Toxicity testing and drug screening using iPSC-derived hepatocytes, cardiomyocytes and neural cells

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<th>Journal</th>
<th>Canadian Journal of Physiology and Pharmacology</th>
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<tr>
<td>Manuscript ID</td>
<td>cjpp-2015-0459.R1</td>
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<tr>
<td>Manuscript Type</td>
<td>Review</td>
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<tr>
<td>Date Submitted by the Author</td>
<td>23-Nov-2015</td>
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<td>Complete List of Authors</td>
<td>Csoıbonyeiova, Maria; Faculty of Medicine, Comenius University in Bratislava, Institute of Histology and Embryology; Polak, Stefan; Faculty of Medicine, Comenius University in Bratislava, Institute of Histology and Embryology; Danisovic, Lubos; Faculty of Medicine, Comenius University in Bratislava, Institute of Medical Biology, Genetics and Clinical Genetics</td>
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<td>Keyword</td>
<td>iPSCs, cardiotoxicity, hepatotoxicity, neurotoxicity, drug screening</td>
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Toxicity testing and drug screening using iPSC-derived hepatocytes, cardiomyocytes and neural cells

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Abstract

Unexpected toxicity in areas such as cardiotoxicity, hepatotoxicity and neurotoxicity is a serious complication of clinical therapy and one of the key causes for failure of promising drug candidates in development. Animal studies have been widely used for toxicology research to provide preclinical security evaluation of various therapeutic agents under development. Species differences in drug penetration of the blood-brain barrier, drug metabolism, and related toxicity contribute to fail of drug trials from animal models to human. The existing system for drug discovery has relied on immortalized cell lines, animal models of human disease, and clinical trials in humans. Moreover, drug candidates that are passed as being safe in the preclinical stage often show toxic effects during the clinical stage. Only around 16% drugs are approved for human use. Research on induced pluripotent stem cells (iPSCs) promises to enhance drug discovery and development by providing simple, reproducible, and economically effective tools for drug toxicity screening under development and, on the other hand, for studying the disease mechanism and pathways. In this review, we provide an overview of basic information about iPSCs, and discuss efforts aimed at the use of iPSC-derived hepatocytes, cardiomyocytes, and neural cells in drug discovery and toxicity testing.

Keywords: iPSCs; cardiotoxicity; hepatotoxicity; neurotoxicity; drug screening
Background

