Use of upcyte® hepatocytes to predict toxicological outcomes

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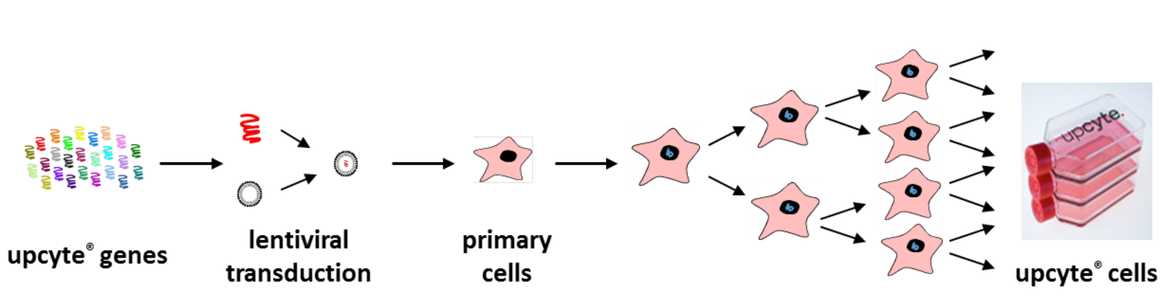
INTRODUCTION

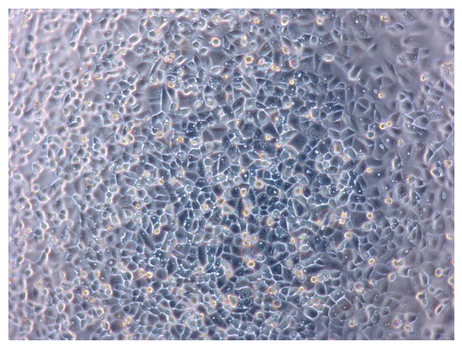
Cultures of primary human hepatocytes (pHH) are routinely used in drug development to evaluate metabolic fate, drug-drug interactions and drug toxicity. However, the supply of pHH is limited by the low and sporadic availability of human liver tissue. To address this, we have developed a technique which causes pHH to proliferate up to 40 population doublings whilst still retaining a metabolically competent phenotype when cultured at confluence ("upcyte hepatocytes"). To assess the utility of upcyte® hepatocytes for toxicity screening, we compared acute toxicity values to pHH and HepG2 cells. Compounds were characterized by TC_{50}, the concentration that caused 50% cell death after 24 hours exposure. Relative hepatotoxicity corresponded well with clinical observations, with aflatoxin B1 (AFB1) showing three orders of magnitude lower TC_{50} value than acetaminophen. Importantly, the TC_{50} profile was not significantly different from primary cells ($p<0.5$) and significantly lower than HepG2 controls ($p<0.01$). To assess the cytotoxicity correlation of upcyte® hepatocytes to that of primary hepatocytes, the TC_{50} of 18 compounds was tested *in vitro* and compared to reported values. For upcyte® hepatocyte from four different donors tested, the TC_{50} profile correlated well with reported values for primary hepatocytes showing an R^2 of 0.99.

Generation of upcyte® hepatocytes

upcyte® - expanded primary cells

The upcyte® technique enables the generation of non-transformed proliferating liver cells from primary human hepatocytes while maintaining adult phenotype. upcyte® cells start to grow from primary cells after transduction with a defined cocktail of lentiviral vectors carrying proliferation inducing genes. upcyte® cells have the ability to proliferate for additional cell doublings, depending on the cell type, without losing functional and phenotypic characteristics of mature cells.

