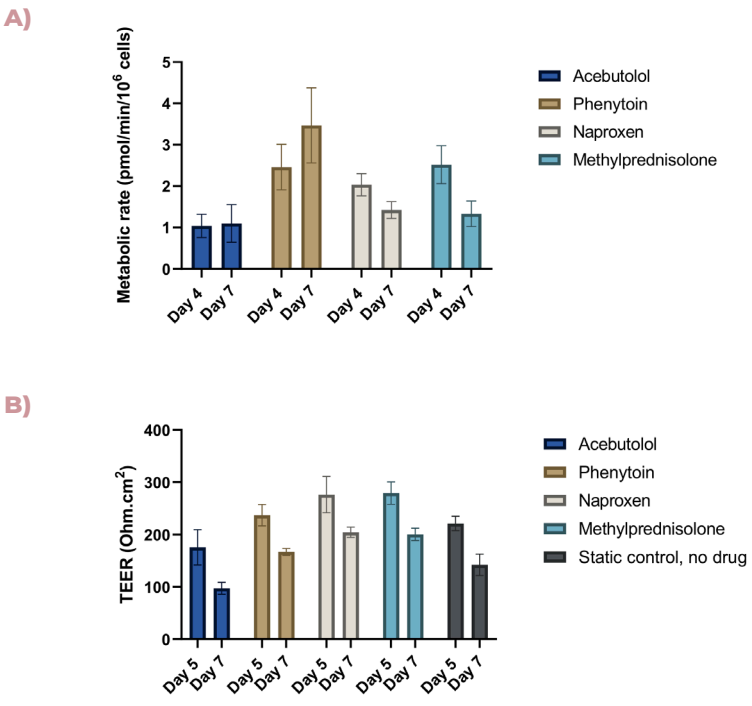
A) Fluid flow in the MPS-TL6 was controlled by the PhysioMimix™ Multi-Organ System. **B)** To estimate oral bioavailability a drug was added as either an oral dose, into the gut compartment, or as an IV dose in the circulating media that flows continuously between liver and gut compartments of the MPS-TL6 plate. **C)** Experimental timeline for bioavailability estimation. PHH were seeded into a porous scaffold to form 3D microtissues within the liver compartment and media was changed at day 1. At day 4, media samples were taken, and QC metrics assessed to ensure functional stability. On day 5, pre-cultured gut Transwells were added into the gut compartment of the MPS-TL6 plate. Gut and liver compartments were then connected by fluid flow before dosing with drugs. Samples were taken over a 48-hour period to obtain drug concentration profiles.

Figure 2. Design and optimisation of oral bioavailability experiments



A) Schematic of mathematical model that describes the concentration of a compound in each compartment of the MPS-TL6 plate. The oral bioavailability of 16 compounds were modelled. **B)** Animal model bioavailability predictions for these 16 compounds were weak[1]. **C)** A mathematical model was run to optimise gut-liver MPS experimental parameters, which includes optimal sampling times and sample frequency. An example is shown for acebutolol with the aim to get as close to its known human bioavailability value. **D)** The concentration of 16 compounds in the small intestine before and after absorption was estimated using a methodology described by Maier *et al.*, that considers the clinical dose of each drug and the resting volume in the small intestine[5].

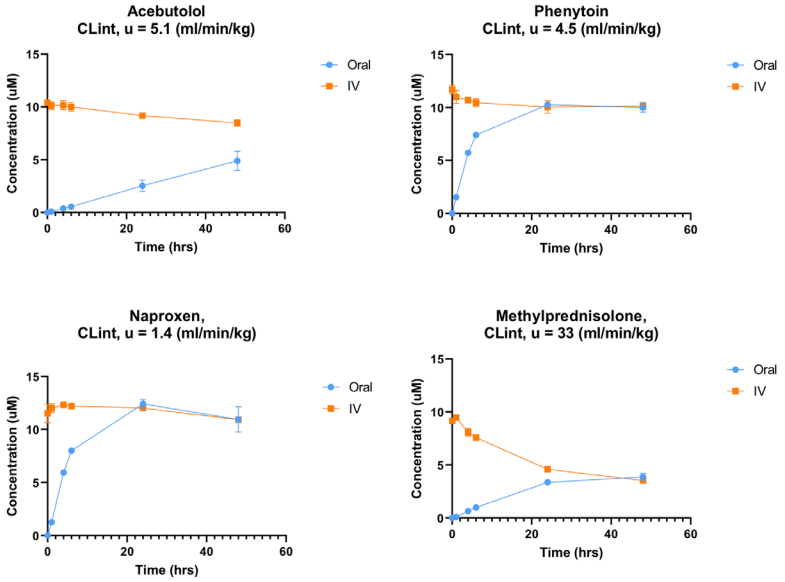
Figure 3. QC metrics of liver and gut functionality were maintained during culture in the MPS-TL6 plate



Four compounds were selected to test oral bioavailability experimentally: acebutolol, phenytoin, naproxen and methylprednisolone. **A)** The metabolic capacity of the liver MPS was assessed using P450-Glo™ CYP3A4 and was maintained throughout the study. **B)** The epithelial barrier stability was maintained in the gut MPS, assessed by taking TEER measurements on the day gut Transwells were added to MPS-TL6 (day 5) and at the completion of the study (day 7).

Figure 4. Correlation to human bioavailability is improved with MPS-TL6 compared to animal models

A)



B)

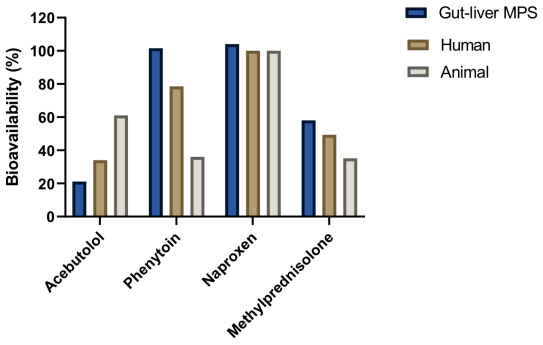
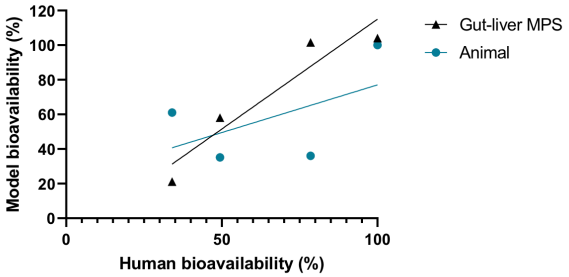


Figure 4. continued...

C)



A) Plots of concentration over time for acebutolol, phenytoin, naproxen, and methylprednisolone for oral and IV dosing routes in the liver compartment. The extent of clearance of each compound is in line with the reported *in vitro* unbound intrinsic clearance rates (CLint, u). **B)** The gut-liver MPS shows improved prediction of human bioavailability for acebutolol, phenytoin and methylprednisolone while naproxen is on par with the animal model's accurate prediction. **C)** The overall correlation to human bioavailability is improved for the four compounds tested experimentally compared to values obtained using animals.

Conclusion

Using drugs with a wide range of bioavailability values, the gut-liver MPS more closely predicted human bioavailability versus animal models for 3 of the 4 compounds tested, and demonstrated equivalent performance for the fourth. Instead of the ethical and cost considerations associated with animal experiments, this *in vitro* approach enables human bioavailability predictions to be made in less than 10 days using a single MPS-TL6 plate per drug. The gut-liver MPS has the potential to provide human relevant bioavailability estimations early in drug development, helping to ensure the success or prevent the failure of drugs in clinical trials.

References

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