Development of new drugs is very complex, expensive and time-consuming process where more than 90% of drug candidates fail in clinical trials due to issues of safety and efficacy (Yu et al. 2013). Conventional drug discovery and development rely on traditional, expensive, animal *(in vivo)* models, such as mice, rat, pig, and monkey. However, such models rarely mirror human disease pathological mechanism. Notably, mice are at least 10x more tolerant to 37% of drugs than humans, while rats and dogs tolerate 4.5-100-fold the concentration of various chemical compounds as humans (e.g. ThioTEPA, Myleran, Actinomycine-D, Mitomycine C, Mythramycine, Fludarabine) (Rajamohan et al. 2013). For example, nonhuman stroke models have been used in the preclinical phase, but failed to predict the responses in humans. Moreover, certain chemicals predicted to be safe from animal studies induce birth defects or serious toxicity in humans. The most notorious example of a drug considered safe after animal testing, but with significant devastating effects in humans is thalidomide, which had no effects on prenatal development in rodents, but caused severe developmental defects in human children whose mothers had taken it during pregnancy to get rid of morning sickness. Thus, animal models alone are unreliable for understanding human disease and for screening drug candidates (Giri and Bader 2015; Kumar et al. 2012; Liu et al. 2013). In recent years, researchers have been forced to find more relevant assessment strategies, including the 3R (replacement, refinement, and reduction) to reduce animal use whenever possible through integrated testing (Anson et al. 2011; Terry et al. 2014). Test methods precisely detecting whether chemical and products can cause injury or disease are necessary for health protection. However, new test methods should not only be more predictive, but also faster, cheaper, and minimize the use of animals (Erkekoglu et al. 2011).
There is growing impulse to move away from animal models for toxicity testing toward cell-based *in vitro* assays, predominantly in preliminary screening. Predictive *in vitro* cell-based assays should reduce or circumvent the dependence on animal models, simplify the assessment of candidate molecules and their metabolites, and facilitate evaluation of the mechanism of toxicity, kinetics, and dose-responses (Sirenko et al. 2014). Combined with newer technology quantifying cell type-specific functions, human cell-based trials could be used to drive structure-activity relationship (SAR) to improve safety profiles. However, most existing *in vitro* assays are below the required standard since they rely on immortalized cell lines or isolated primary cells. Applying human cell-based toxicity assays requires a ready source of human cells exactly reflecting the desired *in vivo* phenotype, and stably maintaining this phenotype in culture. They should be also provided in large scale and at an available cost. Recent technical advances suggest that pluripotent stem cells (ESCs/iPSCs) may ultimately meet these criteria (Scott et al. 2013). Human ESCs derived from early blastocysts are the prototype of pluripotent stem cells that are able of unlimited growth in tissue culture and can differentiate into all cell types in the body (Lian et al. 2010). In contrast to ESCs, iPSCs can be derived from mature differentiated cells by expression of particular pluripotency genes (Oct4, Sox2, Klf4, and c-myc) (Takahashi et al. 2007). Consequently, efforts were made to screen more transcription factors and the iPSCs were produced using a set of TFs: Oct4, Sox2, Nanog, and Lin28 suggesting alternative factors affecting the pathways needed for reprogramming (Nagakawa et al. 2008; Okano et al. 2013). After initial success, the introduction of iPSCs was subsequently shown in many other cell-specific tissues, such as skin fibroblasts, hair follicle, liver and stomach cells, blood cells, amniocytes, etc. (Aasent et al. 2008; Aoi et al. 2008; Hanna et al. 2008; Miyoshi et al. 2010). Genetic material of reprogramming factors has been introduced into the cells via a variety of methods, comprising genome integrating (retroviral/lentiviral/adenoviral/Sendai virus vectors), as well as safer
non-integrating techniques (plasmids, proteins, RNA, the Cre/loxP system, piggyback vectors, minicircle vectors, and small molecules) (Okita et al. 2011; Stadtfeld et al. 2008; Woltien et al. 2011).

Since iPSCs are generated from mature cells, they overlap the ethical and legal issues associated with human ESCs, which require extraction from embryonic tissue. Thus, iPSCs offer a number of advantages over the traditional methods and represent an excellent source of cells suitable for studying human tissue differentiation and function. After initial success, the introduction of iPSCs was subsequently shown in many other cell-specific tissues, such as skin fibroblasts, hair follicle, liver and stomach cells, blood cells, amniocytes, etc. (Aasent et al. 2008; Aoi et al. 2008; Hanna et al. 2008; Miyoshi et al. 2010).

Disease-specific iPSCs can provide a renewable source of patients-specific cells with genetic background sensitive to disease pathology. Drug screening using these cellular models could provide a more reliable assessment of the test compounds (Fig. 1) (Anson et al. 2011; Marchetto et al. 2010). At this point the use of iPSCs in drug screening is in its beginning, and progress toward the development of standardized screening system is still being developed (McGivern and Ebert 2014). Finally, iPSCs may also be used to test developmental, as well as cell-type-specific drug toxicities. Indeed, there are already commercially available human iPSC- derived hepatocytes, cardiomyocytes, and neural cells that may provide the basis for humanized assays to detect side-effects of drugs in a tissue-specific manner (Table 1) (Scott et al. 2013).

**iPSC-derived neurons in drug discovery and toxicity testing**

Neurodegenerative diseases, including Parkinson’s disease (PD), Alzheimer’s disease (AD), Huntington’s disease (HD), and amyotrophic lateral sclerosis (ALS) present an increasing burden for society (Deshmukh et al. 2012). It is widely believed that for most
neurodegenerative disease, there are no effective therapies, partly because of the limited understanding of the mechanism of these disorders. The iPSC technology is useful not only for understanding the mechanism of reprogramming, but also for identifying and suggesting therapies targeting disease course well before cell death and clinical symptoms emerge, which could result to greater success in clinical trials (Cao et al. 2014; Deshmukh et al. 2012). The neurotoxicity test models will allow for studying on one hand the adverse effect on drug candidates and on the other hand the general neurotoxicity in tests well suited for screening of lead compounds. There have been many studies on the potential of iPSC-derived patient-specific cell lines offering not only modeling of molecular pathways, but also providing new targets and a screening platform for the discovery of disease-modifying drugs (Buzanska et al. 2009; Ko and Gelb 2013).

