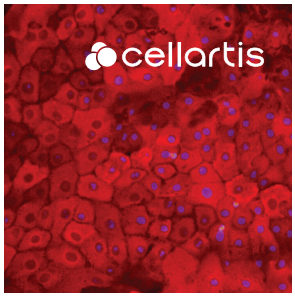

Stem Cell Products

Cellartis® Enhanced hiPS-HEP, a novel cell-based model for toxicity assessment

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Introduction

It takes more than 15 years to develop a drug and withdrawals of candidate drugs late in the drug development process due to unforeseen toxicity is costly for the pharma industry. Hepatotoxicity is one of the major reasons for late attrition of drugs. The current model systems for hepatotoxicity and safety assessment are not satisfactory and there is a need for novel improved cell based models for drug testing.

Cellartis Enhanced hiPS-HEP are human hepatocytes derived from induced pluripotent stem cells which display functional drug metabolizing enzymes in a similar range as cryopreserved human hepatocytes and can potentially serve as an alternative model for assessing hepatotoxicity. In the present application note Cellartis Enhanced hiPS-HEP were exposed to clinically hepatotoxic drugs and cell viability was measured using a WST-1 assay.

Cell culture

Cryopreserved Cellartis Enhanced hiPS-HEP were thawed and plated in 96-well plates according to the technical manual and maintained for eleven days with medium change every other day before start of the toxicity assay.

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Toxicity assay

Cellartis Enhanced hiPS-HEP were exposed to clinically hepatotoxic and non-hepatotoxic compounds (Table 1) with maximum concentration of 500 µM and a 2-fold serial dilution of six doses. The compounds were incubated for 24h prior to cell viability measurement by WST-1 assay (Takara Bio, Inc, Kusatsu, Japan). Dose response curves and TC50 (toxic dose at which 50% of cells are dead) based on the percentage of viable cells of a vehicle control for all compounds were calculated. The mean of four wells per compound and dose (n=4) were displayed in graphs (mean±SEM). The toxicity assay was repeated with three batches of Cellartis Enhanced hiPS-HEP.

Results

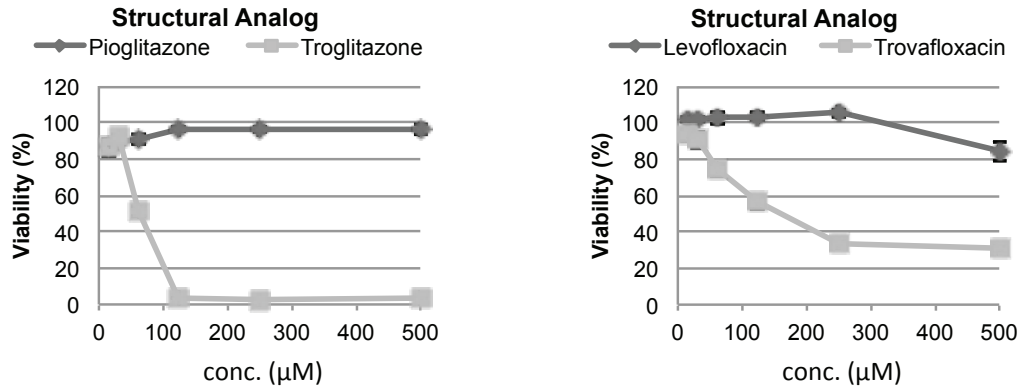

Figure 1. Dose response curves of structural analog pairs, pioglitazone/troglitazone and levofloxacin/trovfloxacin, of which one in each pair is toxic (troglitazone and trovafloxacin) whereas the other one is non-toxic (pioglitazone and levofloxacin). Cellartis Enhanced hiPS-HEP are sensitive to the hepatotoxic compounds troglitazone and trovafloxacin while remaining unaffected by the non-hepatotoxic structural analogs pioglitazone and levofloxacin. N=4; mean±SEM.

