Development of an In Vitro Assay for Chemical-Induced Fatty Liver

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Non-alcoholic fatty liver disease (NAFLD) is a serious health concern affecting an estimated 20% of the U.S. population. Altered lipid homeostasis in the liver, as either steatosis or phospholipidosis, represents major key events for progression to liver injury or diseases such as NAFLD. While components of the metabolic syndrome (obesity, type II diabetes, dyslipidemia, and insulin resistance) are primary contributors to fatty liver disease, certain environmental chemical exposures have been linked to lipid accumulation in the liver. These are proposed to work through a number of modes-of-action (MOA) including mitochondrial impairment, enhanced cytokine production, and altered hepatic lipid secretion (Kaiser et al., 2012).

To establish a human relevant in vitro model for predicting chemical effects on fatty liver disease, a multiplexed high-content screening assay using a 96-well micropatterned co-culture system of primary human hepatocytes was developed to assess chemical-induced steatosis and phospholipidosis. Five reference drug compounds were selected for assay development due to high specificity and well understood MOAs: cyclosporin A and methotrexate (steatosis), propranolol (phospholipidosis), amiodarone (phospholipidosis and steatosis), and caffeine (negative control). Optimization of dosing parameters and kinetics resulted in a 96 hour workflow with a fixed endpoint, 5-channel assay to quantitatively determine hepatocyte-specific neutral lipid accumulation, phospholipidosis, cell counts, and viability. High-content imaging parameters for lipid endpoints were restricted to analyzing lipid droplet size, intensity, and frequency. Anticipated concentration-response curves for the lipid endpoints were observed for most of the reference compounds. In addition, cytotoxicity parameters were validated and performed as expected. Following completion of method development, the next screening phase evaluated the performance of a mixed training set to determine the predictive capacity of the co-culture in vitro platform to identify chemicals contributing to human fatty liver disease.

Implications: Human fatty liver disease is a serious health concern and an emerging area of interest in predictive toxicology. A number of chemical compounds within the U.S. Environmental Protection Agency’s (EPA) Integrated Risk Information System (IRIS) have been observed to induce lipid accumulation in repeat-dose rodent bioassays. However, challenges exist in developing predictive in vitro assays that enable appropriate extrapolation and understanding of relevance of these bioassay responses to human health. The project outlined here used primary human cells in a format suitable for short-term repeat-dosing to quantitatively determine the effects of drug and chemical compounds on altering lipid dynamics. As a common phenotypic outcome following activation of nuclear receptors targeted in the other ScitoVation fit-for-purpose assays, the steatosis assay complements the other members of the assay battery by measuring a functionally relevant indicator of fatty liver disease.

Key words: steatosis, high-content screening, primary human hepatocytes

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This abstract was prepared by the principal investigator for the project. Please see www.americanchemistry.com/lri for more information about the LRI.
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References: