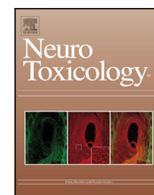
NeuroToxicology 42 (2014) 33–48

Contents lists available at ScienceDirect

NeuroToxicology



Multiparametric High Content Analysis for assessment of neurotoxicity in differentiated neuronal cell lines and human embryonic stem cell-derived neurons

Melinda S. Wilson [1], James R. Graham, Andrew J. Ball *

Science & Technology Group, Bioscience Division, EMD Millipore Corporation, 290 Concord Road, Billerica, MA 01821, USA

ARTICLE INFO

Article history:
Received 17 January 2014
Accepted 26 March 2014
Available online 3 April 2014

Keywords:
Neurotoxicology
Neurotoxicity
High Content Analysis
MTT
LDH
Neurons
Neuronal cell lines
Human embryonic stem cells

ABSTRACT

The potential for adverse neurotoxic reactions in response to therapeutics and environmental hazards continues to prompt development of novel cell-based assays to determine neurotoxic risk. A challenge remains to characterize and understand differences between assays and between neuronal cellular models in their responses to neurotoxicants if scientists are to determine the optimal model, or combination of models, for neurotoxicity screening. Most studies to date have focused on developmental neurotoxicity applications. This study reports the development of a robust multiparameter High Content Analysis (HCA) assay for neurotoxicity screening in three differentiated neuronal cell models – SH-SY5Y, PC12 and human embryonic stem cell-derived hN2™ cells. Using a multiplexed detection reagent panel (Hoechst nuclear stain; antibodies against βIII-Tubulin and phosphorylated neurofilament subunit H, and Mitotracker® Red CMXRos), a multiparametric HCA assay was developed and used to characterize a test set of 36 chemicals. HCA data generated were compared to data generated using MTT and LDH assays under the same assay conditions. Data showed that multiparametric High Content Analysis of differentiated neuronal cells is feasible, and represents a highly effective method for obtaining large quantities of robust data on the neurotoxic effects of compounds compared with cytotoxicity assays like MTT and LDH. Significant differences were observed between the responses to compounds across the three cellular models tested, illustrating the heterogeneity in responses to neurotoxicants across different cell types. This study provides data strongly supporting the use of cellular imaging as a tool for neurotoxicity assessment in differentiated neuronal cells, and provides novel insights into the neurotoxic effects of a test set of compounds upon differentiated neuronal cell lines and human embryonic stem cell-derived neurons.

© 2014 Elsevier Inc. All rights reserved.

1. Introduction

The need to test large numbers of chemicals for their risk of damage to the nervous system is well recognized. In addition to drug safety concerns affecting pharmaceutical development, there are thousands of unclassified chemicals that pose potential environmental neurotoxicity concerns. Consequently, assessment of the potential neurotoxicity of chemicals is increasingly required by regulatory testing schemes (Bal-Price et al., 2012). Traditionally the majority of neurotoxicity testing has been performed using in vivo models, which are expensive, low-throughput, and often lacking in sensitivity (Bal-Price et al., 2012). In recent years there have been calls to develop alternative in vitro neurotoxicity testing stratagems to obtain faster, more accurate and cheaper predictions of neurotoxicity, particularly for the effects of environmental chemicals upon the developing nervous system (Bal-Price et al., 2010; Crofton et al., 2011). With regards to drug safety, several adverse neurotoxic reactions to therapeutics have been reported (Arastu-Kapur et al., 2011; Beijers et al., 2012; Cavaletti et al., 2011), suggesting a need to also evolve preclinical safety pharmacology testing strategies for assessment of new chemical entities and prospective novel therapeutics.

* Corresponding author at: EMD Millipore, 17 Cherry Hill Drive, Danvers, MA 01923, USA. Tel.: +1 978 762 5064.
E-mail address: andrew.ball@emdmillipore.com (A.J. Ball).
[1] Present address: Donald Danforth Plant Science Center, 975 North Warson Road, St. Louis, MO 63132, USA.

http://dx.doi.org/10.1016/j.neuro.2014.03.013
0161-813X/© 2014 Elsevier Inc. All rights reserved.

Cellular models available to the field of neurotoxicology testing have developed over time to include immortal neuronal cell lines (Mundy et al., 2010), transformed neuronal precursor cells (Krug et al., 2013a), primary central nervous system (CNS) cultures (Harrill et al., 2013), co-culture systems (Anderl et al., 2009), and stem cell-derived neuroprogenitor cells (Breier et al., 2010; Harrill et al., 2010; Sison-Young et al., 2012). The challenge remains to characterize and understand differences between each cellular model in their responses to neurotoxicants if scientists are to determine the optimal model, or combination of models, for predictive chemical screening. Primary CNS cultures may appear to be ideal candidates, however they generally require animal sacrifice, may be difficult or expensive to obtain in sufficient quantities, and variability in quality and composition may render them unsuitable for screening purposes (Betts, 2010). Immortal cell lines commonly used to study neurotoxicity include the human neuroblastoma SH-SY5Y and the rat pheochromocytoma PC-12. These tumor-derived cell lines are easy to maintain and expand, with well-defined differentiation conditions to induce a relatively mature neuronal phenotype, making them a useful model for high throughput chemical screening. However, such transformed cell lines may not be adequately representative of native neural cells (Betts, 2010), and in the case of PC-12 cells, their rodent origin may limit their utility for human neurotoxicity screening. Human stem cell-derived neurons are emerging as an appealing alternative to primary cultures and transformed cells (Betts, 2010; Breier et al., 2010; Sison-Young et al., 2012). One example is the hN2™ cell line, which is derived from cells of WA09 human embryonic stem cell origin and which has shown some promise as a potential model for developmental neurotoxicity studies (Harrill et al., 2010, 2011a). Most of these recent advances in model systems and assays for neurotoxicity screening have focused on developmental neurotoxicity using undifferentiated neuronal cells, thus little information is available on the performance of neuronal cell assays in which the cells have been differentiated to more closely resemble mature neurons prior to toxic challenge.

