Application poster: upcyte® hepatocytes for CYP induction studies

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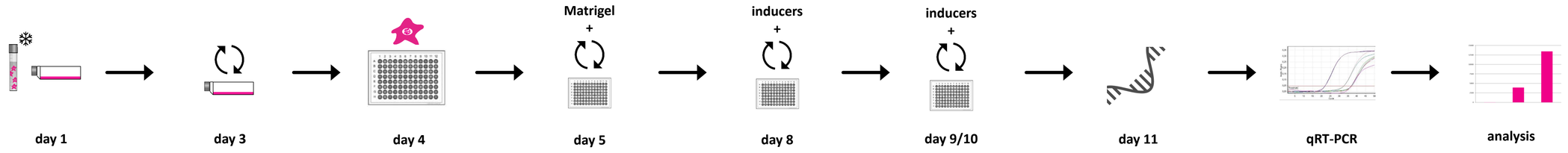
ABSTRACT

Isolated **primary human hepatocytes (pHH)** are used to study: **hepatic metabolism, toxicity and disease pathogenesis**. However, they exhibit several disadvantages, e.g. **short culture longevity** and a **limited quantity of cells** that can be isolated from one donor and a lack of **proliferation capacity**. To overcome this, we have **developed a technique which causes primary human hepatocytes to proliferate** up to 35 population doublings whilst retaining an adult and metabolically competent phenotype with phase I (Cytochrome P450) and phase II activities when cultured at confluence. The **FDA and EMA** recommend (in the drug-drug interaction guidelines concerning *in vitro* CYP induction assessment) using primary human hepatocytes (PHH) and additional data from other cell systems as complementary and supportive info. The recommended endpoints for CYP induction are measurement of **mRNA (FDA) and CYP enzyme activity/mRNA (EMA) for CYP1A2, CYP2B6, and CYP3A** (additional enzymes might be necessary).

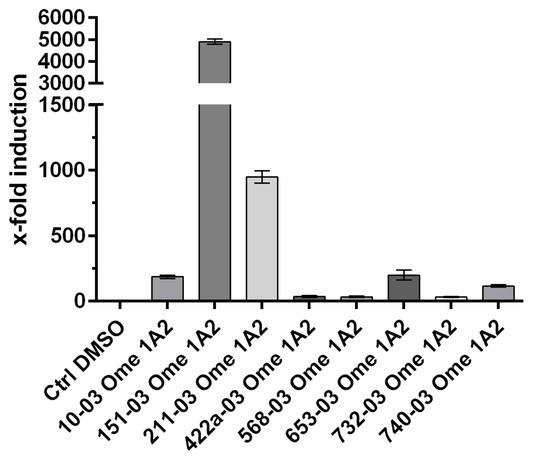
MATERIAL & METHODS



upcyte® hepatocytes of donor: 10-03, 151-03, 211-03, 422a-03, 568-03, 653-03, 732-03 and 740-03 were thawed according to protocol (PFU12) at 10.000 cells/cm² in collagen coated T175 flasks on day 1 in Hepatocyte High Performance Medium (HPM). Medium was changed on day 3 and cells were split on day 4 into 24 well plates with 150.000 cells/cm². On day 5 cells were overlayed with Matrigel. On day 8 medium was changed to HPM containing inducers: 50 µM Omeprazole (Ome; for CYP1A2), 100 µM Phenytoin (Phen, for CYP2B6), 20 µM Rifampicin (Rif, for CYP2C9 & CYP3A4) and 0.1 % DMSO as vehicle control. Medium containing vehicle control or inducers was replaced daily for a further 2 days (day 9 & 10). After 72h of induction RNA was isolated (NucleoSpin® RNA Kit, Macherey-Nagel) and a qRT-PCR was used to quantify levels of selected genes. For all samples the Δct, ΔΔct and the x-fold induction were calculated. GAPDH was used as the reference gene and DMSO control was set to 1. Analysis was performed in Prism.

