Predicting Drug Bioavailability with the Modern-day Toolkit

A central pillar in developing new medicines is understanding the pharmacokinetic/pharmacodynamic relationship (PK/PD). Can the drug reach the target site at a sufficient concentration, engage the target, and produce the desired pharmacological effect? For orally administered medications, prevalent in the pipelines of pharmaceutical companies, an important component of the PK/PD relationship is bioavailability. Bioavailability is defined as the fraction of drug reaching systemic circulation following absorption in the gut and first pass metabolism in the liver. Accurately predicting the bioavailability of orally administered medicines in humans, during pre-clinical development is crucial as it forms the basis for setting safe and efficacious doses in the clinic. This significant parameter is therefore of interest to drug developers and regulatory agencies. When oral bioavailability is insufficient, further optimisation of compound structure, changes to formulation or an alternative route of administration may be required, all of which are grounds to discontinue development.

The Need for Improved Bioavailability Predictions

Studies report that PK/PD issues account for 5–10% of clinical failures. Whilst this percentage has fallen significantly over the past 30 years,[1,2] it remains a concern. Interestingly, a 2014 AstraZeneca study[2] reported that <10% of project teams had a high level of confidence that their drug exhibited "a combination of good drug properties and good pharmacological end points", where good drug properties include bioavailability. So, which factors drove this fall in PK/PD issues and what can we learn going forward to improve bioavailability predictions?

Firstly, our understanding of the physiochemical properties of compounds with appropriate human PK profiles has improved, and this knowledge is being applied earlier in the discovery process.[3] This has been aided by the derivation of absorption, distribution, metabolism, and excretion (ADME) parameters from improved *in vitro* models, such as the use of suspension cultures of primary human hepatocytes to evaluate liver clearance.

A second useful realisation has been the limitation of animal models. Traditionally, animal models have dominated the prediction of bioavailability and have been pivotal in establishing the PK/PD relationship. It is now well established that, while animal models maybe useful qualitative predictors of human bioavailability (i.e., low vs high), they are poor quantitative predictors (i.e., percentage oral bioavailability),[4,5] with no absolute correlation for individual species or all species taken together. In part, this has driven the adoption of *in silico* models, particularly physiologically based pharmacokinetic (PBPK) models. Here, interactions between organs and physiological processes in the human body are described by sets of differential equations that enable animal and human PK and bioavailability to be predicted. In a learn, confirm and refine cycle, PBPK models are first validated against animal data before making human predictions based upon parameters derived from *in vitro* experiments.[6] PBPK models rely on a priori understanding of all the relevant processes, their description in the models and high-quality input data, without which the accuracy of *in silico* predictions is limited.

For bioavailability predictions to improve, more complex *in vitro* models capable of recapitulating tissue level function, such as organ-on-a-chip (OOC) or microphysiological systems (MPSs), are required to address the limitations of animal studies and better inform PKPB models. Importantly for the study of bioavailability, OOCs feature microfluidics to simulate blood flow, which in turn has enabled the interconnection of multiple organs to model systemic effects.[7] Here we briefly review the state of the art in animal and *in silico* models and look at how the prediction of bioavailability can be enhanced by OOCs.

Can Animal Models Accurately Predict Bioavailability?

Animal models dominate when predicting bioavailability, so it is important to question their correlation with humans and investigate the causes of divergence. One seminal study examined the literature and found 184 compounds with both reported human and animal oral bioavailability.[4] Unsurprisingly, the overall correlation between animal and human bioavailability was found to be poor ($R^2 = 0.34$). Mouse ($R^2 = 0.25$), rat ($R^2 = 0.28$), and dog ($R^2 = 0.37$) are the most widely used animal models; however, these species are outperformed by non-human primates (NHP, $R^2 = 0.69$). Despite this, using NHP remains unpopular, given the higher cost and more restrictive ethical considerations. The usefulness of animal models to assess bioavailability is perhaps best reserved for qualitative assessment, given the wide range of values compared to observed human data.

