Examination Of Drug-induced Liver Injury In upcyte® Hepatocytes

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Abstract

Liver toxicity is a major cause of drug failure in clinical trials as well as market withdrawal of approved drugs. In addition, due to species differences in drug metabolism, it is often difficult to extrapolate the results obtained from preclinical animal models to humans. The upcyte® cells are proliferating human hepatocytes that retain important drug metabolizing enzymes, such as cytochrome P450 3A4 (CYP3A4), which make them an attractive model system to examine drug-induced liver injury (DILI). In this study, we examined cytotoxicity in upcyte® cells from four different donors for 12 compounds. The potency for inducing toxicity was examined in the presence of chemical inhibitors of various CYPs. Of the compounds examined, aflatoxin B1 was the most influenced by ketoconazole, a potent and specific inhibitor of CYP3A4. These studies show that using upcyte® hepatocytes to study DILI can potentially reduce attrition due to metabolism-mediated toxicity and improve safety of drug candidates.

Aims and Objectives

This study assessed the utility of assay-ready upcyte® hepatocytes to:

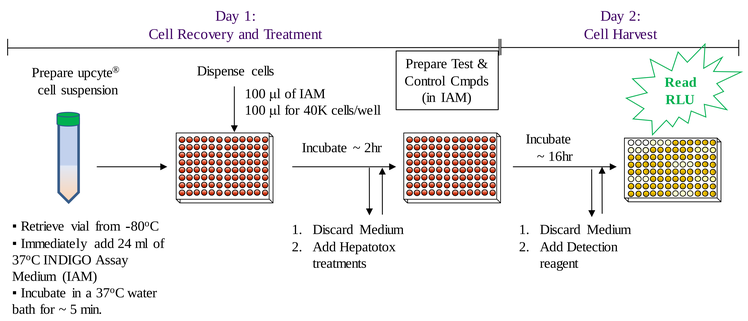
- Conduct rapid *in vitro* assessments of cytotoxicity by model hepatotoxicants, as determined by luminescence-based ATP assay
- Compare four upcyte® hepatocyte lines to study donor-to-donor variability
- Utilize specific cytochrome P450 inhibitors to determine potential bioactivation of model hepatotoxicants in human upcyte® hepatocytes

Materials and Methods

Donor cell lines. Assay-ready upcyte® human hepatocytes (donors 10-03, 151-03, 653-03 and 422A-03) were plated at 40,000 cells per well in collagen-coated, opaque 96-well tissue culture plates in Indigo Assay Media (IAM). Cells were allowed to attach for 2 hours in a 37°C CO_2 incubator. Subsequently, cells were treated with hepatotoxicants, diluted in IAM at concentrations shown in the figures, for 16 hr, in a 37°C CO_2 incubator. Cell Viability was assessed using CellTiter-Glo One Solution (Promega, Madison WI) following the manufacturer's protocol. Luminescence was measured in FLUOstar® Optima multi-mode microplate reader (BMG Labtech, Cary NC). Viability is expressed relative to corresponding vehicle control and plotted with non-linear regression analysis using Prism (GraphPad, La Jolla, CA).

Cytochrome P450 (CYP) Inhibition. Assay-ready upcyte® human hepatocytes (donor 10-03) were plated as described above. Cells were co-treated with CYP inhibitors (CYP3A4, ketoconazole 2 µM; CYP2B6, ticlopidine 0.2 µM; CYP2C9, miconazole 0.2 µM; CYP1A2 α-naphthoflavone (ANF) 2 µM) and select hepatotoxicants for 16 hrs.

Figure 1: upcyte® cell culture and assay workflow-96 well format



Cytotoxicity by model hepatotoxicants in upcyte® human hepatocytes

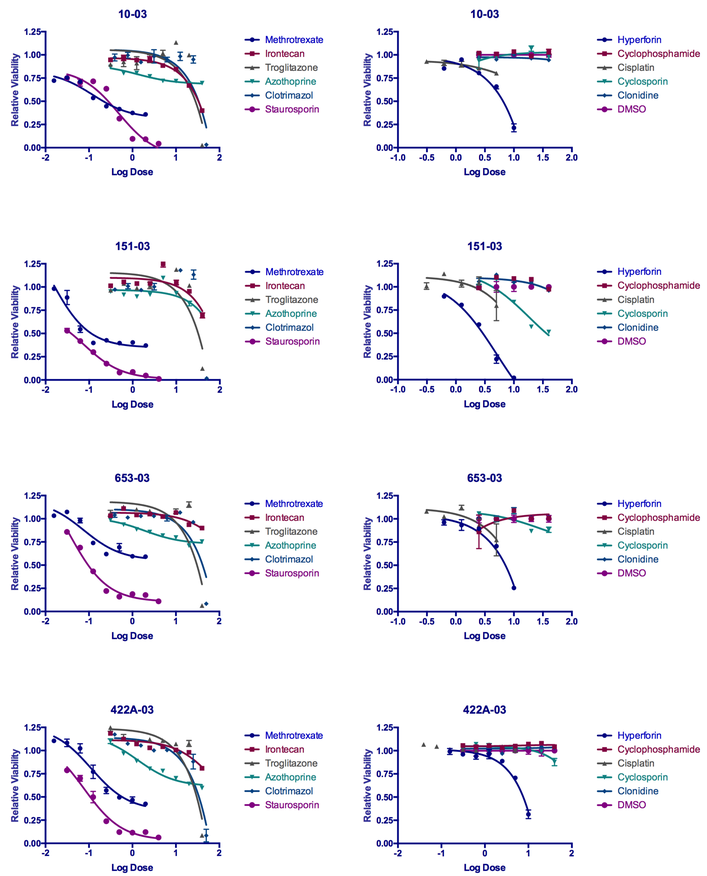


Figure 2: Cytotoxicity in upcyte® human hepatocytes (donors 10-03, 151-03, 653-03 and 422A-03). Cells were treated as described in Materials and Methods. Shown are mean and standard error of mean (SEM) for dose -response curves (n=2, representative experiment performed at least twice). Curves are the result of non-linear regression analysis.