A recent study used iPSC-derived dopaminergic neurons to screen a group of compounds for neuroprotective characteristics as a treatment approach for early stages of PD. One class of these molecules is thought to act by changing dopamine synthesis or reuptake as dopamine itself is considered to be toxic to cells. Other molecules seem to have a protective effect because of their antioxidant properties (N-acetyl cysteine, resveratrol). The authors screened a small number (44) of selected compounds that demonstrated therapeutic effects in rodent systems. However, only 16 provided significant neuroprotection in the rotenone-induced dopaminergic neuron cell death model for PD, supporting the significance of using disease-relevant human neurons for disease assays (Peng et al. 2013). Another group of researchers focused on mitochondrial functions in PD associated with mutation in the PINK1 and LRRK2 (leucine-rich repeat kinase-2) genes. They found a higher sensitivity of these iPSC-derived neurons to chemical toxins valinomycin and concanamycin A. Regarding to this knowledge, iPSC-derived neural cells with these mutations were treated with the Coenzyme Q10, rapamycine or the LRRK2 inhibitor GW5074 during exposure to low concentrations of...
either valinomycin or concanamycin A. The results suggest that iPSC reprogramming technology can help to define groups of patient reacting to different pharmacological treatments (Cooper et al. 2012).

In case of ALS, iPSC have been generated from a number of different familiar forms of ALS, and it has been found that stressors or single-cell longitudinal studies are necessary to see a reduction in motor neuron survival. Yang et al. (2013) used mouse iPSCs carrying a motor neuron reporter gene to assess motor neuron survival after trophic factor withdrawal in the presence of approximately 5000 small molecules in different concentrations. Two compounds (thyrphostin A9 and kenpaullone) not previously detected as motor neuron protective were found. They chose to further characterize kenpaullone because of its protective capacity for not only the ALS motor neurons, but for the wild type motor neurons as well. Interestingly, a follow up study exploring neuroprotection in human derived ALS-iPSCs also demonstrate the effectiveness of kenpaullone compared to two other compounds that had each failed to demonstrated effectiveness in clinical trials. In another study human iPSC-derived motor neurons from patient with ALS were tested with drugs to increase the limited treatment options. Four compounds known to act on RNA metabolism or histone acetylation were screened on this platform, including anacardic acid, which was able to prevent motor neuron death and lower the production of mutant protein. These efforts have now opened the possibility of anacardic acid as a treatment for this incurable neurological disease (Egawa et al. 2012).

Yagi et al. (2011) produced iPSCs from skin fibroblasts of familiar AD patients (preselin-1 or -2 mutations) and succeeded in inducing neuronal cells. These patient-derived neuronal cells produced twice the normal level of the highly toxic Aβ-42. Following treatment of AD iPSC-derived neuronal cells with a γ-secretase modulator revealed production of Aβ-
42 was inhibited, thus this report also gave an example of the potential use of AD iPSCs for testing drug efficacy (Chen and Blurton-Jones 2012).

Demonstration of the interest in iPSCs obtaining new insights related to pathogenesis and treatment was published by Lee et al. (2012) concerning familiar dysautonomia (FD). Using patient specific iPSCs expressing disease-specific biomarkers, authors tested 6,912 candidate molecules and characterized 8 that rescued expression of IKBKAP (inhibitor of κ light polypeptide gene enhancer in B-cells, kinase complex-associated protein), the gene responsible for FD. SKF-86466 was found to induce IKBKAP transcription through modulation of intracellular cAMP levels and PKA-dependent CREB phosphorylation. This molecule also rescued IKAP protein expression and the disease-specific loss of autonomic neuronal markers expression. Their data demonstrate that small molecule discovery using an iPSC-based disease model can identify candidate drugs for therapeutic intervention.

In last decade, there are an increase concerns about neurotoxicity induced in humans by exposure to chemicals. Perhaps one of the most powerful applications of iPSCs in the field of neurotoxicology is the ability to assess the response of neurons and neural progenitors to various toxicants. For example, there is a great interest in examining metabolic markers that predict developmental toxins in pluripotent stem cell-derived neural lineages. Therefore iPSC-derived neural cells offer a unique source for toxicity screens (Liu et al. 2013; McGivern et al., 2014).

