1521-009X/46/11/1556–1561$35.00
Drug Metabolism and Disposition
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https://doi.org/10.1124/dmd.118.084293
Drug Metab Dispos 46:1556–1561, November 2018

DRUG METABOLISM & DISPOSITION

Special Section – New Models in Drug Metabolism and Transport—Commentary

Emerging Models of Drug Metabolism, Transporters, and Toxicity

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Received September 3, 2018; accepted September 14, 2018

ABSTRACT

This commentary summarizes expert mini-reviews and original research articles that have been assembled in a special issue on novel models of drug metabolism and disposition. The special issue consists of research articles or reviews on novel static or microflow based models of the intestine, liver, eye, and kidney. This issue reviews static intestinal systems like mucosal scrapings and cryopreserved intestinal enterocytes, as well as novel bioengineered or chemically engineered intestinal models derived from primary human tissue, iPSCs, enteroids, and crypts. Experts have reviewed hepatic systems like cryopermeabilized Metmax hepatocytes and longer term, hepatocyte coculture system from HμREL, yielding in vivo-like primary and secondary drug metabolite profiles. Additional liver models, including micropattern hepatocyte coculture, 3D liver spheroids, and microflow systems, applicable to the study of drug disposition and toxicology have also been reviewed. In this commentary, we have outlined expert opinions and current efforts on hepatic- and nephrotoxicity models. Ocular disposition models including corneal permeability models have been included within this special issue. This commentary provides a summary of in vivo mini-reviews of the issue, which have discussed the applications and drawbacks of pig and humanized mice models of P450, UGT, and rat organic anionic transporting polypeptide 1a4. While not extensively reviewed, novel positron emissions tomography imaging-based approaches to study the distribution of xenobiotics have been highlighted. This commentary also outlines in vitro and in vivo models of drug metabolism derived from breakthrough genetic, chromosomal, and tissue engineering techniques. The commentary concludes by providing a futuristic view of the novel models discussed in this issue.

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Introduction

Modern drug disposition science employs many in vitro reagents and tools as well as various animal models to understand underlying mechanisms of metabolism, transport, and toxicity. Reagents such as liver microsomes have been a mainstay to study xenobiotic metabolic transformations for decades, and the use of human-derived reagents became prevalent in the 1980s and 1990s (Houston, 1994; Obach, 1999; Riley et al., 2005). In the 1990s, we reaped the fruits of efforts to generate individual drug metabolizing enzymes through cloning and expression (Crespi and Penman, 1997; Venkatakrishnan et al., 2000), efforts to develop human hepatocyte cultures (Carlile et al., 1997), as well as the application of the colon carcinoma cell lines cell system as a model to address drug absorption and efflux transport (Hidalgo et al., 1989; Artursson and Karlsson, 1991). In the 2000s, the development of techniques whereby primary hepatocytes could be cryopreserved converted an otherwise difficult to use reagent into one where laboratories now maintain banks of hepatocytes for routine experiments (Loretz et al., 1989; Li, 1999, 2007; Li et al., 1999; Hewitt and Li, 2015). The science of drug transporters saw a rapid expansion, with the discovery, cloning, and expression of several major transporters involved in drug distribution and clearance (Giacomini, 1997; Zhang et al., 1998; Bi et al., 2006; Cropp et al., 2008; Giacomini and Huang, 2013; Hillgren et al., 2013; Morrissey et al., 2013; Liu et al., 2015a). Coupling all of these advances in reagents with the advent of atmospheric pressure ionization mass spectrometry in the early 1990s, whereby the bioanalysis of samples generated using these reagents could be accomplished with rapid and highly sensitive and selective assays (van Breemen et al., 1997; Hop et al., 2002; Hop, 2006; Soglia et al., 2006), meant that the modern drug disposition laboratory was indeed well-equipped to study the basic science of drug metabolism and disposition as well as to apply these tools to the design and development of new drugs. Furthermore, advances in laboratory automation and computing power have converted in vitro drug metabolism experiments that at one time took days or weeks to complete into activities that can be accomplished in high throughput on hundreds of compounds at a time in miniaturized assay designs (Hop et al., 2008). Nevertheless, these approaches and systems are not without limitations. In vitro systems can approach describing the in vivo situation to a limit, but they always fall short of offering a quantitative, integrated, and comprehensive picture of xenobiotic disposition in vivo. Data from in vitro systems containing human reagents require scaling factors beyond those that would be generated by merely multiplying the size of the in vitro incubation to the size of the in vivo situation. This yields uncertainty regarding whether these in vitro systems are truly reflective of the in vivo situation.