RESULTS & DISCUSSION

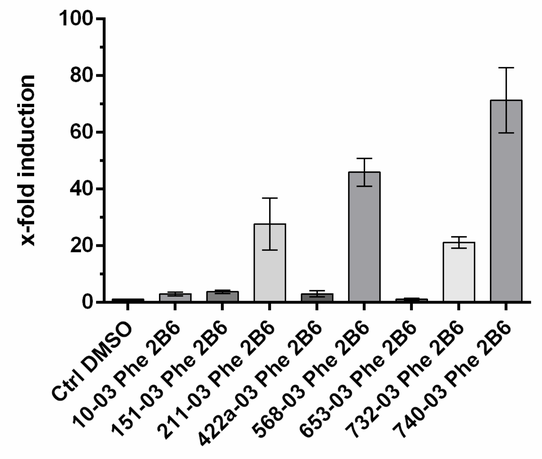
CYP1A2



Graph 1: x-fold induction of CYP1A2 compared to the respective DMSO control (set to 1) for different donors (10-03, 151-03….). Omeprazole was used as inducer. Table 1 below shows the corresponding values incl. standard deviation.

Sample	Ctrl DMSO	10-03 Ome 1A2	151-03 Ome 1A2	211-03 Ome 1A2	422a-03 Ome 1A2	568-03 Ome 1A2	653-03 Ome 1A2	732-03 Ome 1A2	740-03 Ome 1A2
average x-fold	1,0	186,3	4905,6	949,4	36,0	33,5	199,3	31,6	117,1
stdev	0,0	8,4	85,0	32,9	4,1	3,8	27,5	2,1	5,7

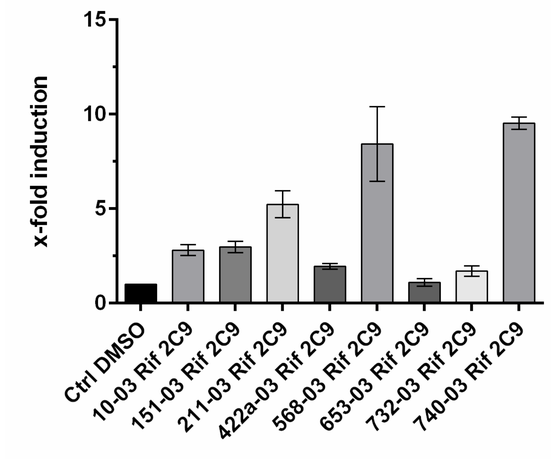
CYP2B6



Graph 2: x-fold induction of CYP2B6 compared to the respective DMSO control (set to 1) for different donors (10-03, 151-03….). Phenytoin was used as inducer. Table 2 below shows the corresponding values incl. standard deviation.

Sample	Ctrl DMSO	10-03 Phe 2B6	151-03 Phe 2B6	211-03 Phe 2B6	422a-03 Phe 2B6	568-03 Phe 2B6	653-03 Phe 2B6	732-03 Phe 2B6	740-03 Phe 2B6
average x-fold	1,0	2,9	3,7	27,6	2,9	45,9	1,1	21,1	71,2
stdev	0,0	0,5	0,4	6,5	0,8	3,5	0,2	1,5	8,1

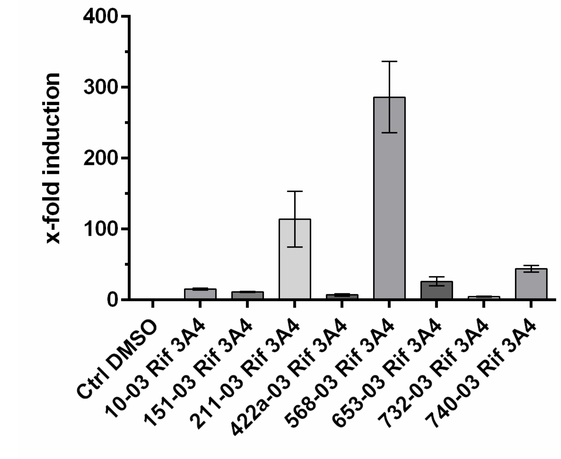
CYP2C9



Graph 3: x-fold induction of CYP2C9 compared to the respective DMSO control (set to 1) for different donors (10-03, 151-03….). Rifampicin was used as inducer. Table 3 below shows the corresponding values incl. standard deviation.

