IN VITRO MULTIPARAMETER HEPATOMOTOXICITY ASSAY DEVELOPMENT IN HUMAN PRIMARY HEPATOCYTES AND HEPG2 CELLS WITH S9 LIVER FRACTIONS

1. Introduction

Drug-induced liver injury (DILI) is a major and difficult-to-predict problem that is a leading cause of drug attrition in clinical trials and postmarket withdrawal. Screening toxicology assays which rely on isolated models and biomarkers directly linked to DILI in humans lack the well-described complexity and metabolic richness of human primary hepatocytes. Therefore, the use of human primary hepatocytes in hepatotoxicity screening has become a standard approach in the pharmaceutical industry. Here, we describe our efforts to develop a novel hepatotoxicity assay using human primary hepatocytes that employs multiple biomarkers. We have generated data that indicate that further testing of a broad range of marketed drugs with and without DILI consequence in man is warranted.

2. Methods

IMMUNOLABELING: The primary human hepatocytes were fixed with 4% paraformaldehyde and immunolabeled with anti-active caspase-3 for detection of apoptosis and anti-HSP70/72 (Enzo Life Sciences) for detection of heat shock protein 70/72.

3. Results

The relative cell count IC50 (half maximal inhibitory constant) value measures cell proliferation. Compound dose-response, one-site model where: y = A + [(B – A)/(1 + ((C/x) ^ D)).

4. Conclusions

The development of comprehensive methodology testing includes repeated evaluation of several compounds in vivo that monitor changes in multiple end points. The development of such a comprehensive, automated fluorescence microscopy and image analysis described herein enables detection of multiple endpoints in multiple conditions for primary human hepatocytes. This is very different from current hepatotoxicity screening for primary human hepatocytes, which relies on relatively simple and less sensitive determinations of cell viability or metabolic activity.

5. Acknowledgements

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6. References


7. Appendix

A. Table 1: Summary of positive signal criteria for each marker.

B. Table 2: A comparison of the percent of attached live cell count IC50, H50F, and TMRE parameters for each compound after 24 hours exposure to human primary hepatocytes (HPH) in vitro.

C. Table 3: A comparison of the relative cell count IC50, H50F, and TMRE parameters for each compound after 24 hours exposure to HepG2 cells in vitro.

D. Table 4: A comparison of multiple datasets (proliferation, apoptosis, and cell cycle) and live and apoptotic compound ratios and H50F exposure to HepG2 cells in vitro in 24 and 72 hr conditions.

E. Table 5: Cell proliferation, apoptosis and H50F/70 parameters for geldofloxacin after 48-hour exposure to HepG2 cells, p<0.05. H50F/70 signal more sensitive marker of early cell death.

F. Figure 1: Representative images of cell proliferation effects on ROS generation and mitochondrial membrane potential in human primary hepatocytes.

G. Figure 2: Representative images of cell proliferation effects on ROS generation and mitochondrial membrane potential in HepG2 cells.

H. Figure 3: Representative images of cell proliferation effects on ROS generation and mitochondrial membrane potential in HepG2 cells.

I. Figure 4: Patterns of apoptosis and cellular stress in HepG2 cells.