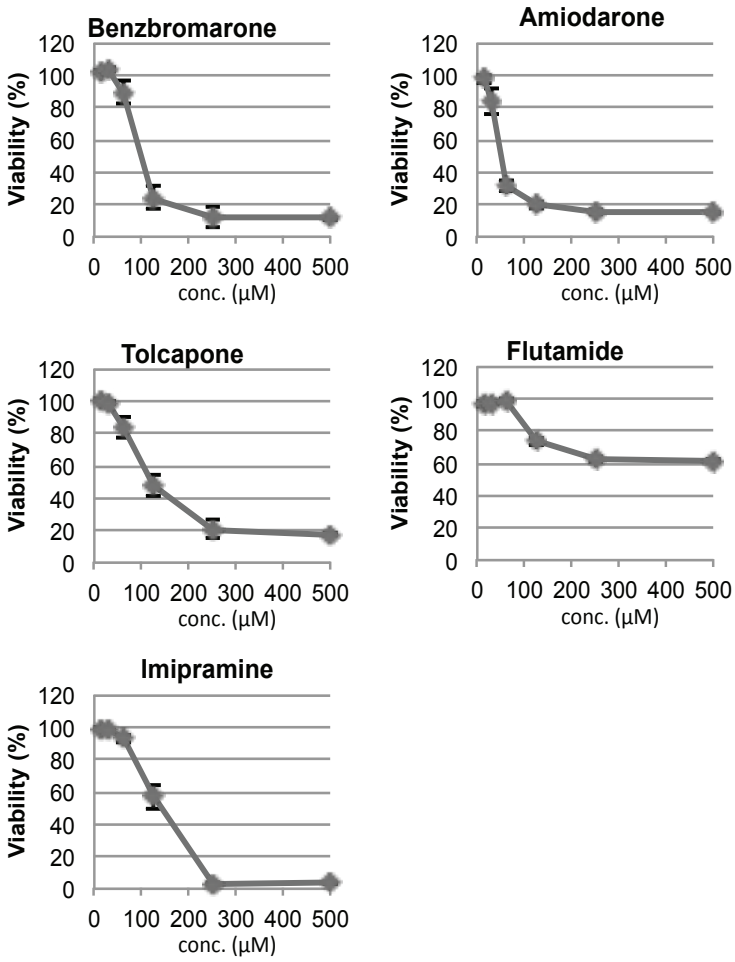

Figure 2. Dose response curves of five hepatotoxic compounds after exposure to Cellartis Enhanced hiPS-HEP for 24h. N=4; mean±SEM.

TABLE 1

Compounds	Status Label (FDA)	Severity (FDA)	Clinical DILI (Xu et al. 2008)	Sandwich PHH = Gold standard (Xu et al. 2008)	Cellartis Enhanced hiPS-HEP	TC50 (µM)
Troglitazone	WD	NA	Positive	Positive	Positive	73
Pioglitazone	-	-	Negative	Negative	Negative	-
Trovafloxacin	WD	NA	Positive	Positive	Positive	118
Levofloxacin	-	-	Negative	Negative	Negative	-
Benzbromarone	WD	NA	Positive	Positive	Positive	90
Amiodarone	BW	8	Positive	Positive	Positive	48
Tolcapone	BW	8	Positive	nt	Positive	113
Flutamide	BW	8	Positive	Negative	Positive	-
Imipramine	AR	3	Positive	Negative	Positive	154