Sample	Ctrl DMSO	10-03 Rif 2C9	151-03 Rif 2C9	211-03 Rif 2C9	422a-03 Rif 2C9	568-03 Rif 2C9	653-03 Rif 2C9	732-03 Rif 2C9	740-03 Rif 2C9
average x-fold	1,0	2,8	3,0	5,2	1,9	8,4	1,1	1,7	9,5
stdev	0,0	0,2	0,2	0,5	0,1	1,4	0,1	0,2	0,2

CYP3A4



Graph 4: x-fold induction of CYP3A4 compared to the respective DMSO control (set to 1) for different donors (10-03, 151-03….). Rifampicin was used as inducer. Table 4 below shows the corresponding values incl. standard deviation.

Sample	Ctrl DMSO	10-03 Rif 3A4	151-03 Rif 3A4	211-03 Rif 3A4	422a-03 Rif 3A4	568-03 Rif 3A4	653-03 Rif 3A4	732-03 Rif 3A4	740-03 Rif 3A4
average x-fold	1,0	15,2	11,6	114,1	7,1	286,3	26,3	4,8	44,1
stdev	0,0	1,0	0,4	27,9	1,1	35,5	4,5	0,5	3,2

It has been described that mRNA and catalytic activity might not correlate due to several different kinds of post-transcriptional control mechanisms, including microRNA (e.g. for CYP3A4; CYP2B6, CYP2E1), factors controlling translation and post-translational insertion in the membranes and phosphorylation [OECD report]. Therefor. it is always recommended to evaluate induction using both methods available. Here we present our mRNA data.

CYP1A2: induction differences were pronounced for CYP1A2 as commonly seen with pHH. Donor 151-03 showed close to 5000x induction after treatment with Omeprazole. Donor 211-03 was close to a 1000x; corresponding well with the low basal mRNA expression comparing all donors [data not shown]. The lowest induction donors: 422a-03, 568-03 and 732-03 showed a range between 30-40x with donor 732-03 having the highest basal mRNA expression. As a comparative value the basal activity of donors 151-03 and 653-03: is around 0-4 pmol/min/mg protein for both donors. In general it was shown that basal mRNA expression of CYP1A2 in upcyte® hepatocytes is low [*Tolosa et al*., 2016; *Schaefer et al*.,2016]. Low mRNA expression and activity explains the distinct induction in some of the donors. pHH used in the same studies showed around 4pmol/min/mg protein [*Tolosa et al*. 2016] and up to 68 pmol/min/mg protein [*Schaefer et al*. 2016, K&K 2018]. Data from BioIVT [Table 5] shows a basal activity of 0.3-40.8 pmol/min/mill cells and an induction of 4-40x for pHH.

CYP2B6: differences for CYP2B6 were less pronounced than with CYP1A2. The lower end showed around 3x (donors 10-03, 151-03 and 422a-03) compared to 46x and 72x (donors 568-03 and 740-03). This corresponds well with high basal mRNA expression for donor 10-03 and the low basal mRNA expression of donors 568-03 and 740-03 [data not shown]. The basal activity is well maintained, ranging from 30-70 pmol/min/mg protein depending on the donor [in house data] up to 230 pmol/min/mg protein for Donor 151-03 [*Schaefer et al*., 2016]. Values for 3x induction of donor 10-03 corresponds with findings from *Tolosa et al.* (using Phenobarbital as an inducer) and Indigo Biosciences [Induction Poster, SOT 2017]. mRNA expression in upcyte® hepatocytes was particularly high for CYP2B6 when measured relative to endogenous internal control b-actin [*Schaefer et al*,. 2016]. Higher basal activity leads to a lower response window and therefore to a lower induction as compared to CYP1A2. Inter-individual differences in hepatic CYP2B6 expression levels and activity in pHH have been reported to vary up to several hundredfold, especially fresh compared to cryopreserved pHH [*Fahmi et al*,. 2016]. In comparison, pHH of BioIVT show a basal activity of 0.1-4.6 pmol/min/mill cells and an induction of 1.8-13.1x [Table 5].

CYP2C9: induction for CYP2C9 was less pronounced and varied from 1,1-9,5x. Donor 653-03, 732-03 and 422a-03 stayed under the 2-fold threshold. Donor 740-03 and 568-03 showed an induction of 9,5x and 8,4x, respectively. For upcyte® hepatocytes the following values were shown: 0.1-3 pmol/min/mg protein [Tolosa *et al.,*2016]; 4.8–29.1 pmol/min/mg protein [K&K, 2018]; 12 pmol/min/mg protein for donor 151-03 [Schaefer *etal.,* 2016]; matching well with internal data of up to 25 pmol/min/mg protein [not shown]. Basal activity for pHH ranges around 2-74 pmol/min/mg protein [Schaefer *et al*. 2016; Tolosa *et al.,* 2016; K&K, 2018]. Data from the literature shows an induction of 1.4-6x for pHH [*Monostory et al*., 2009; Berger *et al*., 2016; Tolosa *et al.,*2016; Sahi *et al*., 2009] reflecting the results of upcyte® hepatocytes well.

