Introduction

Mitochondrial perturbation is a common mechanism of drug-induced toxicity that results in losses of ATP production and membrane integrity changes. These changes are often associated with mitochondrial toxicity versus overt cytotoxicity. Mitochondrial toxicity can be due to a decrease in ATP production with little to no change in membrane integrity, whereas overt cytotoxicity is characterized by the release of cytoplasmic enzymes that can lead to cell death. Here we demonstrate the utility of a multiplexed assay to assess cell mitochondrial function during the discovery phase of drug development.

Overview

The primary function of mitochondria is to generate >90% of a cell's energy, primarily through the process of oxidative phosphorylation. Many drugs are known to induce mitochondrial toxicity by inhibiting mitochondrial function. The effects of mitochondria on drug-induced toxicity are complex and depend on the type of drug and the cell type. Mitochondrial toxicity can be due to a decrease in ATP production with little to no change in membrane integrity, whereas overt cytotoxicity is characterized by the release of cytoplasmic enzymes that can lead to cell death.

Mitochondrial ToxGlo™ Assay

The Precision™ Microplate Pipetting System combines an 8-channel pipetting head and an 8-channel bulk reagent dispenser in one unit. The system is designed to efficiently dispense compounds across a 96-well PP plate, as well as for reagent dispensing. The system is well suited for cell plates, as well as to incubate in a shaker.

Data Analysis

Luminescent ATP detection reagents have excellent sensitivity, linearity, and reproducibility. The assay is an excellent method for analyzing compounds that affect ATP production. The assay reagents are available from Promega Corporation. The assay is an excellent method for analyzing compounds that affect ATP production.

Cell and Compound Preparation

HeLa cells were propagated in a medium containing high glucose DMEM (Promega Catalog #D5010, USA). Cells were cultured in 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin (Pen/Strep) at 37°C for 24 h in a humidified 5% CO2 incubator. For the Mitochondrial ToxGlo™ Assay, cells were serum-starved for 72 h, and then resuspended in the glucose-free medium described above.

Cell Model Effect on ATP Production (Crabtree Effect)

Mitochondria play a central role in cellular metabolism. It is well known that cancer cells rely upon glycolysis when grown using typical high glucose media. Only when glucose is limited is the Crabtree effect observed (Marroquin et al., 1999). Here we demonstrate the utility of a multiplexed assay to assess cell mitochondrial function during the discovery phase of drug development.

BioTek Instruments

The BioTek Instrumentation Cellometer® is a fully automated cell counter and sorter system designed for high-throughput applications. The instrument is capable of counting and sorting cells with excellent sensitivity, linearity, and reproducibility. The system is well suited for cell plates, as well as to incubate in a shaker.

Cell Model Analysis

In Figure 5 – ATP production in cancer-like cells vs. normal hepatocytes, it is shown that cancer cells have a lower ATP production than normal hepatocytes when grown in high glucose media. The Crabtree effect is a phenomenon that occurs when glucose levels are limiting, and is characterized by a decrease in the rate of ATP production.

Conclusions

The ability to detect this effect was tested here using the HeLa cell line and primary hepatocytes. Each cell type was compared to evaluate the ability of the assay to detect differences in ATP production. As previous experiments demonstrating the Crabtree Effect have shown, forcing cancer cell lines to rely upon glycolysis when grown in high glucose media, yields an efficient, yet in vivo-like result.