*In vitro* neuronal differentiation of human iPSCs enables assessment of interaction between early exposure and subsequent risk of neurodegenerative phenotypes in response to acute, chronic, latent toxicant exposure paradigms. Ito et al. (2015) established *in vitro* model for assessing the neurotoxicity of ketamine to human iPSC-derived neurons. Recent studies have shown that ketamine, widely used in anaesthesia, preoperative sedation and analgesia, can have neurotoxic effect in rodents and nonhuman primate (Liu et al. 2011; Paule et al.
Researchers treated human iPSC-derived dopaminergic neurons with various concentrations (20, 100, 500 µM) of ketamine to study their cellular responses. Results showed no effect on cellular morphology of lower doses of ketamine. However, 500 µM ketamine caused neuronal process restriction. Moreover, high dose of ketamine increased ROS production and activated capsase 3/7 activity. Interestingly, mitochondrial dysfunction was observed at 100 µM.

Recently, Pei et al. (2015) published study reporting comparative cytotoxicity of 80 compounds with known neurotoxic effects comprised drugs and pesticides on iPSCs, as well as iPSC-derived neural cells, neurons and astrocytes using the MTT assay. According their results, 50 compounds induced significant cytotoxicity in at least one cell type, four compounds (valinomycin, 3,3’,5,5’-tetrabromobisphenol, deltamethrin, and triphenyl phosphate) displayed cytotoxicity in all tested cell types. Moreover, authors simplified cytotoxicity assay by measuring luciferase activity using lineage-specific luciferase reporter iPSC lines.

iPSC-derived cardiomyocytes in drug discovery and toxicity testing

Heart failure belongs to the most common causes of death in the western countries. In USA about 5.1 million individuals suffer from the heart failure, and about 280 000 die per year. On the basis of 2011 death rate data, more than 2150 Americans die of cardiovascular disease (CVD) each day, an average of 1 death every 40 seconds (Mozaffarian et al. 2015). CVDs include a broad range of disorders such as atherosclerosis, ischemic heart disease, acute myocardial infarction, valvular heart disease, heart failure, cardiomyopathies, arrhythmias, hypertension, and congenital heart disease (Liang and Du 2014). Medical advances at pharmacological and surgical levels have significantly decreased the rate of mortality at the acute stage of the disease, and prolonged life expectancy. The latest advances in research are
promising, although much remains to be learned about pharmacological and cellular treatments for CVDs (Mordwinkin et al. 2013). Rodent models fail to imitate the essential physiological functions of heart because of their faster heart beat than human. Thus, they are inappropriate animal models to test the drugs for arrhythmias in human (Deshmukh et al. 2012).

Considerable efforts are made in the recent years to study the effect of a variety of pharmacological agents on cardiac electrophysiological properties by recording the intracellular action potential via whole-cell patch-clamp or extracellular electrograms via MEA (micro-electrode array). However, there are technical limitations of MEAs, such as number of assayable wells, pre-plating techniques, and length of time in which physiologically meaningful results can be acquired (Guo et al. 2011).

By differentiating patient specific iPSCs into cardiomyocytes (CMs), there is possibility to generate iPSC-based “disease in a dish” models for better understanding disease mechanism and developing new therapeutics (Liang and Du 2014). The use of human iPSC-derived CMs for drug discovery and screening has already proven promising, as human iPSC-CMs have been shown to respond to cardioactive drugs in a similar way as human ESCs (Laustriat et al. 2010; Lian et al. 2010). The advantages of iPSC-derived CMs include their ability to keep the contractile function, thereby providing the homogenous cell culture for testing which ultimately contributes to improved high-throughput drug discovery process. Large-scale generation of CMs from disease-specific human iPSC lines such as patients with heart disease, dilated cardiomyopathy, LEOPARD syndrome, long QT syndrome, and Timothy syndrome hold the potential to serve as a human-based model for both drug development and toxicology screening. This model could provide the pharmaceutical industry with a reliable tool for the pre-clinical testing of candidate anti-arrhythmic and anti-heart
failure pharmacological agents, as well as other fields of medical research for the evaluation of secondary off target cardiac toxicities (chemotherapeutic agents) (Mordwinkin et al. 2013).

