1. Abstract

The purpose of this study was to evaluate Cyp2c9 human hepatocytes (UHH) as an alternative in vitro system for evaluating hepatic drug metabolism and for clearance prediction of metabolically stable compounds. Derived from primary human hepatocytes (PHH) by lentiviral transduction of proliferation stimulating genes, UHH provide a virtually unlimited source of non-tumorigenic primary hepatocytes. Differentiated UHH express adult hepatic markers and maintain functional expression of cytochrome P450 enzymes. Compared to immortalized cell lines, confluent UHH retain expression and functionality of nuclear receptors and drug metabolizing enzymes with regard to major CYP and UGT and at donor-specific level(s).

We assessed the metabolic capacity of UHH in sandwich culture for up to 21 days, focusing on functional in situ enzyme activity and relative mRNA expression of selected phase I and phase II enzymes. Absolute enzyme protein expression was determined by LC-MS/MS quantification. In vitro hepatic clearance (CL) was predicted for a set of slowly and immediately intermediate cleared reference drugs by scaling from in vitro intrinsic clearance (CLint). The in vitro metabolite pattern was semi-quantitatively analyzed exemplarily for alprazolam and mafenide. Data from these experiments were compared to those obtained from sandwich cultures of cryopreserved PHH.

2. Objectives

Focus of the investigations was on the assessment of UHH human hepatocytes and comparison with standard primary human hepatocyte cultures regarding:

- Activity-time profile for key P450s, UGTs, and SULT in sandwich culture
- Utility for CLint prediction of slowly metabolized compounds
- Metabolite pattern for selected reference drugs

3. Methods

3.1 Experimental workflow for expansion and culturing of Uptcyc® human hepatocytes (UHH)

- Expansion for 7 days
- Sandwich culture for up to 21 days

3.2 Analysis of enzyme activity, mRNA, and protein expression, and metabolite pattern

Activities of cytochrome P450s, UGTs and SULTs enzymes were determined in situ via metabolic formation (LC-MS/MS) after incubation with probe substrates. Relative mRNA levels were determined by Taqman RT-PCR. Protein quantification was performed from microsomal preparations by LC-MS/MS quantification of specific tryptic fragments. Metabolite references for drug identification were derived from standards by exact mass (QTOF Orbitrap) according to literature and relative abundance was determined semi-quantitatively by extracted-ion chromatography (XIC).

In vitro CL prediction

References with low to intermediate in vitro CLcomp and as metabolism primary elimination pathway were incubated in 24-well format for up to 120 h. In vitro CLint was scaled from in vitro CLcomp applying the physiologically based in vitro to in vivo direct scaling approach and the well-stirred model.

3.3 UHH in sandwich culture

PHH and UHH were assessed at culture day 7 in 24 well sandwich culture for up to 21 days. Data represent mean ± S.D. of n=2-5 experiments. UHH donor HH31 (A) showed relatively lower levels at start of culture compared to PHH donor Hu1601 (red), with increasing and maintained activity over the study period. Activities in PHH decreased rapidly from start of sandwich culture until day 14 as study end.

4. Results

4.1 Metabolic activity over time in sandwich culture

Enzyme activities (exemplarily shown for CYP2A6, 3A4, 2C9, 2D6, UGT, and SULT) were determined in situ metabolic formation in 24-well sandwich culture for up to 21 days. Data represent mean ± S.D. of n=2-5 experiments. UHH donor HH31 (A) showed relatively lower levels at start of culture compared to PHH donor Hu1601 (red), with increasing and maintained activity over the study period. Activities in PHH decreased rapidly from start of sandwich culture until day 14 as study end.

4.2 Enzyme activity, protein, and mRNA expression

PHH and UHH were assessed at culture day 7 in 24-well sandwich culture. Enzyme activities are depicted as pmol/min/mg, with protein levels as ng/mg of microsomal protein. N/A/ATPase was used as endogenous control for protein quantification. Activity-time profiles were used to generate mean ± S.E.M. (N=2) individual activity curves. Protein and mRNA data mean ± S.E.M. (N=3). Non-specific UGT/SULT activity data was determined by 7-OH-coumarin glucuronidation and sulfation, respectively. CYP2A6 and CYP2B6 data showed most pronounced differences between PHH and UHH.

5. Conclusions

- UHH in sandwich culture showed donor- and enzyme specific expression/activity profiles; levels were found initially lower compared to PHH, however increased over time and were maintained up to 21 days.
- UHH incubations in HPM most accurately predicted in vivo CLcomp for a subset of 11 slowly metabolized reference compounds. Assessed metabolic pathways in UHH predicted better predictions for the 7 intermediate CLcomp tested.
- Prediction performance appeared affected by enzyme activity levels, as indicated by switch of culture medium from HPM to WME for UHH.
- Metabolite pattern for the reference drugs tested were essentially comparable among PHH and UHH sandwich cultures, suggesting similar metabolic degradation pathways.

6. References


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