Introduction

Birth defects are the largest cause of infant mortality and morbidity in the United States, Teratogens, defined as substances that cause one or more fetal abnormalities during development, are responsible for 5-10% of all birth defects. Availability of more predictive developmental toxicity screens would increase pharmaceutical and chemical safety and could reduce the prevalence of birth defects associated with exposure to these compounds. For example, rodent models for developmental toxicity testing do not adequately correlate to human response, resulting in only 62% concordance to human developmental toxicity. To develop an innovative alternative for the in vivo human developmental toxicity of chemicals utilized in Stemina's devTOX assays. We have developed a targeted, rapid, and highly predictive assay of developmental toxicity based on specific biomarker metabolites identified in the K04, LC-MS metabonomics computational devTOX model trained using 23 known human teratogens and non-teratogens. These biomarkers represent different metabolic pathways and show high individual predictivity (70-80%), and combination results in better predictions (85-95%). The targeted biomarker assay produces a metabolic index that is predictive of the potential for human developmental toxicity. When combined with a 9 point curve dose and cell viability analysis, the metabolic index can be used to model developmental toxicity.

Methods

Cell Culture

MCF7 NES cells are maintained on Matrigel (BD Biosciences; San Jose, CA) using Roswell Park Memorial Institute Cell Technologies, Inc. at 37°C, under 5% CO2. NES cells are harvested from T75 plate wells, washed with DMEM/F12 media, reconstituted in mCherry medium containing 10 µg/mL mCherry expression vector (ROCK) inhibitor (EliCaliber), and plated onto Nano-ICF-coated BD-6 well plates at a concentration of 100,000 cells per well in 50 µL of media.

Experimental Treatments

Twenty-four hour after plating, the cells are treated with the test agent dissolved in DMSO. The final concentrations of test agents, generally, create a 9 point dose response curve ranging from 0.36 µM to 300 µM (Figure 2). NES cells are exposed to the test agents for 72 hours, with media changes every 24 hours. The spent media is collected and metabolized through UPLC-MS analysis. Cytotoxicity measurements are performed following media collections using the CellFluor-5 Fluor Cell Viability Assay (Promega).

Sample Preparation

Quenched spent media samples are added to Millipore MilliQ Ultrafiltration 10-kL filters to remove molecules which are greater than 10 kDa. The filtrate is collected and concentrated using a Spectra/Por. The dry samples are reconstituted in a 1:1 mixture of 0.1% formic acid in water: 0.1% formic acid in acetonitrile. Internal standards are added at the quenching and concentration steps to evaluate sample preparation.

Algae Spectrometry

Flavonoid profiles for algal species including Micractinium, Oocystis, and Coscinodiscus are evaluated based on the data presented in a previous publication (Warren et al., 2010). Algal species are identified using the algal database developed by the EPA and National Oceanic and Atmospheric Administration. Each species is analyzed in triplicate and the fluorescence values are averaged, with each sample containing the same number of cells.

Results

Identification of a Targeted Metabolic Threshold of Teratogenicity

QuickPredict: Targeted Metabolic Index to Predict the Dose of Teratogenicity

1. Toxicity is function of chemical agent and exposure level
2. Predict developmental toxicity independent of cell death
3. Identify IC50, NOEL, LOEL of test agent
4. High-throughput targeted assay leads to rapid turn around
5. Compare toxicity profiles of lead pharma compounds in a series

quickPredict is a prediction algorithm which utilizes a ‘omics’ based computational model approach to capture a biochemical phenotype that is able to discriminate developmental toxicants based on known physiological mechanisms. Extension of a combination of these metabolites produces a metabolic index of the potential teratogenicity of a test agent able to predict 95% of the validation set of 27 pharmaceuticals at or below the Cmax concentrations.

Combining a 9 point dose response curve with cell viability and highly predictive metabolic end points provides an agent able to predict 95% of the validation set of 27 pharmaceuticals at or below the Cmax concentrations.

Biological Processes Captured in the Metabolic Teratogen Index

Metabolic Processes

RDS and RNS regulation
Cell cycle regulation
Polyamine metabolism and lipids cycle
Allergic reactions

Role in development

Management of oxidative stress and gasotransmitters
Glutamate homeostasis
Vascularization
NovelTube formation

QuickPredict: Targeted Metabolite Index to Predict the Dose of Teratogenicity

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