Metabolic Capability of Upcyte® Human Hepatocytes in Long-Term Sandwich Culture and Utility for Clearance Prediction

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1. Abstract

The purpose of this study was to evaluate Upcyte® human hepatocytes (UHH) as an alternative *in vitro* system for studying 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-transformed primary hepatocytes. Differentiated UHH express adult hepatic markers and are responsive to prototypical inducers of cytochrome P450 enzymes[1],[2].

We assessed the metabolic capability of UHH in sandwich culture for up to 21 days, focussing 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 vivo* hepatic clearance (CL_H) was predicted for a set of slowly and intermediate cleared reference drugs by scaling from *in vitro* intrinsic clearance (CL_{int}). Data from the above experiments were compared to those obtained from sandwich cultures of cryopreserved PHH.

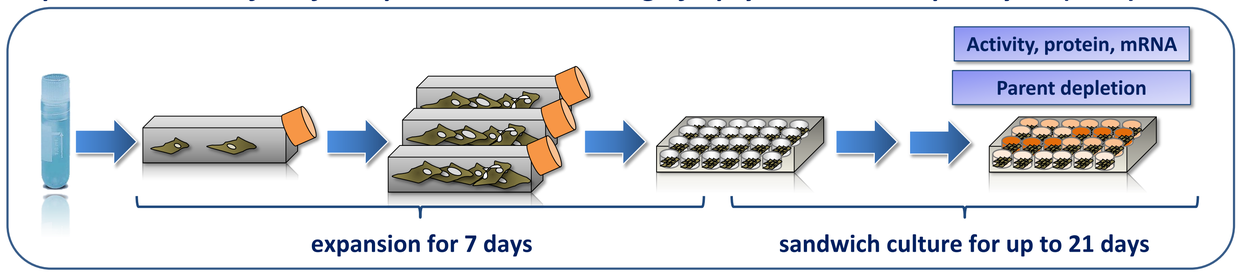
2. Objectives

Focus of the investigations was on the assessment of Upcyte® human hepatocytes regarding:

- **Metabolic capability (P450, UGT, SULT) over time in sandwich culture**
- **Utility for CL_H prediction of slowly metabolized compounds**
- **Head-to-head comparison with standard primary human hepatocyte cultures**

3. Methods

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



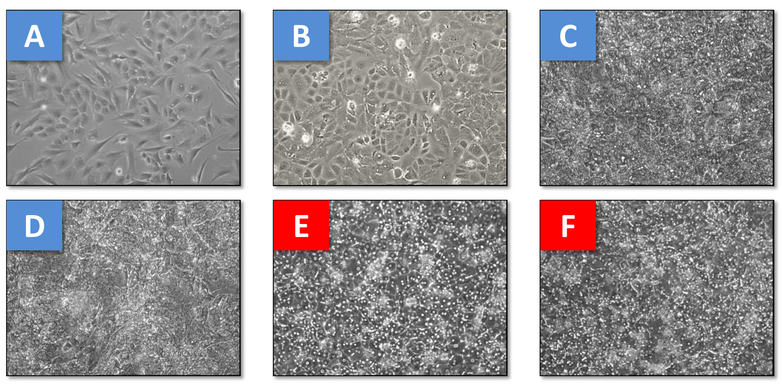
Analysis of enzyme activity, mRNA and protein expression

Activities of cytochrome P450, UGT and SULT enzymes were determined *in situ* via metabolite formation (LC-MS/MS) upon incubation with probe substrates[3]. Relative mRNA expression levels were determined by Taqman RT-PCR[3]. Protein quantification was performed from microsomal preparations by LC-MS/MS quantification of specific tryptic fragments[4].

