Published in final edited form as:

Expert Opin Drug Metab Toxicol. 2016 May ; 12(5): 475–477. doi:10.1517/17425255.2016.1162292.

New technologies in drug metabolism and toxicity screening: organ-to-organ interaction

Abhinav Bhushan[1], **Nicole J. Martucci**[2], **Berk Usta**[3], and **Martin L. Yarmush**[*,3,4]

[1] Department of Biomedical Engineering, Illinois Institute of Technology, Chicago, IL 60616

[2] Department of Biomedical Engineering, Binghamton University, Binghamton, NY 13902

[3] Center for Engineering in Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114

[4] Department of Biomedical Engineering, Rutgers University, Piscataway, NJ 08854

1. Introduction

Pharmaceutical drugs are necessary to cure or slow down the progression of diseases. The promising chemical and biological candidates that go through the drug development process are evaluated first for toxicity and then for efficacy against the target disease[1]. Before these promising drug candidates can be administered to humans, they go through extensive testing through in silico models, in vitro cell based assays, and animal models at different stages of preclinical drug development. The goal of these stages is to predict as much of the human relevant drug metabolism and toxicity as possible so that compounds that may cause these adverse events can be identified[2]. We present below some of the new technologies that can increase our confidence in predicting the human metabolism and toxicity. Here we will focus the discussion on emerging technologies that incorporate cells and proteins of human origin for the use in pre-clinical studies.

2. Humanized mouse models

Genetically humanized mice are animals in which a mouse gene has been replaced by a portion of a human gene. Genetically humanized mice have been used to evaluate the impact of various xenobiotic receptors, transporters, and CYPs to the metabolism, disposition, and toxicity of a myriad of drug compounds [3]. One of the main advantages of using the genetically humanized mouse is the ability to maintain the models through breeding, as the replaced human gene carries over to the fetus. These mice models have shown to be useful in various applications for drug metabolism, disposition, and toxicity because they have one or a few selected humanized proteins. This allows the direct contribution of the selected human protein to be traced, such as the cytochrome P450s (CYPs). In the case of the liver, the genetically humanized mouse models can also predict human hepatotoxicity through the

[*]Corresponding author, ireis@sbi.org.

Declaration of Interest

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

use of xenobiotic receptors and CYPs[4]. Specifically, through the role of CYP2E1, humanized mouse models have predicted acetaminophen toxicity to the liver[4]. Additionally, genetically humanized mouse models have been used to study the metabolism of PhIP (2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine) through the use of CYP1A2[5].

The genetically humanized mice are limited when a need for a complete expression of human genes is desired. A recent technology that overcomes this limitation of the genetically humanized mice is the chimeric liver humanized mouse models that aims to achieve complete repopulation of the mouse liver with human hepatocytes. These mice are programmed to avoid rejection of the transplanted human hepatocytes as the mouse liver cells are ablated. There are currently three primary models of the chimeric liver humanized mice undergoing development - uPA/SCID model, the FRG model, and the TK-NOG model[6]. These models differ in the way they achieve repopulation of the liver by human cells; however, the end result for each model is similar, i.e., humanization of the liver. A major advantage chimeric models have over the genetically humanized models is that all genes, proteins, and pathways are of human origin. This allows the toxicity of a compound towards the human cells to be assessed. Chimeric mouse models have resulted in the successful prediction of two known toxic compounds previously not recognized as toxic by traditional preclinical studies, fialuridine[7] and bosentan[8].

Both the genetically and chimeric humanized mouse models have a lot of promise in predicting human metabolism and toxicity for drugs. The present models have primarily focused on the metabolic component, i.e., the liver. As these models become more complex and are adopted, we see great potential to study organ-organ interactions, which will of course depend, up to some extent, upon the organs that have been humanized. The predictability of clinical drug toxicity, metabolism, and success rates in drug development will significantly benefit by means of genetically and chimeric liver humanized mouse.

3. In vitro microphysiological cell constructs

The liver is the primary organ that metabolizes a drug compound into its metabolites. Often, drugs are not seen to be efficacious or toxic to the human body until after the compound has been metabolized. These metabolites can often be toxic to other organs such as the heart or the kidney. Such cross-organ toxicity can traditionally only be evaluated in animal models. A new technology of microphysiological multi-organ systems is being developed that can mimic these toxic tissue-tissue interactions[9]. These multi-organ constructs can simulate human metabolism of the liver, convert the drug of interest into its metabolites, and not only quantify the therapeutic actions but also the potential toxic side effects the metabolites have on other organs. The advances in pharmaceutical development that could be seen through the use of these multi-organ constructs is enormous, having the potential to recognize toxicity of drugs to humans before they enter clinical trials.