Cardiovascular toxicity is a major cause of drug withdrawal during clinical development, accounting for up to 33% of drug failure. It can lead to the formation of reactive oxygen species (ROS), apoptosis, altered contractibility, change in cardiac rhythm, and changed cardiac gene expression (Khan et al. 2013). Also anti-cancer therapeutics such as anthracyclines (doxorubicine) exhibit cardiotoxicity through mechanism which is independent of its DNA binding ability, potentially form through increase ROS activity. Small molecule kinase inhibitors (KIs) are another class of anti-neoplastic drugs with reported cardiotoxic effects (dilated cardiomyophaty). Current in vitro toxicity screens depend on the artificial expression of single cardiac ion channels in genetically transformed cell lines, such as Chinese hamster ovary (CHO) or human embryonic kidney (HEK) cells, which do not accurately model appropriate genetic, cellular, or biochemical characteristics of the human heart. The use of CHO and HEK cells to assess cardiotoxicity is impaired by genetic aberrations of these cells and the failure of ectopically expressed channels to accurately model the same channels found in human CMs (Cheng and Force 2010; Ferreira et al. 2008; Liang et al. 2013). A more feasible implementation of iPSCs in drug toxicity testing at this time is on large grids and assayed for toxicity in a manner analogous to high-throughput screens for drug discovery. Using this approach, iPSC technology can be integrated into the current paradigm for drug development as part of safety testing in the early phases of clinical trials. Human iPSC-CMs recapitulate many of the in vivo cardiomyocytes functions and may be an ideal system for assessing multiple facets of toxicity. Because the mechanisms responsible for cardiotoxicity are poorly understood, and often unpredictable, it is necessary to test systems closely mimicking the in vivo condition with appropriate measurable endpoints (Anson et al. 2011; Ko and Gelb 2014). Recently, Mathur et al. (2015) presented a cardiac
microphysiological system (MPS) with the features essential for an ideal in vitro biological model to predict cardiotoxicity: a) cells with human background; b) physiologically relevant tissue structure; c) computationally predictable perfusion mimicking human vasculature; and, c) multiple models for analysis. MPS is able to keep human iPSC-derived cardiac tissue viable and functional over multiple weeks. Researchers applied this system to test cardiac response with four model drugs (Isoproterenol, E-4031, Verapamil, Metoprolol). Their results suggest that this human iPSC-derived cardiac-MPS could significantly improve the ability to prognosticate on drug efficacy and toxicity in vitro, decreasing both the cost and duration of bringing a new drug candidate to market.

Many of the drugs selected for evaluation in human iPSC-CMs are known to reduce Na\(^+\) and/or K\(^+\) ion fluxes slowing the rate of depolarization or repolarisation respectively, and lead to prolonged QT interval. Quinidine, a class of IA antiarrhythmic agent (Na\(^+\) channel blocker), prolongs the QT interval. Several reports have shown that treatment of human iPSC-CMs with quinidine resulted in prolonged field potential duration (Khan et al. 2013; Zeevi-Levin et al. 2012). Human iPSC-CMs represent dose and time dependent arrhythmias when challenged with drugs (astemizole, cisapride, dofetilide, erythromycin, flecainide, quinidine, sotalol, terfenadine and thioridazine) known to cause human Ether-a-go-go (hERG) inhibition, QT prolongation and TdP (Torsade de pointes). Drugs that block hERG channels or cause QT prolongation but do not cause TdP clinically (ranolazine, alfuzosin, verapamil, moxifloxacin) do not cause arrhythmias in human iPSC-CMs at therapeutic dose (Moretti et al. 2010).

Yokoo et al. (2009) differentiated human iPSCs into cardiomyocytes, and compared the effects of drugs on iPSC-CMs and on cardiomyocytes derived from ES cells, as well as with empirical results obtained in clinical settings. They investigated the effects of 8 drugs (procainamide, mexiletine, flecainide, propranolol, amiodarone, verapamil, adrenaline,
isoproterenol) on two indicators (beating frequency and contractility), and found that drugs affect the frequency of beating and contractility of iPSC-CMs in much the same way as they do in clinical settings. Cardiomyocytes derived from ESCs also responded to drugs in the same way as iPSC-CMs. Thus, these results suggest that patient-specific iPSCs could be used to select for the best drug to treat arrhythmic disease at the individual level.

