ABSTRACT: Adverse drug reactions in the liver are one of the major causes of atition in drug development with both small and large molecules. Novel therapeutic modalities such as antibodies and other biologics can be taken up by non-parenchymal cells, such as phagocytic cells in the liver (mainly Kupffer cells) and can limit drug exposure and cause liver toxicity. Therefore, evaluating Kupffer-cell uptake can serve as an indirect screen for liver toxicity. However, due to lack of suitable organotypic in vitro models, Kupffer cell uptake has been difficult to study. Here, the use of primary heterotypic 3D rat liver microtissues for assessment of drug-induced Kupffer-cell activation is demonstrated. Morphological characterization of rat liver microtissues demonstrated the presence of ED1 and ED2-positive Kupffer cells and the hepatocytes. Electron-microscopy confirmed layering of Kupffer cells between hepatocytes in the rat liver microtissues. Lipopolysaccharide (LPS)-treatment of the rat liver microtissues resulted in increased uptake of acetylated LDL (ac-LDL), whereas incorporation of ac-LDL was difficult to study. Here, the use of primary heterotypic 3D rat liver microtissues for assessment reduced when the phagocytosis inhibitor gadolinium chloride was applied, indicating ac-LDL uptake by Kupffer cells is dependent on phagocytosis. An increase in IL-6 secretion was also observed at 48 and 120 hrs post-treatment with LPS, indicating Kupffer cell activation. Together these data demonstrate the suitability of heterotypic rat liver microtissues for assessing Kupffer cell activation and functionality by external stimuli, and highlight the potential of 3D rat liver models for evaluating liver toxicity.

BACKGROUND: InSphero’s 3D rat liver heterotypic microtissue model is a 3D co-culture model-based assay to assess liver Kupffer-cell activation and functionality. Rat liver microtissues (rLiMT) are generated by co-culture of primary rat hepatocytes and non-parenchymal cells. The hepatocytes remain polarized, differentiated and active. With a viability of 95%, the canaliculi network ensures proper clearance of bile-acids into the medium. InSphero’s rLiMT, hepatosphere. The hepatocytes remain polarized, differentiated and active. With a viability of 95%, the canaliculi network ensures proper clearance of bile-acids into the medium.

Characterization of Non-Parenchymal Cells in rLiMT

1. rLiMT stained positive for markers of monocyte (ED1+) and macrophage (ED2+) lineages.
2. ED1+(undifferentiated macrophages) and ED2+ (differentiated macrophage) are scattered-out the spheroids.
3. Morphology of both ED1+ and ED2+ cells are consistent to the morphology of these cells in vivo.

Microscopic analysis of rLiMT

Development of Dil-ac-LDL as a functional marker of NPC function

1. Lipopolysaccharide (LPS) stimulates ac-LDL accumulation in specific cells in a concentration-dependent manner.
2. Gadolinium chloride reduces ac-LDL accumulation significantly.
3. Co-exposure of LPS and Gadolinium chloride reduces ac-LDL staining.
4. Incubation of ac-LDL up to at least 72h is required to observe significant ac-LDL uptake.

Development of DiI-AcLDL as a functional marker of NPC function

Fig 1: Microstructure of InSphero’s rLiMT, Immunofluorescence staining with endothelial cell marker ICAM-1 (red) and bile canaliculi marker DPPVI (green). DAPI is shown in blue. Provided by Swedik Hamnem and Prof. Jan G. Haaf, Roche Diagnostics, Germany.

Fig 2: Expression of monocyte and macrophage markers (ED1 and ED2) in rLiMT. Upper panel: Sections of rat liver representing experimental control. Lower Panel: InSphero’s rLiMT. Microtissues at day 3 were fixed with PFA (2%; RT) and paraffin-embedded before sectioning the tissues. Anti-ED1 and Anti-ED2 antibodies were used to detect presence of undifferentiated and differentiated macrophages respectively using DAB (brown staining) as the chromogen. The sections were counterstained with the nuclear stain hematoxylin.

Fig 4A: Microscopic analysis of rLiMT in GravityTRAP® plates with and without LPS treatment. DAPI: Red: Di-ac-LDL. Tissues were pre-treated for 120h with LPS and Gadolinium chloride before addition of YOYO-1 Di-ac-LDL for 30 min, followed by fixation with 4% PFA for 20 min and DAPI-staining in GravityTRAP® plate (n=3). Left panels, images were captured with non-confocal fluorescence microscopy. Right panels, Quantification of ac-LDL signal was done by image analysis of ac-LDL fluorescence in rLiMT after different treatments. The fluorescence intensity of the region of interest (ROI) microwell was quantified with ImageJ software individually for each microtissue. Mean and S.D. was calculated from at least 3 individual microtissues in each treatment group.

Effect of LPS treatment on the markers of Parenchymal and Non-parenchymal cell function

1. Interleukin-6 (IL-6) secretion is stimulated by LPS treatment, but is not abolished in presence of Gadolinium chloride.
2. Albumin secretion is not significantly affected by LPS and/or Gadolinium chloride treatment and indicates good hepatocellular viability.

Conclusions

- The InSphero rat liver microtissues consists of functional parenchymal and non-parenchymal cells that closely resemble liver composition in vivo.
- The model offers the unique opportunity to study pharmacodynamics that is dependent on parenchymal and non-parenchymal cell interactions e.g. inflammatory DILI.
- A unique advantage of this model is the ability to do histological assessment of the microtissues to assess pathological changes (which is a sensitive visual end-point for assessing drug induced liver injury).
- Next Steps: Using the model to study the drug toxicity caused by small and large molecules.

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