The multi-organ constructs are able to mimic the in vivo microphysiology by connecting several in vitro organ-on-a-chip culture models together through a physiologically relevant fluidic stream. One advantage of using these devices is the ability to use human cells

compared to normal toxicological animal studies. This potentially allows for a higher predictivity of toxic compounds since it is mimicking the human metabolism instead of animal metabolism. Other advantages of using the multi-organ constructs is the cost efficiency, which allows one to test many combinations of compounds of varying concentrations, without having to purchase a large amount of animals.

Many labs have been successful with their development of multi-organ microdevices. One team developed constructs comprising liver-intestine[10], liver-skin[10], and a liver-neurosphere[11]. Using a multi-organ chip platform, these researchers used the liver to metabolize the drug of interest, troglitazone, and used either a reconstructed human intestine as a barrier model or a human skin biopsy to mimic the human vasculature. By applying repeated doses of troglitazone, Maschmeyer et al. were able to quantify oral and systemic administration routes in humans[10]. The developed liver-neurosphere microdevice was capable of long term performance – application of 2,5-hexanedione, a neurotoxin, to the liver-neurosphere co-culture induced high apoptosis within the system previously not seen with single tissue culture systems[11].

The same group has also developed a four organ construct comprising small intestine, liver, skin, and kidney[12]. The primary human small intestinal model was used to provide barrier function between the apical side of the intestine and blood flow, while the basolateral side of the intestine was able to distribute the substance to a liver model. They added renal proximal tubule cells to separate the blood flow circuit from the second excretory circuit. A skin biopsy was added to be used as an alternative absorption route and to analyze toxicity due to a drug and its metabolites. Measuring the concentration of glucose and lactate in the media supernatants, they were able to quantify the metabolic activity of the tissues. Through gene expression analysis and immunohistochemistry, they showed that each of the organs' characteristics and capabilities were comparable to physiologically relevant values.

Tasking a different approach, Bale et al. demonstrated a novel low-volume two-chamber micro fabricated platform for evaluating drug metabolism and toxicity[13]. The high media to cell ratio often seen in many multi-organ constructs can cause dilution of the metabolite that is formed, which reduces the toxic or therapeutic effects that are being studied. The microdevice developed here utilized a permeable membrane, which eliminated the need for any fluidic flow, creating a low media to cell ratio platform. Tegafur-uracil, whose metabolites act as a chemotherapeutic drug, was applied to the two chamber device comprising human liver and human breast cancer cells. Within 24 hours, the resultant toxicity and metabolic conversion were able to be measured and seen to be similar to results *in vivo*. The significance of this device is in the low media to cell ratio, creating a microscale device unlike many other devices currently on the market. The high volume to cell ration in the current marketable devices often miss important toxic compounds because they do no mimic physiologic conditions closely enough. This new microdevice detailed by Bale et al. provides a novel platform for screening drugs for toxic effects.

4. iPS-derived cells

Primary human hepatocytes have long been considered the gold standard for liver metabolism and toxicology assessments because they retain their phenotypic functions for several weeks. However, the lack of general availability of primary human hepatocytes and significant lot-to-lot variability often make them difficult to use. There have been several ongoing efforts to derive human hepatocytes from induced pluripotent stem cells (iPSC) and to use these cells for drug development. Recently, Ware et al. developed a micropatterned co-culture using commercially available induced pluripotent stem cell-derived human hepatocyte-like cells (iMPCCs)[14]. Treated for six days with 47 different drugs, the iMPCCs increased the response to drug toxicity compared to the conventional methods. Through the use of iPSC taken directly from the patient, iMPCCs can show patient specific responses to certain drugs of interest, and lead to the development of personalized medicine[15]. Several groups are expanding the use of iPS derived cells to represent cells from other organs such as heart, kidney, and brain. This is very promising because as these cell transformations become routine, one can start to assemble different cell constructs together to mimic organ-organ interactions investigate drug metabolism and toxicity and even obtain a personalized outcome.

5. Expert Opinion

The chimeric mice models have made it possible to study the role of human enzymes in metabolizing drugs as well as toxicity, which could decrease the cost of developing drugs and expedite clinical trials. With further advancement in the technology, we could begin to see these models being applied to toxicity of other organs and truly develop into a potent tool to study organ-organ interactions in drug metabolism and toxicity. Advances in the in vitro technologies through the development of microphysiological systems that use human origin cells are exciting because it is becoming possible to study toxicity, metabolism, and organ-to-organ interactions without conducting animal studies. One challenge that the field faces is to develop appropriate culture media or a universal media to culture all of the organs together; most cells have specific concoction of media that is best for their growth – connecting these cell constructs together to be cultured under one media makes it very challenging. Another challenge is to maintain physiological ratio of cells between the organs and relative to the media or blood. Microfluidics offers these cultures an advantage of reducing the media volumes several fold to improve the cell to media ratio, however, tailoring it to different organs that are connected together will be an important barrier to be addressed. With the addition of robust cell sources such as iPSC derived tissue specific cells, these microphysiological systems are expected to become even more versatile and reliable. The iPSC technology is growing exponentially; however, the iPSCs themselves are different from stem cells, and the cells derived from iPSCs are different from the primary cells. These differences still need to be worked out especially in terms of how they affect drug metabolism and toxicity. iPSCs however represent a very exciting future because they can provide the fastest route to personalized assessment of drug metabolism and toxicity. The ultimate goal of these technologies is to be able to predict the metabolism and toxicity of drug compounds under development before the first-in-human studies. Taken together, these

promising new technologies show great promise towards predicting human metabolism and toxicity and thereby accelerating translation of drug compounds to the clinic.