In vivo CL_H prediction

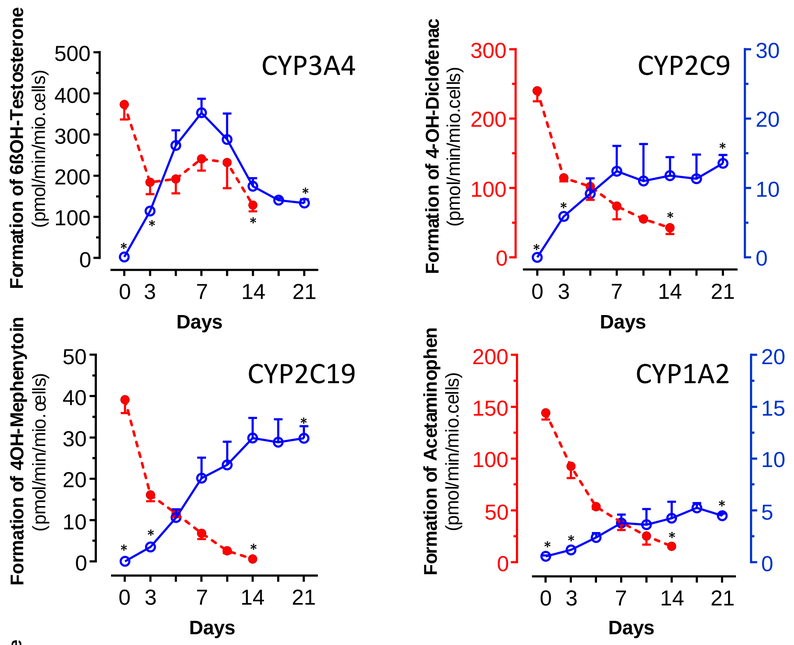
Reference drugs with low to intermediate *in vivo* $CL_{nonrenal}$ and metabolism as primary elimination pathway were incubated in 24-well format for up to 120 h. *In vivo* CL_H was scaled from *in vitro* CL_{int} applying the physiologically based *in vitro-in vivo* direct scaling approach and the well-stirred model[3].

4. Results



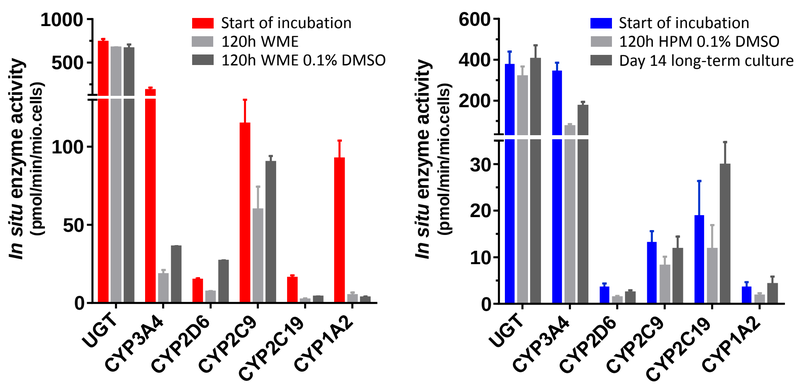
Morphology of UHH and PHH cultures

Light microscopy images of UHH 151-03 (blue) in expansion culture at 6 h (A) and 48 h (B) after initial plating; in confluent sandwich culture prior to incubation start at culture day 7 (C), and 5 days later without medium change (D). PHH Hu1601 (red) sandwich culture prior to incubation start at culture day 3 (E), and 5 days later without medium change (F).



Metabolic activity over time in sandwich culture

Enzyme activities were determined by *in situ* metabolite formation in 24-well sandwich culture for up to 21 days. Data represent mean ± SD of N=2-5 experiments. UHH donor 151-03 (blue) showed relatively lower levels at start of culture period compared to PHH donor Hu1601 (red), with increasing and maintained activity over the study period. Activities in PHH decreased readily from start of sandwich culture until day 14 as study end.