https://doi.org/10.1124/dmd.118.084293.

ABBREVIATIONS: CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-CRISPR associated protein 9; P450, cytochrome P450; DDI, drug-drug interactions; DMEs, drug metabolizing enzymes; PSCs, pluripotent stem cells; UGTs, uridine glucuronysyl transferases.

In addition, whole animal models, which have been used for years to attempt describing the disposition of xenobiotics in human, have their failings in that the identities, activities, and expression levels of various drug metabolizing enzymes and transporters do not mirror that of human. Thus, while animals offer integrated multiorgan physiologic systems that are similar to humans, they differ from humans at the molecular level.

All of this notwithstanding, we believe that we are on the threshold of a new wave of breakthroughs in approaches and technologies to study drug metabolism and disposition. In the past, advances that were made, attempted to break down complex systems into more simple, understandable components such as individual drug metabolizing enzymes and transporters. In this issue, many of the new approaches described tend to be more integrative and complex in an attempt to recapitulate intact in vivo systems. Efforts at genetic and tissue engineering have been ongoing and meeting with increasing success over the past decade. These include things such as micropatterned hepatocyte coculture systems (Wang et al., 2010; Schwartz et al., 2014; Nguyen et al., 2015), microphysiological systems coupled with engineered microflow systems, and humanized rodents, among many others. This was the important motivation behind assembling this special issue. We have commissioned mini-review articles from leading experts on these systems and models that can be used to study drug metabolism and disposition. Additionally we have included original research articles that were submitted to the journal over the past several months that use new models to study drug disposition. Whether these new systems become as routine as microsomes or hepatocytes remains to be seen; each of these systems will require additional testing and validation before graduating to the drug metabolism scientist's toolbox. There certainly have been advances in the past that have not panned out in the long term for drug screening (e.g., liver slices, Fa2N4 immortalized hepatocytes) and some of the advances described in this issue may suffer the same fate in the future. But it is our hope that these all will emerge as successful, as they would greatly enable our ultimate pursuit of advancing pharmacotherapy to the betterment of human health. Below is a brief summary of the contents of this issue.

In Vitro Models

Intestine. Intestine as an organ has been of significant interest to drug metabolism scientists, but no validated in vitro or animal methods to predict prospectively intestinal disposition events like targeted drug delivery, intestinal DDI, or gastrointestinal toxicology are available to date. Intestinal subcellular fractions like microsomes and mucosal scrapings have been applied as predictive tools but with limited success. Recently, intestinal epithelial models derived from human primary sources have received significant attention. Li et al. (2018a) reported processing and optimization of mucosal epithelium and cryopreserved enterocytes (Ho et al., 2017) to study intestinal metabolism, P450 induction, and gastrointestinal toxicity. Similarly, Iwao and colleagues have reported differentiating human iPSCs into functional enterocytes (Onozato et al., 2018). In this issue, an intestinal mini-review outlines applications and current limitations of such emerging human derived models bioengineered from intestinal crypts, mucosal extracts, iPSC-derived organoids, and human intestinal tissue (Sawant-Basak et al., 2018). It is recommended that biochemical characterization followed by transcriptomic, proteomic, and functional end-points of these novel models will increase confidence in using them to study intestinal drug disposition and toxicology exerted by small molecules.