One of the fundamental challenges in drug discovery is extrapolating PK parameters from animals to humans and bioavailability is not unique in this. Differences in physiology and metabolic capacity between humans and rodents are the main contributors to the disparity. For example, the gut microbiome population differs substantially in rodents and rats do not have a gall bladder, one of the main constituents of the hepatobiliary system. Expression of enzymes that drive drug metabolism varies not only between rodents and humans but between mice and rat strains. Differences also manifest in absorption kinetics, expression of transporters in the gastrointestinal tract and extent of plasma protein binding, all important when determining PK parameters.[5]

Regardless, animals remain an important part of pre-clinical drug discovery. Unlike standard *in vitro* models, often cultured as a single cell type on plastic, animal physiology is akin to humans in many ways. It is complex with circulation, an immune system and organs that are interlinked. Therefore, the challenge for OOC technologies is to more accurately model the complexity of a human *in vitro* to improve pre-clinical drug discovery predictions.

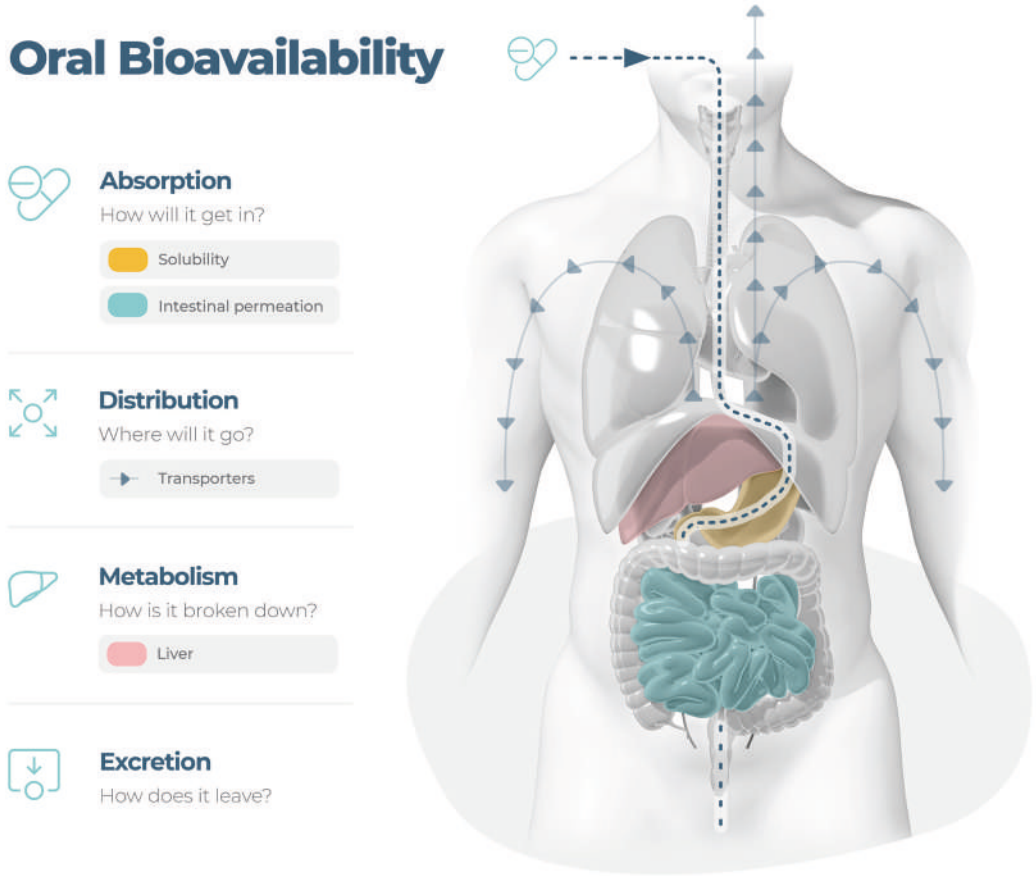

Figure 1 – Human oral bioavailability. Orally administered drugs are first solubilised by the stomach, then absorbed via the gut to be metabolised by the liver. Metabolites are then distributed through the body via transporters. Solubilised drug molecules that have not been absorbed through the gut are excreted out of the body.