To perform neurotoxicity assessment, in vitro cell-based cytotoxicity assays have been frequently utilized, in particular assays such as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and LDH (lactate dehydrogenase) release have found widespread application in studies quantifying neuronal cell death (Bal-Price et al., 2008; Lobner, 2000; Wogulis et al., 2005). The MTT assay measures the metabolic activity of cells and is simple in application and analysis, leading to its widespread use to assess neuronal cell viability. The LDH assay measures the release of LDH from cells in which the plasma membrane is damaged and is widely used to assess drug-induced cytotoxicity. Similar to MTT this assay is simple and amenable to higher throughput applications. However both the MTT and LDH assays are broad cytotoxicity assays which can provide little information about the mechanisms of action of toxicity; additionally little is known about their ability to detect neuron-specific toxic events, particularly in differentiated neuronal cells.

It is generally accepted that in vitro neurotoxicity screening methods benefit from the inclusion of neuron-specific endpoints (Bal-Price et al., 2008, 2010, 2012; Crofton et al., 2011). High Content Analysis (HCA) technology has emerged as a technology well-suited for high-throughput assessment of neuron-specific endpoints (Anderl et al., 2009; Culbreth et al., 2012; Dragunow, 2008). Using HCA, neuronal cells can be exposed to potential toxicants, fluorescently stained for neuronal-specific markers, and images acquired and quantified using image analysis algorithms. It has been demonstrated that automated algorithms for neurite analysis compare closely to results obtained by manual tracing, with the same measurement precision for treatment effects but with throughput that is orders of magnitude higher than with manual methods (Ramm et al., 2003). Pioneering work has extensively validated this technique for analysis of developmental neurotoxicity (Breier et al., 2008; Culbreth et al., 2012; Harrill and Mundy, 2011; Harrill et al., 2010, 2011a,b, 2013; Mundy et al., 2010; Radio and Mundy, 2008; Radio et al., 2008, 2010), and its utility has also expanded into studies of neurotoxicity induced by pharmaceutical therapeutics in mature, differentiated neuronal cells (Arastu-Kapur et al., 2011). To date, HCA has primarily been used to study the effects of neurotoxicants on neuron count and neurite outgrowth, as these are well-established endpoints for neurotoxicity studies; however HCA also enables the detection of multiple morphological, intensity and subcellular event localization measurements. This creates the possibility for highly multiplexed imaging-based toxicity assays (Anderl et al., 2009; Giuliano et al., 2010); a capability of HCA which has not yet been widely exploited for neurotoxicity assessment.

This study had three main objectives. Firstly, to determine if development of a robust multiparameter HCA assay for neurotoxicity screening in multiple differentiated neuronal cell models is feasible. Secondly, to compare HCA-based neurotoxicity assessment with data from MTT and LDH cytotoxicity assays using a panel of test compounds. Thirdly, to compare and contrast the responses of widely used neuronal cell lines with human embryonic stem cell-derived neurons in their responses to a panel of test compounds. To address these objectives, a multiplexed HCA-based neurotoxicity screening assay was developed using a combination of four detection reagents per well – antibodies against βIII-Tubulin and the phosphorylated form of the neurofilament subunit NF-H (pNF-H), the DNA-binding Hoechst 33342 nuclear dye, and Mitotracker® Red CMXRos. This assay was run using a test set of 36 chemicals in differentiated SH-SY5Y, PC-12, and hN2™ cells, and results compared with those obtained under the same conditions with the MTT and LDH assays. The overall aim was to learn lessons which may be applied to future testing paradigms in mature neurons, particularly for neurotoxicity assessment of pharmaceuticals and potential environmental hazards.

2. Materials and methods

2.1. Materials

The human neuroblastoma SH-SY5Y and rat pheochromocytoma PC-12 cells were obtained from ATCC (Manassas, VA). hN2™, a human embryonic stem cell (hES) derived neuronal cell line, was purchased from ArunA Biomedical, Inc. (Athens, GA). Ham's F-12 medium, heat-inactivated horse serum and fetal bovine serum (FBS) were obtained from Life Technologies (Carlsbad, CA). F12-K medium was purchased from Thermo Fisher Scientific, Inc. (Waltham, MA). Eagle's Minimum Essential Medium (EMEM) was purchased from ATCC (Manassas, VA). AB2 basal neural medium and ANS neural supplement for hN2™ culture were purchased from ArunA Biomedical, Inc. (Athens, GA). Human recombinant Leukemia Inhibitory Factor (LIF), recombinant human β-nerve growth factor (NGF), recombinant human brain derived neurotrophic factor (BDNF), L-glutamine and dimethyl sulfoxide (DMSO) were from EMD Millipore (Billerica, MA). Penicillin-streptomycin (PS), retinoic acid (RA) and the growth substrates poly-L-lysine (PLL), laminin and calf skin collagen were purchased from Sigma–Aldrich (St. Louis, MO). Table 1 provides full details of all test compounds used in this study. Briefly, all test compounds were obtained from either EMD Millipore (Billerica, MA) or Sigma–Aldrich (St. Louis, MO) with the exception of Bortezomib, which was purchased from Selleck Chemicals (Houston, TX). MTT Cell Growth Assay Kit was from EMD Millipore (Billerica, MA). LDH

Download English Version:

https://daneshyari.com/en/article/2589622

Download Persian Version:

https://daneshyari.com/article/2589622

Daneshyari.com