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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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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, apically, 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/II drug and lipid metabolism

Cell culture
- HepG2/C3A or HepaRG were grown to >80% confluence on collagen-coated plates and treated in triplicates for 24 h with prototypical inducers rifampicin (CYP3A4) and omeprazole (CYP1A2) [10-5] and AHR activator phenobarbital [10-4].

Cell phenotype
- Cell phenotype was assessed by light-microscopy under phase contrast
- Presence and abundance of Cyp2 was assessed via dual immunofluorescent and nuclear staining (CYP3A4/ phosphoH2A DAPI)
- CYP1A2/3A4 Activity
- CYP1A2/3A4 activity was determined using Promega Pico-Glo™-Luminosifier, and read on a GloMax Multi-plus 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 bolus. 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, phosphoH2A, 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±6.6% [p<0.01] and testosterone 66.5±7.6% vs. 2.6±0.2% [p<0.001]
- as well as more refined metabolic breakdown of phenacetin and testosterone

CONCLUSIONS

Only HepaRG cells retain differentiated morphological/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 (predictive) 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.