Once achieved, these new technologies will likely reduce costs and experimental time versus animal studies, whilst also circumventing ethical constraints.

Despite their promise, it is unlikely that *in vitro* technologies such as OOC will completely replace animal studies in the short term, as regulators will still demand safety studies before clinical trials in humans. However, in 2021, the European Parliament passed a resolution to phase out animal testing in research, regulatory testing, and education. The immediate challenge therefore is to make OOC models as good – or better – at predicting PK parameters, bioavailability, and toxicology versus animals to reduce the numbers used. This goal is also in line with the commitment made by the Association of the British Pharmaceutical Industry in a 2015 report[10] to the 3Rs principle of animal research: replacement, reduction, and refinement.

The Integral Role of *In Silico* Models

Over the past few decades, the use of *in silico* models has grown and they now form an integral part of the drug discovery development process. In submissions to regulators, *in silico* models are used to extrapolate PK parameters and predict PK profiles for a drug candidate. They also offer the possibility to explore diverse clinical questions, such as interactions with over-the-counter medications or the role genetics and aging play in the behaviour of a drug, which cannot easily be investigated in animals.

The simplest *in silico* models used to predict PK profiles are built using compartments or "building blocks" that describe the relationship between the plasma concentration of a drug with time.[6] As models become more complex, the number of compartments increase, with each compartment representing an organ or tissue. The compartments are interconnected by flow rates, which resemble circulating blood flow. These PBPK models use *in vitro – in vivo* extrapolation (IVIVE) techniques to predict drug plasma and tissue concentration, as well as bioavailability. One of the advantages of PBPK is that models can be updated throughout drug development in a "learn, confirm, and refine" approach as experiments move from *in vitro* to *in vivo* then into clinical trials. Once validated in humans, the model can be used to investigate dosing regimens, likelihood of drug-induced liver injury and population effects such as age, race, and genetics.

There is no doubt about the importance of *in silico* modelling in drug discovery, but there is also a need for continued improvement. The predictability of the model is dependent on the quality of the input parameters. Given PBPK relies heavily on data from early *in vitro* and animal studies, there is motivation to integrate models that better predict ADME, safety and efficacy profiles, with OOC likely to play an increasingly prevalent role. For low clearance compounds, *in vitro* methods using liver microsomes lack the sensitivity to measure their metabolic rate and as drugs become more metabolically stable, transporter-mediated PK becomes more important. However, PBPK models often lack system parameters, such as transporters and abundance of enzyme in different organs, that make transporter-mediated drugs more challenging to predict.

As next-generation PBPK develops, it is likely to utilise emerging technologies to improve the *in silico* prediction of candidate drugs. OOC has the potential to considerably improve

