Human hepatic HepaRG cells maintain high intrinsic CYP450 activity/metabolism and significantly outperform standard HepG2/C3A cells used in drug pharmacology applications

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BACKGROUND

Tissue Modelling for drug testing

- Conventional *in vitro* human hepatic models for drug testing are based on the use of standard cell lines or primary human hepatocytes (PHHs)
- However, limited availability, inter-donor functional variability and early phenotypic alterations of PHHs *in vitro* restrict their use; whilst cell lines such as HepG2/C3As lack a substantial and variable set of liver-specific functions, specifically, CYP450 activity
- Human HepaRG cells are an alternative organotypic co-culture model of hepatocytes and cholangiocytes that maintains *in vivo*- like liver-specific functions, including intact Phase-I-III drug and lipid metabolism

Objectives

- In this study we compared phenotypic and functional parameters including CYP450 activity and metabolism between HepG2/C3A and human HepaRG cells to assess their value as hepatic models for pre-clinical drug testing
- This approach is aimed at further understanding the suitability of more physiologically-relevant *in vitro* human models to replace or reduce animals in drug pharmacology and toxicology applications

METHODS

Cell culture

- HepG2/C3A or HepaRG were grown to >80% confluence on collagen-I-coated plates and treated (in triplicates) for 24 h With prototypical inducers rifampicin (CYP3A4) and omeprazole (CYP1A2); [n=3]

Cell phenotype

- Cell phenotype was assessed by light-microscopy under phase contrast
- Presence and abundance of CYPs was assessed via dual immunofluorescent and nucleic staining (CYP3A4/ phalloidin/ DAPI)

CYP1A2/ 3A4 Activity

- CYP1A2/3A4 activity was determined using Promega P450-Glo™-Luminescence; and read on a GloMax Multi+ plate reader.
- HepG2/C3A and HepaRG activities were validated by inhibition with specific inhibitors on technical replicates

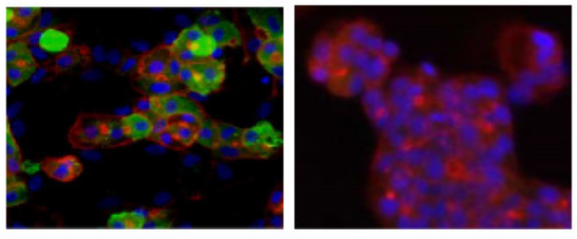
CYP1A2/ 3A4 Metabolism

- CYP1A2/3A4 metabolism was assessed by challenging the cells with 50 µM testosterone or phenacetin boluses. Supernatant and cell samples were taken after 2 hours of incubation at 37°C.
- Relative turnover and metabolic breakdown were measured via HPLC (Testosterone) and LC-MS/MS (Phenacetin)

RESULTS

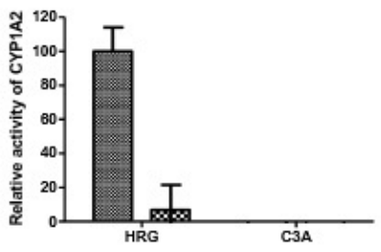
Hepatic phenotype

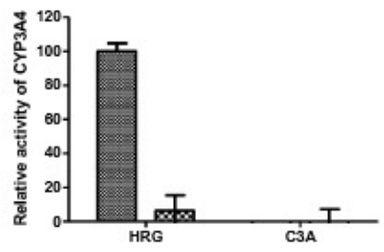
- HepaRG [left panel] and HepG2/C3A [right panel] showed markedly different abundance of CYP3A4, as indicated by immunofluorescent staining against a marker panel consisting of CYP3A4/ phalloidin/ DAPI staining.



CYP1A2/ 3A4 Activity

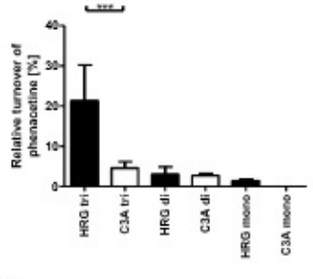
- HepaRG CYP1A2 and 3A4 activity was manifold higher than that measured in HepG2/C3A cells [grey bars; p<0.001]. In fact relative luminescence measured in HepG2/C3A cells was at levels of blank controls.
- Specificity of CYP induction was confirmed with specific inhibitors [spotted grey bars]: Fluvoxamine [CYP1A2] & Ketoconazole [CYP3A4]

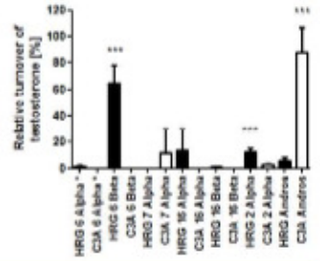




CYP1A2/ 3A4 Metabolism

- HepaRG showed significantly higher turnover of phenacetin 28.5±4.4% vs. 11.7±0.6% [p<0.01] and testosterone 56.5±7.6% vs. 2.0±0.2% [p<0.001]
- as well as more refined metabolic breakdown of phenacetin and testosterone





CONCLUSIONS

Only HepaRG cells retain differentiated morpholigical/ phenotypic features relevant to drug testing strategies

Analytical techniques including LC-MS/MS allow HepaRG cells to be interrogated for drug reactive metabolite formation – allowing intrinsic (predicitive) drug clearance rates to be calculated

HepaRG cells may represent a more physiologically-relevant pre-clinical platform for CYP450 activation/ inhibition, safety pharmacology, as well as drug-drug interaction studies