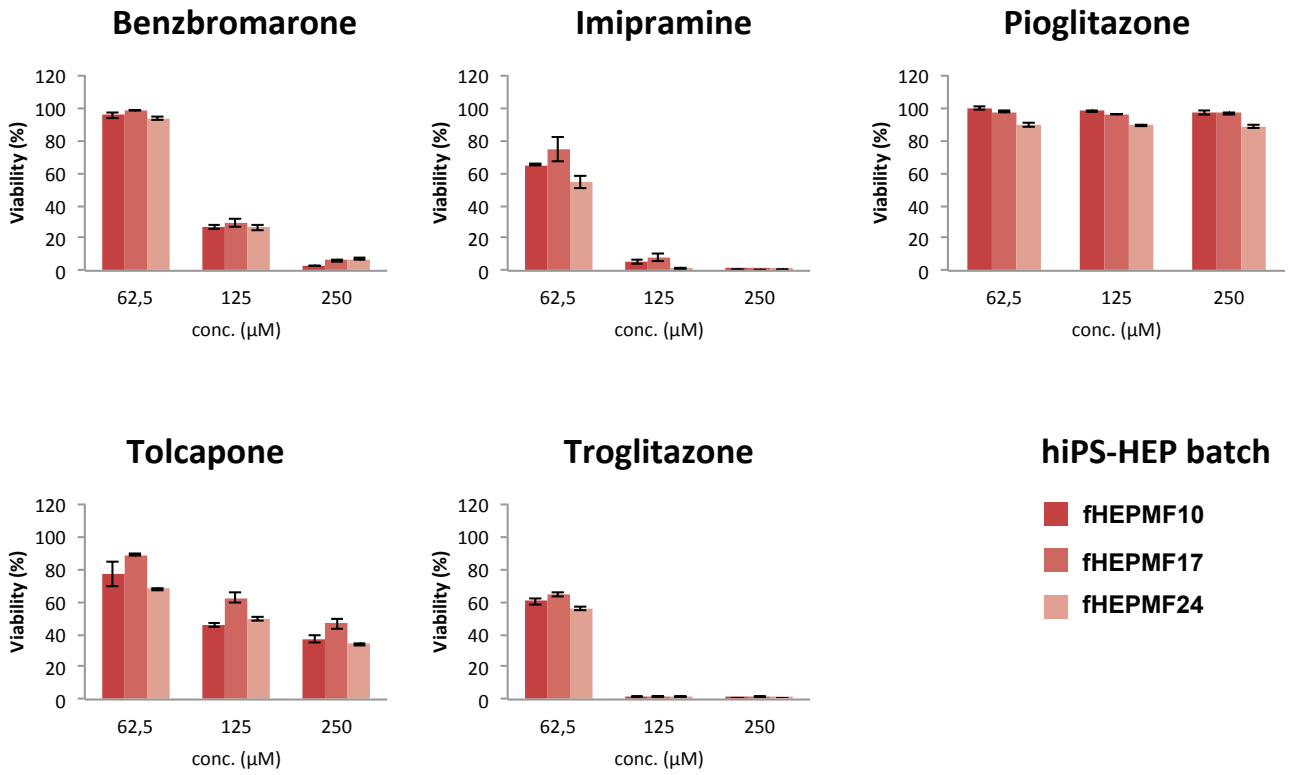

Figure 3. Three batches of Cellartis Enhanced hiPS-HEP display consistent response to hepatotoxic and non-hepatotxic drugs. Data from batches fHEPMF10, fHEPMF17 and fHEPMF24 are presented N=3;mean±SEM.

Conclusions

In conclusion, Cellartis Enhanced hiPS-HEP expressing drug metabolizing enzymes, correctly distinguish between hepatotoxic and non-hepatotoxic structural analogs of drugs e.g. troglitazone from pioglitazone and trovafloxacin from levofloxacin. The data demonstrates that Cellartis Enhanced hiPS-HEP are sensitive to clinically hepatotoxic compounds. According to the study by Xu et al. (2008), sandwich cultures of cryo human primary hepatocytes are insensitive to imipramine and flutamide in contrast Cellartis Enhanced hiPS-HEP response as clinically expected. The response to hepatotoxic and non-hepatotoxic compounds is consistent between batches of Cellartis Enhanced hiPS-HEP.

References

Xu et al. Cellular Imaging Predictions of Clinical Drug-Induced Liver Injury. Toxicol Sci **105**(1), 97–105 (2008).