In addition to the interplay of DMEs and transporters, it is now acknowledged that the microbiome plays a role in the disposition of endogenous and exogenous agents. However, the role of the microbiome is not yet identified during the drug candidate optimization or selection stages and is often a post hoc observation once the candidate is administered to humans. Microbial disposition of drug substances may contribute to the inter-individual variability in pharmacokinetics within a population or may be a key determinant of bioavailability (prodrugs). As a result, it appeared timely to us as editors to capture this as an emerging tool in the study of drug metabolism and toxicity. In this issue, a report is included in which an in vitro system was designed to mimic the drug metabolizing capabilities of human colonic microbiome. Several drugs were incubated in this system and shown to undergo various reduction reactions such as *S*-oxide reduction, nitro reduction, ring opening of benzisoxazole, and reductive cleavage of an azo bond, similar to transformations that had been previously reported to occur in vivo (van de Steeg et al., 2018). In addition, Bisanz et al. (2018) reviewed the state-of-the-art of microbial metabolism of small molecules. The authors have guided the readers on screening new therapeutics in in vitro microbial assays, ex vivo incubations in/or preparations from human stool samples, and in vivo models derived by colonizing microbes in laboratory animals that are raised in "sterile" conditions. The authors also recommended how to determine underlying mechanisms of microbial transformations using expression or "-omics"-based approaches.

Liver. The liver is an extensively studied clearance organ in humans, as it expresses most major DMEs and transporters. Many reagents needed for in vitro approaches to study drug metabolism in the liver have been available for decades. Most commonly used are subcellular fractions, such as liver microsomes, which possess the cytochrome P450 and UGT enzymes. Cryopreserved primary human and animal hepatocytes became available, and because these possess all hepatic drug metabolizing enzymes as well as transport functions, cryopreserved hepatocytes have become commonly employed to study drug disposition in vitro. But these systems have shortcomings. Hepatocytes in suspension drug metabolism assays may lose viability quickly, and this limits metabolic incubation times to approximately 4 hours. In simple and sandwich culture systems, hepatocytes demonstrate downregulation of various drug metabolizing enzymes, which makes the use of long-term cultures an unviable approach for drug metabolism studies. However, advances have been made and are continuing to be made to develop longer term hepatocyte culture systems. In this issue, Li et al. (2018b) reported the use of permeabilized cofactor supplemented cryopreserved hepatocytes, which require simplified storage and usage conditions compared with conventional cryopreserved human hepatocytes. While some examples of the use of these systems in drug metabolism studies exist in the literature (Wang et al., 2010; Ballard et al., 2014; Vivares et al., 2015; Tsamandouras et al., 2017a,b), Burton et al. (2018) reported a new system in this special issue, wherein the HμREL hepatocyte coculture system was evaluated successfully for the ability to yield in vivo-like drug metabolite profiles, even including metabolites arising from serial metabolic systems running up to 7 days of incubation. Such improvements coupled with tissue engineering sciences, microfabrication, and coculture approaches may have met the challenge of conducting in vitro drug metabolism studies that can be carried out for days. Such systems may then be suitable for measuring the metabolic clearance of drugs and drug candidates that have very low intrinsic clearance rates (i.e., scaled values of under 1 mL/min/kg body weight in human). Furthermore, the coculture of hepatocytes with other cell types present in the liver has greatly enhanced the performance of hepatocyte cultures for drug disposition measurements and the introduction of microfluidic flow of medium has even further enhanced these systems (Bhatia and Ingber, 2014). In this special issue, three reviews cover various aspects of these novel engineered systems (Bale and Borenstein, 2018; Caetano-Pinto and Stahl, 2018; Underhill and Khetani, 2018). Underhill and Khetani (2018) describe the advances that have been made in constructing these systems including micropattern hepatocyte coculture, 3D liver spheroids, and microflow