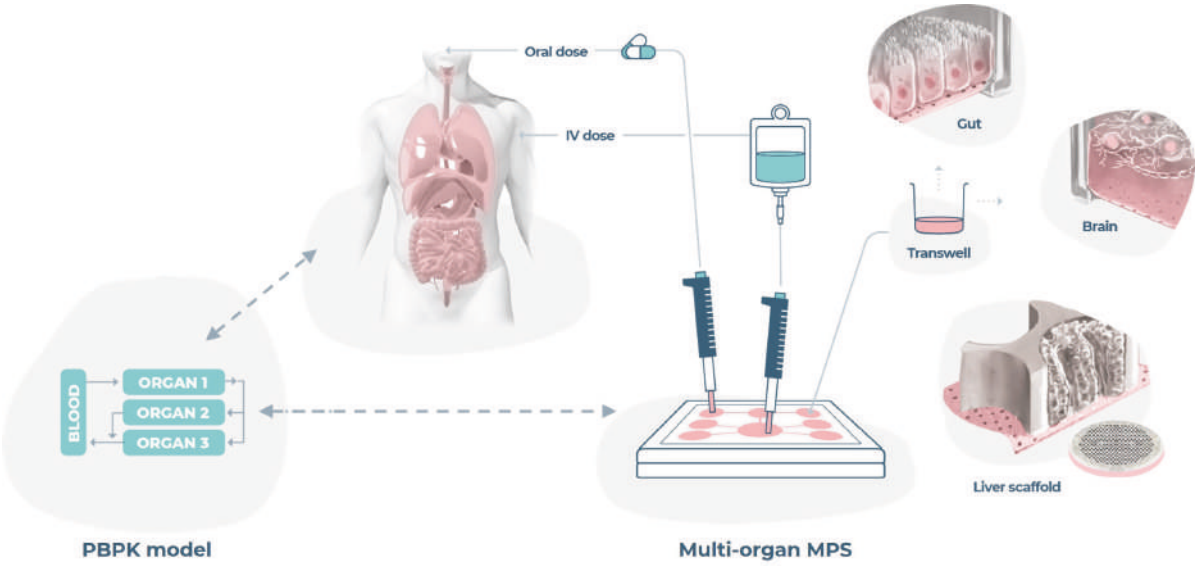

Figure 2 – Combining PBPK modelling and Organ-on-a-chip for human-relevant bioavailability

the quality of data inputted into PBPK, as its models meet the challenges of recreating the complexity of human physiology and disease. In parallel, computational tools such as artificial intelligence and machine learning have the capability to analyse large, complex datasets such as chemical properties, patient, and *in vitro* data. Given a "training dataset" for known drugs, these tools could be used in a complementary manner to predict the parameters that feed into PBPK models before *in vitro* experiments begin and, as technology advances, predictions of bioavailability and critical safety parameters (such as DILI and possible side effects) may be possible at the early, compound selection stage of drug discovery.

Organ-on-a-chip for Human-relevant Bioavailability Predictions

Developments in tissue engineering and microfluidics have facilitated the design of OOC technologies which aim to recapitulate the functional unit of an organ or tissue at a smaller scale. OOC can be viewed as a bridge between traditional *in vitro* tissue culture on 2D plastic and *in vivo* animal models. There are several requirements that OOC should include: (1) fluid flow that mimics blood in vessels; (2) similar functionality to *in vivo* tissues; (3) use of human cells and where possible multiple cell types and (4) reproducible and long-term cell culture. The design of an OOC platform will depend on the end application and user. For example, screening the effect of drug–drug interactions on bioavailability, which is a concern to regulators and can be impractical to undertake *in vivo*, will require OOC platforms with sufficient throughput to run multiple replicates and many combinations in parallel.

Liver-on-a-chip represents a great example of where OOC provides improvements over standard *in vitro* methods. Primary human hepatocytes, when cultured in suspension or 2D, have short-lived activity of cytochrome P450s, the group of enzymes that metabolise many drugs. The combination of fluid flow and formation of a 3D microtissue in the liver-on-a-chip from CN Bio (Cambridge, UK) has enabled improved functionality of hepatocytes *in vitro*, with measured metabolic activity for at least 28 days.[11] Other OOC companies also provide models of the liver: for example, Emulate (Cambridge, US) embed cells of the liver into a classic chip-type device, about the size of a credit card. The platform by TissUse GmbH (Berlin, Germany) is on a similar microscale yet allows the interconnection of different organs into systems. In contrast, CN Bio use a larger scale, open-well design with embedded micropumps that also enable single and interconnected multi-organ studies. Providing easy access to media and cells, this latter multi-organ system is well suited to bioavailability assays.

For determining ADME parameters such as bioavailability *in vitro*, a multi-OOC model with fluidically linked gut and liver compartments is required. This enables an oral drug to be dosed into the gut compartment, where it passes through the intestinal barrier before entering the liver compartment for metabolism by hepatocytes.[8,9] Medium samples taken over time are used to generate plots of drug concentration over time that resemble PK profiles, with an area-under-the-curve measurement made to estimate oral bioavailability. Multi-OOC also allows for the study of potential crosstalk between these organs; for example, the metabolites produced by liver metabolism of prodrugs used to treat cancer may have toxic effects on intestinal cells.