Human iPSC-CMs from patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) were characterized by their calcium handling in response to catecholamines. Dantrolene (a drug effecting on malignant hyperthermia) was also found to reduce the abnormal delayed after depolarization seen in CPVT human iPSC-CMs, reinforcing its possible therapeutic use for CPVT (Ko and Gelb 2014).

iPSC-CMs with long QT syndrome mutations were tested with a panel of ion channel blockers to establish the similarity of their responses to the electrophysiological signature of LQTS. Treating LQTS human iPSC-CMs with potassium channel blockers exacerbated the LQTS phenotype, whereas a calcium channel blocker had moderating effects, correlating with the clinical effects of these drugs on LQTS patients (Itzhaki et al. 2011).

iPSC-derived hepatocytes in drug discovery and toxicity testing

Drug-induced liver injury (DILI) is a severe pathological condition belonging to the most frequent reasons for withdrawal from the market of approved drugs. DILI represents the major cause of acute liver failure and of liver transplantation in Western countries (Lu et al. 2015; Wobus et al. 2011). Most hepatotoxic drugs cause liver injury infrequently and in most cases after long-term treatment. Thus, a number of new drugs need to be efficiently screened every year to estimate their potential for toxicity (Yu et al. 2014). Intrinsic hepatotoxicity following acetaminophen (APAP; paracetamol in Europe) overdose accounts for the majority of cases of drug-induced acute liver failure in the United States and the United Kingdom. The
mechanisms by which drugs and their metabolites cause liver toxicity are complex and only partially understood. Mechanisms for DILI include cell stress imposed by reactive metabolites, direct targeting of mitochondrial function, inhibition of P-450, and immune reactions triggered by drugs or their metabolites (Guguen-Guillouzo et al. 2010; Russmann et al. 2009).

A number of in vitro models have been established for hepatotoxicity testing. For example, precision-cut liver slices include all cell types of the liver in their natural architecture and have xenobiotic metabolism capacity (Elferink et al. 2011). However, this model is possibly not well suited for high-throughput studies. Another model involving immortalized lineages (HepG2 and HepaRG) present major limitations in terms of supply and relevance of metabolic reactions respectively (Laustriat et al. 2010). Primary hepatocytes have a central role in metabolism that can be investigated in vitro. They make possible to study of enzymes involved in drug transformation, to identify their metabolites and to predict interactions. However, primary human hepatocytes posses sporadic and limited accessibility, high intrinsic variability, phenotypic instability, and during culturing display a rapid loss of metabolic function (Berger et al. 2015; Guguen-Guillouzo et al. 2010; Laustriat et al. 2010). There have been great advances in liver stem cell biology, but adult liver stem cells are rare within tissue, making their isolation and expansion difficult for large scale applications (Czyz et al. 2003). There has been also significant focus on generating hepatic endoderm from ESCs. However, there have been remarkable issues related to heterogeneous populations of ESC-derived hepatocytes. Hay et al. (2008) revealed the important role of Wnt3a in critical stages of human liver development. They found out that Wnt3a together with ActivinA have an effect on human ESCs, which produced a viable and predictable model of human hepatic differentiation in vitro. Results of this study present a liver differentiation model, which can circumvent the problems associated with processing primary hepatocytes.
Human iPSC-derived hepatocytes show great promise because of their ability to have a primary tissue-like phenotype, consistent and unlimited availability, and potential to establish genotype-specific cells from different individuals. These reasons make iPSCs an exciting alternative *in vitro* model system to explore the role of genetic diversity in DILI. Since cells from patients with many different metabolism phenotypes must be tested to establish safety, human iPSC-derived hepatocytes from this wide range patients, are expected to improve the drug discovery process and may lead to personalized drug administration (Chun et al. 2010; Anson et al. 2011; Yu et al. 2014; Godoy et al. 2015). For example, iPSC-hepatocytes generated from individuals with different CYP polymorphisms would be of great value for the study of drug metabolism and toxicity prediction of new drugs (Asgari et al. 2010).