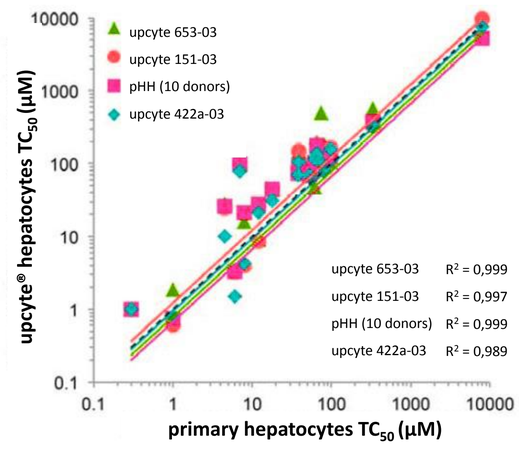




Morphology of confluent upcyte® hepatocytes

RESULTS

upcyte® hepatocytes in toxicity testing

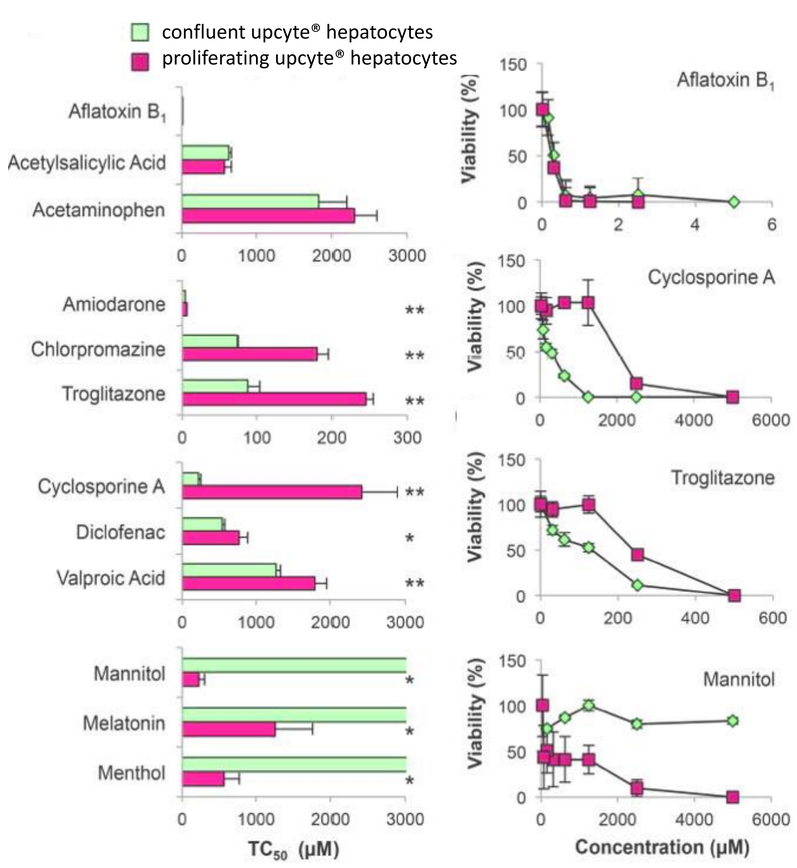


TC_{50} correlation between upcyte® hepatocytes and pHH using 18 compounds

Graph comparing the TC_{50} of 18 compounds in differentiated hepatocytes from donors #653-03, #151-03 and #422a-03 against TC_{50} values of primary human hepatocytes of 10 donors. Toxicity was measured using the MTS assay. All donors showed an R^2 correlation of 0.99 (n=3). Values presented in Log scale.

TC_{50} comparison vs HepG2 and pHH

TC_{50} values of six chemical compounds obtained from 24h dose-response studies in upcyte® hepatocytes, HepG2 cells and pHH. Normalized TC_{50} toxicity profile of upcyte® hepatocytes was not significantly different from the profile of pHH ($p=0.466$, $n=4$), whereas HepG2 profile was significantly different ($p=0.030$, $n=3$). Values presented in Log scale.



Difference between proliferating and confluent upcyte® hepatocytes

(left) TC_{50} values (nine toxic and three control compounds) of metabolically functional confluent upcyte® hepatocytes were compared with proliferating cells (n=4). The TC_{50} values of AFB1, acetylsalicylic acid and acetaminophen were not affected by cell differentiation & density. The other six of the nine hepatotoxic compounds showed significantly higher toxicity in confluent hepatocytes than in proliferating hepatocytes, suggesting that partly metabolic activation was required for the toxic effect. Surprisingly, the three control compounds (mannitol, melatonin, menthol) showed higher toxicity in proliferating than in confluent hepatocytes.