The translation of data generated by OOC to parameters relevant in PBPK modelling is a challenge that needs to be met to ensure relevance in pre-clinical drug development. To achieve this, mathematical modelling can be utilised, starting with differential equations that describe the concentration of a compound over time in an OOC system. In a multi-OOC, mechanistic models consider operational characteristics such as compartment volumes, flow rates and the scaling of hepatic cells that are seeded to that of a human adult liver.[8] Once model analysis is performed, PK parameters such as hepatic and intestinal clearance rates as well as information on biodistribution can be obtained.

As OOC adoption increases in the pharmaceutical industry, there is a need for continued improvement. Where possible, OOC should utilise primary cells that better recapitulate the functionality of tissues and are more responsive to toxicological effects versus cell lines derived from cancer tumours. The inclusion of circulating and tissue-resident immune cells in OOC models would make disease models such as cancer more responsive to immunotherapies. For bioavailability, addition of the microbiome that is known to bioaccumulate and metabolise

certain drugs would provide an improved estimation of the amount absorbed through the small intestine and available for hepatic metabolism.

Conclusion

Significant strides have been made in reducing clinical attrition due to PK/PD and bioavailability issues, but problems remain. Animal models continue to be used in bioavailability research, but their limitations are serious and difficult to overcome. The development and adoption of PBPK modelling and more latterly OOC, particularly multi-organ OOC, point the way forward. Improvements in prediction will be found using these technologies both separately and in concert. The three areas in which multi-organ OOC systems can offer benefits are (i) direct measurement of bioavailability, particularly those compounds and modalities that are not easily described in PBPK models, (ii) derivation of high-quality ADME parameters required by PBPK models and (iii) as a platform to validate PBPK models.

Now that expertise in PBPK modelling has expanded and commercially produced multi-organ OOC systems have been widely adopted, we should be questioning the use of animals in bioavailability testing. By providing scientists with more translationally relevant data that drives down failures due to PK/PD issues, will multi-organ OOC and PBPK models reduce and ultimately replace animals in this area? The early signs are promising, but all models – be they *in vitro*, *in vivo* or *in silico* – have limitations, and require a clear context of use to ensure successful application. As multi-organ OOCs are added to the toolkit, success will be achieved by defining the unique points at which they add value and placing them in a framework with other technologies to achieve a robust and well understood methodology for predicting bioavailability.

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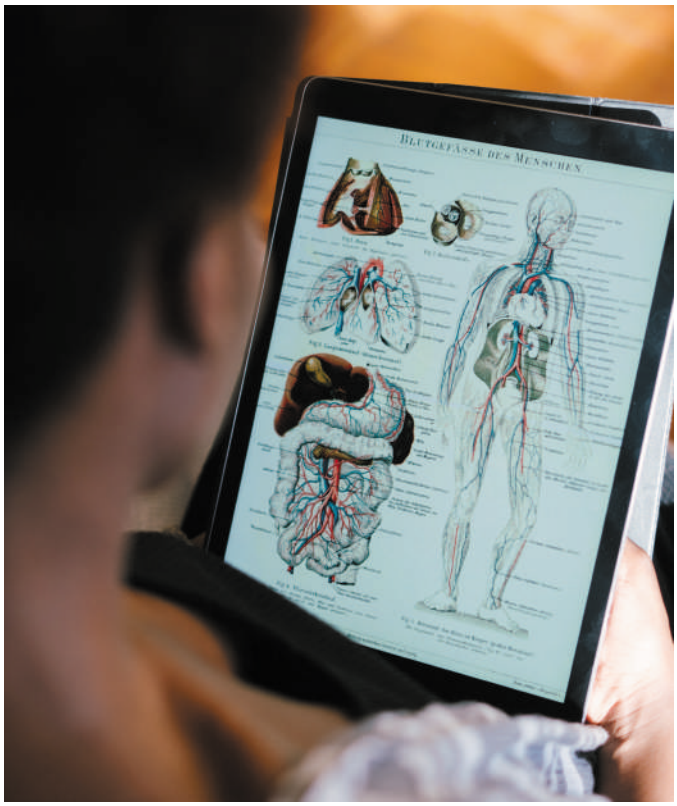

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