Characterization of iPSC-derived hepatocytes has revealed primary hepatocytes-like features: expression of albumin, urea production, glycogen storage, the formation of bile canalicular networks, and expression and induction of major cytochrome P450 isoforms. Several assays have been developed for measuring general and mechanism-specific hepatotoxicity (Choi et al. 2013). Work of Sullivan et al. (2010) demonstrated for the first time the efficient generation of hepatic edodermal lineage derived from iPSCs of both sexes and two ethnicities. Differentiated hepatocytes exhibited hepatic morphology and expressed hepatic markers (Albumin, E-cadherin). Demonstration of endodermal origin was proved by expression of alpha fetal protein, HNF4, and CYP7A1. Si-Tayeb et al. (2010) described procedure that support efficient generation of highly differentiated human iPSC-derived hepatocytes exhibiting crucial liver functions. Such cells can integrate into parenchyma of liver *in vivo* and therefore they could be immediately useful for drug screening and toxicity testing assays.
Rashid et al. (2010) published that proteasome inhibitor (MG132) treatment of human iPSCs-derived hepatocytes from patients with α-1-antitrypsin (ATT) deficiency exacerbated the disease phenotype, revealing a disease-specific accumulation of α-1-antitrypsin. This result demonstrated capability of the disease specific iPSC-derived hepatocytes for modeling key pathological feature of ATT deficiency in vitro and may be suitable for future drug screening assays. In another study, Choi et al. (2013) used ATT-deficiency iPSCs for screening the clinical ready drug library and identified five clinical drugs to reduce ATT accumulation in these iPSC-derived hepatocytes. In addition, the authors used transcription activator-like effector nuclease (TALEN) technology for gene targeting in ATT-deficiency iPSCs demonstrating the high efficiency of this cost-effective targeting technology. Holmgren et al. (2014) used human iPSC-derived hepatocytes to study hepatotoxicity in response to chronic drug exposure, cells were exposed to relevant concentrations of hepatotoxic compounds for 2, 7, and 14 days. Monitored was also cell morphology, viability and the induction of steatosis and phospholipidosis. According the results during long-term exposure, the iPSC-derived hepatocytes showed increasing sensitivity to amiodarone and aflatoxin B1, which are highly dependent on CYP3A activity to generate toxic metabolites. Steatosis was induced by troglitazone. This study demonstrates the usefulness of human iPSC-derived hepatocytes in long-term toxicity evaluation and chronic toxicity testing in vitro.

Group of Medine et al. (2013) developed an interdisciplinary approach of generating highly stable and sensitive hepatocytes models derived from pluripotent stem cell lines (ESCs, iPSCs). Researches performed on these metabolically active hepatocytes populations comprehensive analysis of hepatocellular toxicity in response to well-defined pharmacological compounds. Based on their results, pluripotent stem cell-derived hepatocytes could model toxicity predictably representing a key progress in the field of toxicity testing.
In another study focusing on hepatocytes modeling, Szkolnicka et al. (2014) demonstrated the possibility to model the potential from DILI using human ESCs and iPSCs-derived hepatocytes. They developed a serum-free, and scalable approach to hepatocellular differentiation during which human pluripotent stem cells underwent a series of morphological changes. Human ESCs, as well as iPSCs populations exhibited downregulation of pluripotent transcripts and differentiation toward the endoderm. These cell populations could have a direct application in human modeling. Moreover, they could become a significant source in the field of cell-based therapies.

Recently, Sirenko et al. (2014) developed imaging and analysis methods (high-content imaging-based assay using iPSC-derived hepatocyte cell model) providing tools for characterization of multiple toxicity phenotypes using live cells. Their results of testing commercially available hepatotoxic compounds showed significant promise for compound screening and safety evaluation during drug development.

iPSCs-derived hepatocytes are expected to be utilized also in the field of drug screening. However, the hepatocyte differentiation efficacy and hepatic function of iPSC-derived hepatocytes were not sufficient to perform iPSC-based drug discovery. Takayama et al. (2015) decided to improve the method of hepatocytes differentiation from human iPSCs by overexpression of hepatocytes-related transcription factors (forkhead box protein A2 - FOXA2 and hepatocyte nuclear factor 1 alpha - HNF1α) during differentiation process and culturing cells in 3D conditions using Nanopillar plate. Researchers reached more than 80% efficacy of differentiation into albumin-positive iPSC-derived hepatocytes. These results offer the new possibility of efficient differentiation method as a useful tool in drug development.

Another interesting approach in animal-free drug development using iPSC-derived hepatocytes was published by Faulkner-Jones et al. (2015). The study reported the first investigation into the bioprinting of human iPSCs following postprinting differentiation into
hepatocytes-like cells (HLCs). HLCs showed no differences in hepatocytes marker expression and similar morphology when compared to a non-printed control. Moreover, authors verified that their valve-based printing process does not influence the pluripotency.

Conclusion

Patient-derived iPSCs do not only play role in drug discovery and in preclinical toxicity testing for the benefit of patients with neurodegenerative, cardiovascular, and liver diseases but also bring hope for generation of replacement cells for transplantation therapies. In the context of existing drug testing platforms, such as animal studies, human clinical trials, animal iPSCs, and ESCs, human iPSCs provide advantages that can enhance the current approaches to drug discovery. Moreover, the ability of human iPSCs to simulate organ systems may allow studying the effects of drug’s metabolites on various cell types. However, there are still a number of obstacles that need to be overcome. The major one is the low efficiency of iPSCs formation, caused by poor viral infectivity, heterogenous genomic interaction of viral vectors, random limitations of transgene expression, and cell-type-specific resistance to reprogramming (Cao et al. 2014). Another obstacle for the therapeutic use of iPSCs is teratoma formation. It has been demonstrated in the murine system that iPSC-derived chimeras frequently develop tumors. In order to reduce the risk tumor formation, the use of small molecules only represents the most recent promising improvement in reprogramming efficacy (Lu et al. 2013). Following this idea, Hou et al. (2013) achieved the first successful reprogramming using only small molecule compounds.

Other limitations include numerous technical problems, such as the time-consuming nature of experiments, large clonal variation of iPSCs, and heterogeneity of differentiated cells. The creation of scalable culture systems that require less maintenance and increase yield would be helpful. An important task in using iPSCs is producing large amounts of cells with
low heterogeneity that behave in a consistent way. Moreover, it is essential to optimize the protocols for the differentiation of iPSCs into neuronal, cardiac, and hepatic lineages, because the efficiency of differentiation is still low (Ko et al. 2014). Such protocols will have to be easily scalable, with the use of robotic platforms, or bioreactors. Production of more mature cell types from iPSCs can be beneficial for the use as a model system for adult human organs in pharmacological and toxicological studies. Alternatively, industrial sources of specified cell are also available. This possibility removes concerns for the end user over consistent maintenance and differentiation protocols (McGivern et al. 2014).

Nowadays innovative method involving bioprinting of iPSCs and differentiation to hepatocytes-like cells was published by Faulkner-Jones et al. (2015). The ability to bioprint iPSCs while maintaining their pluripotency and differentiate them into specific cell types is opening a new opportunity for producing organs from patient-specific cells which could be used for animal-free drug development.

While the application of iPSC-derived cells in toxicity testing and drug development represents the most advanced and practical use of pluripotent stem cells, the acceptance of major pharmaceutical companies to adopt a new approaches and replace the well-established test methods is rather slow process (Dick et al. 2010).

Acknowledgements

This work was supported by the Grant VEGA no. 1/0153/15 and UK no. 36/2015.

References


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Table 1. Examples of drug testing in iPSC-derived disease models

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<td>PS1, PS2</td>
<td>Compound E, compound W</td>
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<td>Familial: APP</td>
<td>DHA, dibenzoylmethane, NSC23766</td>
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<td>Kenpaullone, dexpramipexole, olesoxime</td>
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<td>Motor neurons</td>
<td>TDP-43 Q343R, TDP-43 M337V, TD-43 G2985</td>
<td>Trichostatin A, spliceostatin A, anacardic acid and garcinol</td>
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<td><strong>Familiar dysautonomia</strong></td>
<td>Neural crest</td>
<td>IKBKAP8 2507 + 6T &gt; C</td>
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<td>precursors, neurons</td>
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<td>Tested 6,912 small molecule compounds for rescued expression of IKBKAP</td>
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<td>VPA, tobramycin</td>
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<td>Cardiomyocytes</td>
<td>RYR2 S406L</td>
<td>Dantrolene</td>
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<td>CASQ2 D307H</td>
<td>Isoproterenol</td>
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<td>KCNH2 (HERG) A614V</td>
<td>Isoproterenol hydrochloride, nifedipine, ranolazine dihydrochloride, pinacidil monohydrate, cisapride, E-4031</td>
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<td>Z allele SARPINA1 E342K</td>
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<td>ATP7B R778L</td>
<td>Curcumin</td>
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**Fig. 1.** Induced pluripotent stem cells and their potential for toxicity testing and drug screening.