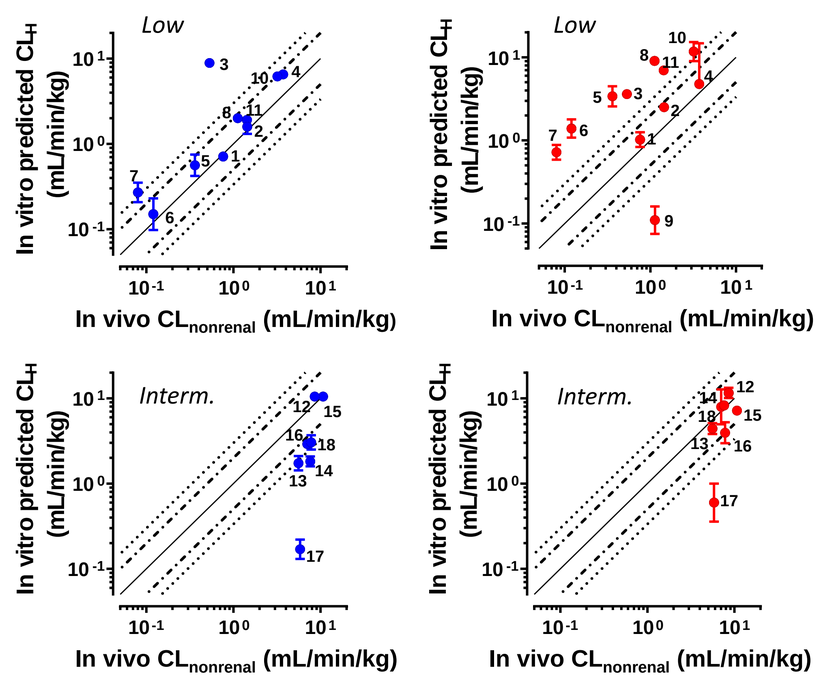
Metabolic activity under extended depletion assay condition

Enzyme activity was monitored over a maximum period of 120 h without medium change, as used in this study for substrate depletion assays of metabolically stable compounds. Data shown represent mean ± SD (N=2-5). UHH 151-03 (blue) were cultured in HPM medium recommended by the vendor, originally containing 0.1% DMSO. PHH Hu1601 (red) were cultured in standard Williams´ Medium E (WME) with and without 0.1% DMSO. PHH showed a marked decrease in most of the tested P450 enzyme activities over 120 h. Decrease was less pronounced in WME containing 0.1 % DMSO (especially for CYP3A4 and CYP2D6), comparable to UHH cultured in HPM with 0.1 % DMSO. Data for UHH at culture day 14 with daily medium change are shown for reference.

	UHH (151-03)			PHH (Hu1601)		
	Activity	*mRNA*	*Protein*	*Activity*	*mRNA*	*Protein*
CYP1A2	3.80 ± 0.78	0.11 ± 0.10	<1.0	67.4 ± 50.3	115 ± 10	56.7 ± 2.0
CYP2B6	227 ± 92	919 ± 265	11.9 ± 1.9	3.60 ± 2.11	9.02 ± 0.89	1.66 ± 0.17
CYP2C8	11.2 ± 6.0	49.5 ± 19.1	3.13 ± 1.05	21.1 ± 1.1	25.2 ± 3.5	3.15 ± 1.72
CYP2C9	12.0 ± 4.6	111 ± 45	8.80 ± 2.08	73.9 ± 18.9	65.3 ± 8.5	182 ± 51
CYP2C19	17.7 ± 4.4	17.1 ± 8.4	4.88 ± 0.11	6.84 ± 1.42	4.58 ± 0.94	2.71 ± 0.35
CYP2D6	3.18 ± 0.56	6.87 ± 4.95	1.26 ± 0.29	12.0 ± 3.6	62.6 ± 8.43	7.88 ± 0.70
CYP3A4	339 ± 37	329 ± 141	45.1 ± 8.2	241 ± 98	102 ± 15	50.8 ± 10.0
UGT1A1	111 ± 17	508 ± 106	96.2 ± 2.7	65.0 ± 0.1	347 ± 60	42.5 ± 4.3
UGT1A9		8.06 ± 1.93	1.73 ± 0.15		36.3 ± 7.8	1.99 ± 0.86
UGT2B7	426 ± 18	39.4 ± 2.8	1.86 ± 0.30	768 ± 93	221 ± 33	7.04 ± 0.15
UGT2B15		4.05 ± 1.00	2.19 ± 0.15		51.3 ± 12.0	15.9 ± 2.0
SULT	15.4 ± 0.4	n.a.	n.a.	20.6 ± 0.7	n.a.	n.a.
Na/K-ATPase			8.11 ± 0.43			11.9 ± 0.3

P450 and UGT enzyme activity, protein and mRNA expression

UHH and PHH were assessed at culture day 7 of 24-well sandwich culture. Enzyme activities are depicted as pmol/min/mio. cells, mRNA levels as 10^3-fold of endogenous control β-actin, and protein as fmol/µg of microsomal protein. Na/K-ATPase was used as endogenous control for protein quantification. Activity values represent mean ± SD (N=2-5 individual experiments), mRNA and protein data mean ± SEM (N=1). Non-specific UGT/SULT activity data was determined by 7-hydroxycoumarin glucuronidation/sulfatation. CYP1A2 and CYP2B6 data showed most pronounced differences between UHH and PHH.



