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Comparative neurotoxicity screening in human iPSC-derived neural stem cells, neurons and astrocytes

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ABSTRACT

Induced pluripotent stem cells (iPSC) and their differentiated derivatives offer a unique source of human primary cells for toxicity screens. Here, we report on the comparative cytotoxicity of 80 compounds (neurotoxicants, developmental neurotoxicants, and environmental compounds) in iPSC as well as isogenic iPSC-derived neural stem cells (NSC), neurons, and astrocytes. All compounds were tested over a 24-h period at 10 and 100 μM, in duplicate, with cytotoxicity measured using the MTT assay. Of the 80 compounds tested, 50 induced significant cytotoxicity in at least one cell type; per cell type, 32, 38, 46, and 41 induced significant cytotoxicity in iPSC, NSC, neurons, and astrocytes, respectively. Four compounds (valinomycin, 3,3′,5,5′-tetrabromobisphenol, deltamethrin, and triphenyl phosphate) were cytotoxic in all four cell types. Retesting these compounds at 1, 10, and 100 μM using the same exposure protocol yielded consistent results as compared with the primary screen. Using rotenone, we extended the testing to seven additional iPSC lines of both genders; no substantial difference in the extent of cytotoxicity was detected among the cell lines. Finally, the cytotoxicity assay was simplified by measuring luciferase activity using lineage-specific luciferase reporter iPSC lines which were generated from the parental iPSC line.

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1. Introduction

The human brain is enormously complex and undergoes dramatic changes in cell number, overall size, and morphology during development. The complex pattern of development is carefully orchestrated with timed morphogenetic movement, stage specific regionalization, and cell lineage segregation (Dobbing and Sands, 1973; Ourednik et al., 2001). While other developing organs exhibit a similar pattern, the sheer number of neurons, the complexity of the wiring, and the disproportionately large number of genes that are expressed in the brain render its development potentially more susceptible to environmental influences. However, of the more than 80,000 compounds in commerce, only 11 have been identified as human developmental neurotoxicants while more might remain undiscovered (Grandjean and Landrigan, 2014).

Despite the fact that rodent-based developmental neurotoxicology models have relatively low sensitivity, low throughput, and high cost, they have been the primary approach for detecting potential human neurotoxicants. However, given the enormous difference in size and complexity of the human brain as compared to the brain of rodents, many of the developmental pathways are different or are regulated with additional factors (Clancy et al., 2007; Deacon, 1997; Van Dam and De Deyn, 2006). For example, the sets of genes that control forebrain expansion and regulate human cell fate are largely absent in rodents. Also, fibroblast growth factor (FGF) has different effects on myelination in humans and rodents (Hu et al., 2009), and compounds that are toxic to rodent cells may have no effect on human cells or vice versa (Malik et al., 2014; Xia et al., 2008).

In response to increased concerns about neurotoxicity induced in humans by exposure to chemicals during development, the scientific community is developing alternatives that will reduce the use of traditional laboratory animals while addressing the demand for increased and more relevant testing. In addition, more than 30,000 chemicals without adequate toxicological information are estimated to be in use in the United States and Europe (Schmidt, 2009), and the task of testing thousands of chemicals systematically with classical animal tests exceeds our present capabilities. In 2008, in response to the US National Academy of Sciences' report on "Toxicity Testing in the 21st Century" (NAS, 2007), a collaboration was established between the National Institute of Environmental Health Sciences (NIEHS)/National Toxicology Program (NTP), the U.S. Environmental Protection Agency's (EPA) National Center for Computational Toxicology (NCCT), and the National Human Genome Research Institute (NHGRI)/National Institutes of Health (NIH) Chemical Genomics Center (NCGC) (Collins et al., 2008). In mid-2010, the U.S. Food and Drug Administration (FDA) joined the collaboration, known informally as Tox21. The objective of this partnership is to shift the assessment of chemical hazards from traditional experimental animal toxicology studies to one based on target-specific, mechanism-based, biological observations largely obtained using in vitro assays, with the ultimate aim of improving risk assessment for humans and the environment. Additionally, the new European legislation on chemicals – Registration, Evaluation, Authorization and Restriction of Chemicals (ReACH) – explicitly mentions the possibility of using both experimental (in vitro) and non-testing (structure-activity relationships, read-across, categories) alternative methods (ReACH, 2015).

The recent advance in pluripotent stem cell (PSC)-based technology and the ability to generate truly large numbers of allelically diverse cells and use uniform methods to differentiate them into all of the major types of cells offer a potential new tool for improved understanding of chemically-induced adverse reactions. This is especially useful for developmental neurotoxicity, because neural cells differentiate early during development and this process is relatively easy to recapitulate in vitro via rosette formation and isolation of neural stem cells (NSC), which can subsequently be differentiated into neurons and glia. Several groups including our own have developed protocols to differentiate NSC, neurons, astrocytes, and oligodendrocytes from PSCs (Liu et al., 2013; Shaltouki et al., 2013; Swistowski et al., 2009, 2010). In addition, we and others have developed lineage specific markers and reporter lines which facilitate high content screening (Efthymiou et al., 2014) to allow us to obtain much more information from a single assay. We have utilized these tools to perform high throughput screens at different stages of development using purified cell populations (Han et al., 2009; Peng et al., 2013).

Here, we present the results obtained from testing a 80-compound library comprised of drugs (e.g., valproic acid) and pesticides (e.g., aldicarb, rotenone) with known neurotoxic potential as well as environmental compounds with unknown neurotoxic potential (e.g., flame retardants, polycylic aromatic hydrocarbons [PAHs]) for their cytotoxic effect on isogenic cells at four stages of neural differentiation (iPSC, NSC, neuron, astrocyte) using the MTT assay. This assay measures the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan as a measure of cell viability (Berridge et al., 2005; Morgan, 1998). When tested at 10 and 100 μM, 32 (40%) to 46 (58%) of the compounds induced significant cytotoxicity in the four cell types, with cell-type specificity. The results were confirmed by the re-testing of four selected compounds that were cytotoxic to all four cell types, and the testing was extended using rotenone to additional iPSC lines of both genders. Finally, we show that the cytotoxicity assay can be simplified by measuring luciferase activity using lineage-specific luciferase reporter iPSC lines that we generated from the parent iPSC line.

2. Results

2.1. *Generation of purified populations of neural cells from iPSC*

We have previously reported methods of generating a homogeneous population of NSC and subsequently differentiating them from multiple human ESC/iPSC into pure populations of neurons and astrocytes that are suitable for high throughput assays (Efthymiou et al., 2014; Han et al., 2009; Liu et al., 2013; Shaltouki et al., 2013). In this study, we generated NSC, neurons, and astrocytes from the well-characterized integration-free iPSC