Development of Fit-For-Purpose Multifunctional Liver Co-Culture Systems

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In both the regulatory and commercial arenas, the goal is to move away from time consuming, costly in-life rodent studies and toward safety assessment strategies that rely on testing species-relevant cells in vitro. The liver has been a major focus of these efforts, yet there are currently no in vitro alternatives for hepatotoxicity testing accepted by regulators, and the assays that do exist typically utilize short-lived hepatocyte monolayer cultures that lose phenotypic markers and metabolic competence. While hepatocytes have been the primary component of in vitro hepatotoxicity assay development, the non-parenchymal cells (NPCs) (i.e., hepatic stellate cells, Kupffer cells, and liver sinusoidal endothelial cells) also play a critical role in the progression of liver pathologies. An ideal in vitro liver model for hepatotoxicity testing would include multiple cell types and be able to support hepatocyte viability, phenotypic maintenance, and metabolic competence for an extended time in culture to allow for repeated exposures and long-term dosing. To that end, the goal of this project is to develop organotypic co-culture systems that can recapitulate responses observed in vivo, as well as maintain metabolic capability and extended viability. We have focused on developing two-dimensional 96-well plate-based and three-dimensional alginate bead-based co-culture systems that include primary rat hepatocytes, stellate, Kupffer, and endothelial cells. These models can support hepatocyte viability in vitro out to eight days in 2D and 28 days in 3D. Importantly, markers of hepatocyte differentiation and polarity are maintained in co-culture, but hepatocyte monocultures with no other cell types lose phenotypic markers after prolonged culture. The effects of well-studied hepatotoxicants, including acetaminophen and phenobarbital, were characterized in the 2D mono- and co-culture models using phenotypic and transcriptomic analyses, and the co-culture model resulted in responses that were more representative of the in vivo phenotypes. Together these data demonstrate that the organotypic model can faithfully recapitulate rodent in vivo liver phenotypes observed in response to canonical hepatotoxicants and suggest that the co-culture model could be useful for testing the effects of compounds in vitro as an early stage alternative to in-life studies.

Next Steps.

- We are continuing to refine the characterization of the 2D and 3D mono- and co-culture models using both the phenotypic and transcriptomic response to well-characterized hepatotoxicants after repeat dosing (out to 7 days in vitro).
- We are utilizing the 2D and 3D primary rat hepatocyte culture models to explore the effects of metabolism-mediated toxicity in vitro.

Implications. Hepatotoxicity is a frequent endpoint leading to the regulation of many chemicals. Since most chemicals are assessed in animal models prior to human exposures, it is important to consider that key species differences between rodents and humans may result in distinct mechanisms of action. Ultimately, the goal of vitro alternatives is to predict human health impacts, but because existing regulatory decisions are principally based on rodent data, our rodent in vitro model that can re-capitulate in vivo responses is valuable for 1) establishing confidence in the alternative approach and 2) early stage testing to reduce or replace in vivo testing.

Collaborations: None.

Key words: hepatocyte, non-parenchymal cell, co-culture, nuclear-receptor, metabolism, 3D models

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