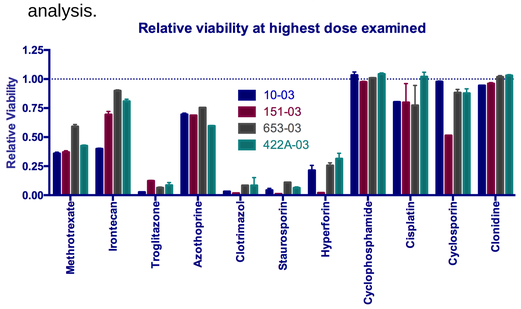


Figure 3: Cytotoxicity in upcyte® human hepatocytes (donors 10-03, 151-03, 653-03 and 422A-03) at the highest dose of each compound in Figure 2. Shown are mean and SEM (n=2, representative experiment performed at least twice).

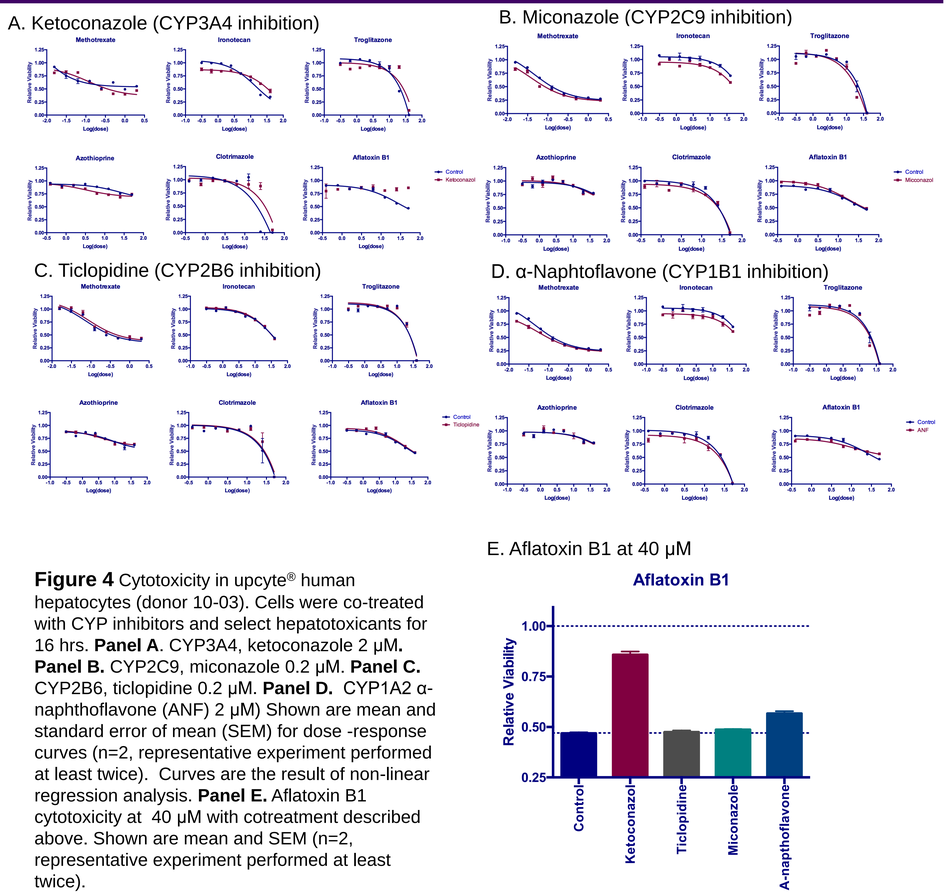


Figure 4 Cytotoxicity in upcyte® human hepatocytes (donor 10-03). Cells were co-treated with CYP inhibitors and select hepatotoxicants for 16 hrs. **Panel A**. CYP3A4, ketoconazole 2 µM**. Panel B.** CYP2C9, miconazole 0.2 µM. **Panel C.** CYP2B6, ticlopidine 0.2 µM. **Panel D.** CYP1A2 α-naphthoflavone (ANF) 2 µM) Shown are mean and standard error of mean (SEM) for dose -response curves (n=2, representative experiment performed at least twice). Curves are the result of non-linear regression analysis. **Panel E.** Aflatoxin B1 cytotoxicity at 40 µM with cotreatment described above. Shown are mean and SEM (n=2, representative experiment performed at least twice).

Results and Conclusions

- upcyte® hepatocytes are sensitive to a variety of known hepatotoxicants, including those that require bioactivation (i.e. Aflatoxin B1)
- There were modest donor-to-donor differences in cytotoxicity for the majority of the compounds tested, with some exceptions. Most notable is the sensitivity of donor 151-03 to hyperforin and cyclosporine A and donor 10-03 to irinotecan.
- The ability of upcyte® cells to bioactivate compounds was explored using specific cytochrome P450 inhibitors. In most instances, inhibiting these particular CYPs had little effect on toxicity (IC_{50} or maximal effect). Of the inhibitors examined, ketoconazole had the most pronounced response, consistent with the role of CYP3A4 metabolizing a wide array of xenobiotics. As shown in Figure 4, inhibiting CYP3A4 dramatically affected the toxicity of aflatoxin B1.
- Taken together these studies show the utility of upcyte® cells for screening compounds for hepatotoxicity, in part due to the high basal activity of several drug metabolism enzymes. Also, the availability of assay-ready upcyte® cells from various donors allow for rapid assessment of variability in the human population for hepatotoxic responses.