Acknowledgments

The authors were supported in part by NIH grant DK095984.

Bibliography

Papers of special note have been highlighted as:

* of interest

** of considerable interest

1. Pritchard JF, Jurima-Romet M, Reimer ML, Mortimer E, Rolfe B, Cayen MN. Making better drugs: Decision gates in non-clinical drug development. Nature Reviews Drug Discovery. 2003; 2(7):542–553. [PubMed: 12815380]
2. Rautio J, Kumpulainen H, Heimbach T, et al. Prodrugs: design and clinical applications. Nature Reviews Drug Discovery. 2008; 7(3):255–270. [PubMed: 18219308]
3. Jiang XL, Gonzalez FJ, Yu AM. Drug-metabolizing enzyme, transporter, and nuclear receptor genetically modified mouse models. Drug Metab Rev. 2011; 43(1):27–40. [PubMed: 20854191]
4*. Cheung C, Yu AM, Ward JM, et al. The CYP2E1-humanized transgenic mouse: role of cyp2e1 in acetaminophen hepatotoxicity. Drug Metabolism and Disposition. 2005; 33(3):449–457. [PubMed: 15576447] [Paper reports on introducing into a mouse a human gene that produces active enzyme capable of metabolizing drugs.]
5. Cheung C, Loy S, Li GX, Liu AB, Yang CS. Rapid induction of colon carcinogenesis in CYP1A-humanized mice by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and dextran sodium sulfate. Carcinogenesis. 2011; 32(2):233–239. [PubMed: 21081470]
6**. Scheer N, Wilson ID. A comparison between genetically humanized and chimeric liver humanized mouse models for studies in drug metabolism and toxicity. Drug Discovery Today. 2015 [A very good introduction to the different humanized mice models.]
7. Xu D, Nishimura T, Nishimura S, et al. Fialuridine induces acute liver failure in chimeric TK-NOG mice: a model for detecting hepatic drug toxicity prior to human testing. PLoS Medicine. 2014; 11(4):e1001628. [PubMed: 24736310]
8. Xu D, Wu M, Nishimura S, et al. Chimeric TK-NOG mice: a predictive model for cholestatic human liver toxicity. Journal of Pharmacology and Experimental Therapeutics. 2015; 352(2):274–280. [PubMed: 25424997]
9**. Esch EW, Bahinski A, Huh D. Organs-on-chips at the frontiers of drug discovery. Nature Reviews Drug Discovery. 2015; 14(4):248–260. [PubMed: 25792263] [A good source of information on microphysiological systems.]
10. Maschmeyer I, Hasenberg T, Jaenicke A, et al. Chip-based human liver-intestine and liver-skin co-cultures--A first step toward systemic repeated dose substance testing in vitro. European Journal of Pharmaceutics and Biopharmaceutics. 2015; 95(Pt A):77–87. [PubMed: 25857839]
11. Materne EM, Ramme AP, Terrasso AP, et al. A multi-organ chip co-culture of neurospheres and liver equivalents for long-term substance testing. Journal of Biotechnology. 2015; 205:36–46. [PubMed: 25678136]
12. Maschmeyer I, Lorenz AK, Schimek K, et al. A four-organ-chip for interconnected long-term co-culture of human intestine, liver, skin and kidney equivalents. Lab on a Chip. 2015; 15(12):2688–2699. [PubMed: 25996126]
13. Bale SS, Sridharan GV, Golberg I, et al. A novel low-volume two-chamber microfabricated platform for evaluating drug metabolism and toxicity. TECHNOLOGY. 2015; 03(04):155–162. [PubMed: 26925437]

14. Ware BR, Berger DR, Khetani SR. Prediction of Drug-Induced Liver Injury in Micropatterned Co-cultures Containing iPSC-Derived Human Hepatocytes. Toxicological sciences. 2015; 145(2):252–262. [PubMed: 25716675]

15**. Kimbrel EA, Lanza R. Current status of pluripotent stem cells: moving the first therapies to the clinic. Nature Reviews Drug Discovery. 2015; 14(10):681–692. [PubMed: 26391880] [An excellent review on iPSC technology.]