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systems. The review by Bale and Borenstein (2018) discusses developments in microscaled technologies that are being optimized to capture human-relevant in vitro systems. These novel hepatic microfluidic technologies differentiate from existing cryopreserved hepatic assays in that unlike existing single cell-type (hepatocytes) static suspensions, these systems are fabricated to coculture different hepatic cell types including Kuppfer cells and stellate cells, resulting in a more complete liver model. In addition, these systems are typically normalized to physiologic endpoints like plasma albumin, metabolic functions, and markers of cellular health. The authors suggest that such integrated coculture systems could allow investigators to study complex underlying mechanisms of nutrient transport, drug-disease interactions (Harvey and Morgan, 2014), and the interplay of metabolism-transport phenomena, all of which are limited by the lack of important cell types in the traditional cryopreserved hepatocyte suspension assays.

These advances in bioengineered liver technologies are viewed as successful and may shape the future of predictive sciences in drug disposition. While the expression of P450s in these systems is an appropriate validation of how they compare with liver microsomes and human hepatocytes, an application of such a novel human-derived hepatic system relies on the physiologic relevance of transporter expression and relative activity compared with that in healthy human tissue. Caetano-Pinto and Stahl (2018) comprehensively reviewed the expression profiles and functional activity of clinically relevant efflux and uptake transporters in human primary cell-derived in vitro cell cultures including organoids and spheroids as well as microphysiological systems of the liver. The authors have extended this review to models of intestine, kidney, blood-brain barrier, and tumor cells.

The liver is also a locus of toxicological events. However, liver toxicity is not yet fully predictive despite several in vitro (Aleo et al., 2014; Shah et al., 2015) and in vivo models tested to date. This issue provides an expert review of different in vitro assays, including cytotoxicity assays, reactive metabolite assays, and mitochondrial dysfunction assays and their ability to predict idiosyncratic drug induced liver injury (Kenna and Uetrecht, 2018). The authors also recommend generating a greater mechanistic understanding of DILI and developing more validation of immune tolerance assays to improve the prediction of idiosyncratic drug induced liver injury with both existing and new in vitro toxicology assays.

Eye. While there are no validated, high-throughput ADME (Absorption, Distribution, Metabolism, and Excretion) screening models available to study the ocular disposition and toxicity of drugs, in this issue, Yamaguchi and Takezawa (2018) reported an in vitro model mimicking the corneal epithelial and endothelial cells via a collagen vitrigel membrane. This model, as designed, captures barrier functions of the cornea and can be applied to predict corneal permeability of compounds in drug screening. In addition to permeability assessments, understanding the disposition, distribution, and toxicology of molecules across the corneal epithelium is of importance. In this issue, Dumouchel et al. (2018) reviewed additional in vitro ocular models derived from primary systems to study the disposition and toxicity of small molecules. This mini-review discusses the advantages and disadvantages of ocular cell lines, ocular tissue homogenates and tissue sections, and ocular subcellular fractions, with the caveat that extensive pharmacokinetic data on human ocular disposition are not available.

Kidney. Renal elimination of drugs is predicted from preclinical models by allometric scaling as validated models of renal disposition and nephrotoxicity do not exist. Species differences in renal transporters were reported previously (Dresser et al., 2000; Shen et al., 2015). It is therefore no surprise that the development of new primary cell-derived kidney models have received significant attention. In this special issue, Bajaj et al. (2018a) reviewed microphysiological and bioprinted models of kidney with an emphasis on the origin of kidney cell sources from which these models have been derived and optimized. The authors discussed how these emerging models may have the potential to change our approach to predict renal clearance and renal DDIs.

As mentioned above, in addition to renal disposition and DDIs, nephrotoxicity has also received significant attention during early and later stages of drug development and regulatory review. In this issue, Bajaj et al. (2018b) also reported differentiation of human PSCs into 3D structures, capturing the proximal tubule cell types, and subsequently evaluated this model as a predictive assay for nephrotoxicity. In this system, solute linked carriers and ATP binding cassette transporters were expressed at physiologically relevant levels, but renal transporters were expressed at lower levels. Functionally this system can distinguish nephrotoxins from non-nephrotoxic compounds and offers a starting point to study nephrotoxicity using human-derived kidney cells.