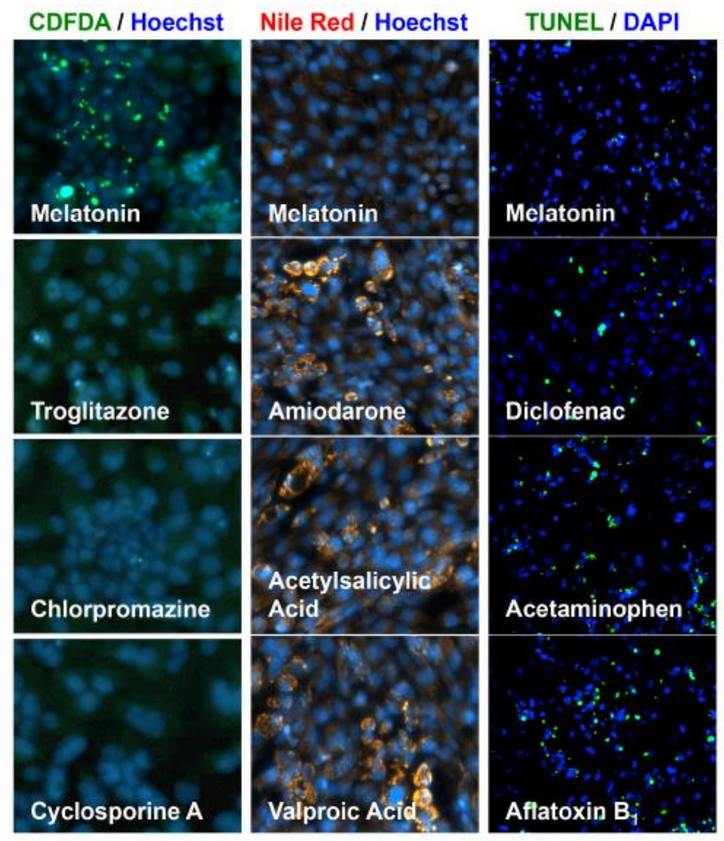
(right) Dose dependent toxicity curves of upcyte® hepatocytes treated with various compounds are shown for representative members of each group. All presented data from donor #653-03.

upcyte® hepatocytes predict toxicological outcome (cholestatis, steatosis, apoptosis)

The nine toxic compounds tested (the same compounds as in the figure on the left) have various known pathological effects in patients, including cholestasis, steatosis and apoptosis. It was studied whether these effects could be detected in upcyte® hepatocytes exposed to TC_{20} concentrations of each drug. Fluorescence quantification was used as following: **loss of bile acid production** (cholestasis) was evaluated by **CDFDA** staining, **lipid accumulation** (steatosis) by **Nile Red** staining, and **apoptosis** by **TUNEL** labeling of positive nuclei.

The cells were exposed for 24-48h to cholestasis- (*troglitazone, chlorpromazine and cyclosporine A*) , steatosis- (*amiodarone, acetylsalicylic acid or valproic acid*) or apoptosis-causing (*diclofenac, acetaminophen and AFB1*) drugs or negative control (*melatonin*). Loss of hepatic bile secretion and apoptosis was visible after 24h, lipid accumulation after 48h.

All panels present data from donor #653-03.



All data from 2015 Levy *et al.* – Nature Biotechnology (NBT-doi:10.1038/nbt.3377) - long term culture and expansion of primary human hepatocytes

CONCLUSION

In general upcyte® hepatocytes maintain albumin production, phase I and II gene expression and CYP450 activity and show functional epithelial polarization (data not shown here). The toxicological responses of upcyte® hepatocytes were similar to those of primary human hepatocytes and could predict different toxic outcomes such as cholestatis and steatosis.
The upcyte® technology allows for the generation of large batches of cells (up to 12 x 10^9 cells per donor) enabling a reproducible and standardized experimental setting. The ability to generate cells from multiple donors will enable the study of idiosyncratic (type B) toxicity as well as population heterogeneity in metabolic activities, gene expression and antiviral responses. In conclusion, our results demonstrate that expandable upcyte® hepatocytes from multiple genotypes can be used effectively for toxicological screening assays.