CYP3A4: enzymes of the CYP3A family are of particular importance as they are alone involved in the metabolism of more than 50% of human drugs. Induction ranged between 5-15x on the lower end compared to 44x, 114x and 286x (donors 740-03, 211-03 and 568-03, respectively). Values for 15x induction of donor 10-03 corresponds with findings from *Tolosa et al* [2016]. The reported basal activity of around 340 pmol/min/mg protein for donor 151-03 and 30 pmol/min/mg protein for donor 653-03 [values for day 7, *Schaefer et al*.] compared to pHH (240 pmol/min/mg protein). From comparing the different donors we found very low basal mRNA expression for donor 568-03 [data not shown]. The reported spectrum of CYP3A4 activities of pHH varies between less than 1 pmol/min/mg protein to higher than 1000 pmol/min/mg protein [*Gomez-Lechon et al*., 2004; *Gerets et al*, 2012; K&K 2018] offering a large induction window. BioIVT's internal data shows basal activity of 0.7-45.5 pmol/min/mill cells and an induction of 3x-45x [Table 5]. Mentioned by *Kenny et al*., evaluating data from 581 individual experiments for rifampicin-induced CYP3A4 mRNA, with median fold-induction values ranging from 7.1- to 75x across 15 donors [*Kenny et al.,* 2016]. Regulatory agencies recommend **2-fold change** relative to vehicle control, with concentration dependence, tested **in 3 different donors** to identify a positive *in vitro* inducer for CYP3A4 [*Kenny et al*, 2016].

LITERATURE

2016 Tolosa *et al*., [doi: 10.1093/toxsci/kfw078]
2016 Kenny *et al*., [doi:10.1124/dmd.118.081927]
2012 Gerets *et al*., [doi: 10.1007/s10565-011-9208-4]
2018 Yokoyama *et al*., [doi: 10.1248/bpb.b17-00913]
2016 Berger *et al*., [doi: 10.3389/fphar.2016.00443]
2018 Kammerer & Kuepper [DOI 10.3233/JCB-179012]
2016 Schaefer *et al*., [doi:10.1124/dmd.115.067348]
2016 Fahmi *et al*., [doi: 10.1124/dmd.116.071076]
2010 Jennen et al., [doi:10.1093/toxsci/kfq026]
2009 Monostory *et al*., [doi: 10.1124/dmd.108.023887]
2009 Sahi *et al*., [DOI: 10.1002/jbt.20264]

SUMMARY & CONCLUSION

		CYP1A2	CYP2B6	CYP2C9	CYP3A4
basal activity	pHH BioIVT (pmol/min/mill cells)	0.3-46.8	0.1-4.6	no data available	0.7-45.5
	upcyte (pmol/min/mg protein)	0.4-317	8-227	0.1-92	21-339
fold induction	pHH BioIVT	4.0x-40.1x	1.8x-13.1x	no data available	3.1x-44.9x
	upcyte	33.5x-4905.6x	1.1x-45.9x	1.1x-9,5x	4.8x-286.3

Table 5 – summary of the basal activity and x-fold induction of pHH (n=54, measured on day 5 after seeding) and upcyte® hepatocytes (n=8). For hepatocytes 1mg of protein is estimated to be 1 Million cells.

All of the donors were responsive to the inducers. The basal activity and fold induction of upcyte® hepatocytes is comparable to primary human hepatocytes. The right donor needs to be chosen carefully: a donor with low fold induction might not be sensitive enough and a donor with a big fold induction might give an exacerbated response and false positive results. upcyte® hepatocytes offers an alternative cell model for induction screenings which is easy to handle, relatively cheap and available in large lot sizes. Further steps would include testing different inducers for each CYP enzyme, a concentration dependent induction as well as transferring the assay to 384well plate for screening purposes.

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