1: alprazolam, **2**: prednisolone, **3**: diazepam, **4**: voriconazole, **5**: tolbutamide, **6**: meloxicam, **7**: warfarin, **8**: glimepiride, **9**: theophylline, **10**: riluzole, **11**: oxazepam, **12**: midazolam, **13**: atazanavir, **14**: diclofenac, **15**: lidocaine, **16**: risperidone, **17**: flecainide, **18**: atomoxetine

Clearance prediction performance of UHH and PHH cultures

Correlation of *in vitro* predicted CL_H and *in vivo* $CL_{nonrenal}$ disregarding plasma protein binding for 18 reference drugs in sandwich cultured UHH (blue) and PHH (red). Incubations were performed for 96-120 h. Data represent mean ± SD of N=3 experiments for UHH (151-03), and mean ± SD for three PHH donors (BD371, HC3-31, Hu1601) with each N=1 experiment, respectively. Correlation plots depict subsets of low (#1-11, upper panels) and intermediate (#12-18, lower panels) clearance drugs. The solid line represents conformity, dotted lines 2- and 3-fold error range, respectively. Theophylline was excluded from the UHH plot as depletion could not be reliably determined. Good prediction performance was seen with UHH 151-03 for the set of low CL drugs (*in vivo* $CL_{nonrenal}$ ≤5mL/min/kg), for which PHH generally overpredicted $CL_{nonrenal}$. In contrast, UHH showed a trend towards underprediction of the intermediate cleared reference drugs (5-15 mL/min/kg).

		UHH		PHH		
		151-03 (N=3)	653-03 (N=1)	BD371 (N=1)	HC3-31 (N=1)	Hu1601 (N=1)
Low CL drugs (N=11)	% within 2-fold	73	36	18	27	27
	% within 3-fold	73-82	64	36	36	27
Interm. CL drugs (N=7)	% within 2-fold	29-43	0	57	71	86
	% within 3-fold	43-71	29	86	86	86
All drugs (N=18)	% within 2-fold	56-61	22	33	44	50
	% within 3-fold	67-78	50	55	50	50

Accuracy of CL_H prediction for different UHH and PHH donors

Overview on prediction accuracy for two UHH and three PHH donors tested in this study. Prediction for the 11 low CL drugs tested was most accurate and reproducible using UHH 151-03. Overall prediction accuracy for the entire 18 reference drugs was found comparable for UHH 151-03 and the three PHH donors used in this study.

5. Conclusions

- → **Activity and expression of enzymes tested in sandwich cultured UHH 151-03 were initially lower compared to PHH, however increased over time and maintained levels up to 21 days**
- → **Extended incubation periods of up to 120 h were feasible for CL prediction of 18 low and intermediate cleared reference drugs using UHH sandwich cultures**
- → **UHH incubations most accurately predicted *in vivo* $CL_{nonrenal}$ for a subset of 11 slowly metabolized reference drugs; cultured PHH provided better predictions for the 7 intermediate CL compounds tested**

6. References

[1] Burkard, A *et al*. (2012) Generation of proliferating human hepatocytes using Upcyte® technology: characterization and applications in induction and cytotoxicity assays. *Xenobiotica* **42:** 939-956.
[2] Levy, G *et al*. (2015) Long-term culture and expansion of primary human hepatocytes. *Nat.Biotechnol.***33:** 1264-1271.
[3] Schaefer, M *et al*. (2016) Upcyte® Human Hepatocytes: a Potent In Vitro Tool for the Prediction of Hepatic Clearance of Metabolically Stable Compounds. *Drug Metab Dispos* **44**: 435-444.
[4] Schaefer, O *et al*. (2012) Absolute quantification and differential expression of drug transporters, cytochrome P450 enzymes, and UDP-glucuronosyltransferases in cultured primary human hepatocytes. *Drug Metab Dispos* **40**: 91-103.