In Vivo. While new technologies like tissue engineering and microfluidics appear to be advancing our science on the in vitro front, there are also advances being made to improve in vivo animal models, particularly to help make the data generated in these models more mechanistic and less descriptive. Genetic manipulation of the drug metabolizing enzymes and transporters in rodents can almost be considered commonplace, with models such as the multidrug resistant protein triple knockout mouse model considered as a well characterized mainstay of drug research to address questions of absorption and brain penetration (Uhr et al., 2007; Ose et al., 2008; Kodaira et al., 2010). In other models, not only are animal genes coding for drug disposition proteins knocked out, but the corresponding orthologs of human genes are knocked in. Furthermore, investigators have attempted to humanize fully specific organs in rodents, an exciting prospect that has shown some mixed success (Ohtsuki et al., 2014). In addition to genetically manipulated animals, natural variants such as the Eisai hyperbilirubinemic rat,which lacks the biliary multidrug resistance protein 2 transporter, can serve as a valuable tool in drug research (Chu et al., 2006). In addition to modified/mutant animals, research into specific species that may offer a better picture of drug disposition in the human is always of interest. Certainly on an evolutionary relationship, it might be expected that various species of monkey would offer a more human-like picture of drug metabolism and disposition (De Bruyn et al., 2018). However, there are many examples where this is not the case. To this end, one article in this special issue discusses the advantages and disadvantages of porcine species as models of human drug disposition (Tang and Mayersohn, 2018). As with any animal species, similarities and differences to humans with regard to drug disposition will exist. The authors have humorously titled their article with the colloquialism "a pig in a poke?", questioning whether the use of pigs to study drug disposition is valid. In their summary they make the case that the pig as a model of human drug disposition is actually quite good, and they call for its increased use so that more data can be gathered as validation.

In vivo whole body imaging in laboratory animals and in humans to visualize drug distribution is another emerging and powerful technique to understand how the body deals with xenobiotics (Eyal et al., 2009; Sugiyama, 2009; Takashima et al., 2012; Hume et al., 2013; Ke et al., 2013; He et al., 2014; Takashima et al., 2015). Drug transporters can have profound impact on the concentrations of drugs in various tissues. Certainly the use of positron emission tomography using carbon-11–labeled drugs has offered insight into distribution phenomenon exemplified by recent examples, including a pravastatin analog for liver (Kaneko et al., 2018) and verapamil for the brain (Liu et al., 2015b). In this special issue, we have included an article wherein investigators used a naturally fluorescing compound pheophorbide A to visualize breast cancer resistance protein activity in the mouse (Yasuda et al., 2018). This is appealing because it does not require the use of cyclotron-derived radioisotopes, and the effect of breast cancer resistance protein inhibitors in vivo can be probed by visualizing the differences in pheophorbide A distribution.

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In contrast to the modest success achieved from the study of drug metabolism and distribution in naive laboratory animals, P450 humanzed mice, although investigated extensively, have met with mixed success. Bissig et al. (2018) reviewed the subject and summarized the applications of humanized P450 mouse models. The authors reviewed the differential regulation in pregnane X-receptor and retinoid X-receptor pathways between mouse and human, leading to differences in drug metabolism and DDI outcomes in these humanized mice that may be different from humans. Incomplete humanization can also contribute to the lack of translatability of these models. In addition, studying genetic variation (P450 polymorphism in these models) has been shown to be challenging due to the lack of complete characterization of these developed systems. Bissig et al. (2018) suggested humanization of individual genes/proteins to generate chimeric mice as an alternate approach. However, very high variability, incomplete humanization, compromised immune system, and incidence of adverse drug reactions can limit the use of these models. The current relatively low scalability and high cost add to the limited applications and usage of such humanized systems for drug screening and development.

