A Multiplex, Automated Approach to Screen for Mitotoxicity in Human Hepatocytes and HepG2 Cells

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ABSTRACT

Many drugs and environmental chemicals have been implicated in mitochondrial toxicity leading to cellular dysfunction and disease. Mitochondrial dysfunction plays a role in the etiology of numerous cellular and metabolic disorders. The mechanisms of mitochondrial bioassays are complex due to a multiple number of factors such as evaluating mitochondrial replication and disruption of electron transport chains, and by organs-specific susceptibility. The aim of the multiplex approach primarily targets the evaluation of both liver and non-liver cell lines for determining the mitotoxicity profile of different drugs and environmental chemicals. To overcome these challenges, we have developed an automated system to measure mitochondrial integrity approaches associated with stress.

MATERIALS AND METHODS

Cell Preparation. HepG2 cells were propagated in a standard hormone-supplemented medium consisting of Dulbecco’s Modified Eagle Medium (DMEM), 10% fetal bovine serum, 1% non-essential amino acids, and 1% Penicillin-Streptomycin (Life Technologies). HepG2 cells were seeded and incubated in 12-well plates (20,000 cells/well) for 24 hours at 37°C. After 24 hours, the culture medium was replaced with 1 ml of serum-free DMEM and the plates were returned to the incubator for 48 hours. The culture medium was again replaced with 1 ml of serum-free DMEM and the plates were returned to the incubator for an additional 24 hours. The assay was initiated by addition of 0.3 ml of the test substance to each well.

Crabbe’s Effect on Cellular ATP Levels

Studies, including those described by Winnick, have shown that differences between cancer cell models and normal primary cells in ATP is achieved by using different cell lines. The ATP levels may be determined using the CellTiter-Glo System (Promega, Madison, WI). This system provides a luciferase-based ATP assay that is highly sensitive and specific for the detection of ATP in cells. The assay is based on the principle that ATP can be detected as a light signal when combined with luciferase.

Concentration Response Curve

Concentration-dependent responses to cytochalasin B were investigated. Three known toxins, antimycin, CCCP, and digitonin were tested using HepG2 and primary hepatocytes models. A known reduced of cellular resistance, digitonin, was evaluated as a mitochondrial control. The resulting ATP levels were determined using a microplate reader and the ATP levels were normalized to the control levels.

Time Response Curve

The assay was also further tested using compound incubations from 0 to 6 hours to study time-dependent response data. To further demonstrate the potential of this assay, several primary hepatocytes models were tested with compounds that demonstrated significant responses at the 2-hour time point.

C O N C L U S I O N S

The multiplex assay provides an easy way to distinguish between compounds which regularly effect mitochondrial function, and those that effect the cell by other mechanisms. The results from the CellTiter-Glo System and the ATP assay demonstrate that the multiplex assay can be used to evaluate the mitotoxic potential of different drugs and environmental chemicals. The multiplex assay provides a rapid and accurate method for evaluating the mitotoxic potential of different drugs and environmental chemicals.