In this special issue, in addition to P450 humanization of mice, Chen and Tukey (2018) reviewed UGT humanized mice. The authors discussed the humanization of mice with the UGT1 locus (hUGT1 mice), and the application of the UGT1A1 gene has provided a unique tool to study the onset of hyperbilirubinemia and the implications for neonates. Most recently Yamasaki et al. (2018) generated a novel P-gp humanized mouse model and reported that human multidrug resistant protein 1 mRNA was detected (lower than physiological amounts) and was functionally lower in activity than in humans in various tissues of the humanized mice, including in the brain capillary fraction and plasma membrane fraction of intestinal epithelium.

Apart from significant advances made in developing primary cell-derived systems, one of the most rapidly growing bioengineering tools in all fields of medicine is clustered regularly interspaced short palindromic repeats (CRISPR) and their associated (Cas) nucleases or CRISPR-Cas. In drug metabolism, this technology has been applied both in vitro and in vivo. In vitro, Dorr et al. (2017) were the first ones to report using CRISPR-Cas9 to successfully edit the CYP3A5 allelle in the human hepatocyte cell line *HuH-7*. Most recently, two mutant sulfotransferase 4A1 mouse strains were generated utilizing CRISPR–Cas9 technology (Garcia et al., 2018). In vivo, Sano et al. (2018) evaluated organic anion transporter 1a4-humanized mice to predict the net flux of probe organic anion transporter 1a2 substrates, but met with limited success, because in this model the mRNA increase did not result in protein expression and a <2-fold change in brain-to-plasma ratios of probe substrates in knock-in animals compared with that determined in wild-type animals (Sano et al., 2018). In this issue, Karlgren et al. (2018) extensively reviewed all of these and other reports where this technology has been used by drug metabolism scientists in vitro and in vivo. In this review, the authors describe several novel applications of this technology to determine the role of drug metabolism enzymes and transporters in xenobiotics disposition, the study of interindividual variability using CRISPR-Cas9, and human applications of CRISPR-Cas9 with associated safety issues that will need to be addressed prior to its broader application.

Finally, this issue has described how genetically diverse mouse populations can be applied to study toxicology attributed to genetic polymorphisms (Mosedale, 2018). More specifically, the author reviewed the application of diverse mouse populations to understand and predict adverse drug reactions and DILI. The review includes discussion of how such genetically modified mouse strains can improve the translatability of nonclinical safety studies and how outcomes pooled from such nonclinical toxicology studies followed by building a population model may result in an early assessment of adverse drug reactions of new compounds associated with genetic variants. This mini-review also discussed applications and limitations of a mouse diversity panel and mouse populations composed of newer recombinant lines and associated challenges.

Conclusions and Future Directions

In conclusion, the most favorable and desirable outcome of this special issue would be to further the development of models and advance accurate prediction methodologies and tools to predict prospectively the fate of new chemicals in humans. Recapitulating human physiology in a laboratory setting will enable investigating fundamental biochemical mechanisms of xenobiotic disposition and lead to a greater understanding of drug-drug and drug-disease interactions. Such investigations will reduce uncertainty in predictions of pharmacokinetics and toxicology. Alternatively, such investigations when performed retrospectively may uncover mechanisms of DDIs, toxicity, and disposition of small molecules that would otherwise be unexplainable by current systems and reagents. Such outcomes can be achieved by these models once qualified against pre-specified clinical markers (reverse translation of functional and biochemical end-points). Systems that are validated could be applied to predict disposition and toxicity in humans in the near term and to support regulatory dossiers for new drug candidates in the longer term. As alluded to in the introduction, the tools and technologies that were new in the 1980s and 1990s have become routine in present day drug disposition research. It is highly likely that the new tools and technologies described in this special issue will be viewed as routine drug disposition approaches in the coming decade.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Sawant-Basak, Obach.

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