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In vitro **Toxicology**

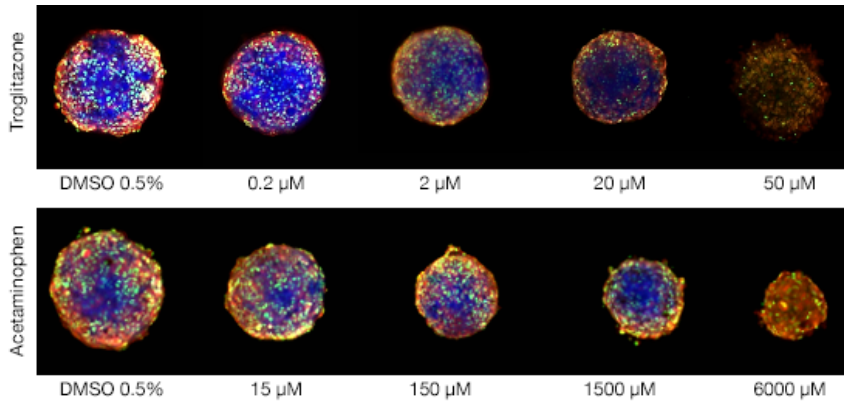
3D Hepatotoxicity Assay using HepaRG Spheroids

Background Information

- Drug-induced hepatotoxicity is a leading cause of attrition during drug development. *In vitro* three-dimensional (3D) cell cultures allow better recapitulation of the complex *in vivo* microenvironment than traditional 2D monolayer models.
- 3D models also permit long term compound exposures allowing a closer replication of clinical dosing strategies.
- Glutathione depletion, reactive oxygen species (ROS) formation, mitochondrial disruption and cellular ATP depletion are key mechanisms involved in drug induced hepatotoxicity.
- Confocal HCS allows the simultaneous detection of each cell health parameter within a 3D spheroid in combination with a measure of cellular ATP content.

Figure 1

Representative 3D confocal high content screening (HCS) images of known hepatotoxins, troglitazone and acetaminophen, labelled with Syto11 (green) to detect DNA structure, monochlorobimane (mBCL) (blue) to detect GSH content, dihydroethidium (DHE) (yellow) to detect ROS formation and MitoTracker deep red (red) to detect mitochondrial function.



Nuclei GSH Content ROS formation Mitochondrial function

Protocol

Spheroid
Cryopreserved HepaRG™ cells

Analysis Platform
Confocal Cellomics ArrayScan® XTI (Thermo Scientific)

Test Article Concentrations
8 point dose response curve with top concentration based on 100x C_{max} or solubility limit. 3 replicates per concentration*

Test Article Requirements
150 µL of a DMSO* solution to achieve 100x C_{max} (200 x top concentration to maintain 0.5% DMSO) or equivalent amount in solid compound

Time Points
14 days (336 hrs)*

Quality Controls
Negative control: 0.5% DMSO (vehicle)

Positive controls: L-buthionine sulfoximine (GSH depletion and ATP) and rotenone (ROS formation and spheroid size)

Data Delivery
Minimum effective concentration (MEC) and AC_{50} value for each measured parameter (spheroid count, spheroid size, DNA structure (DNA), mitochondrial mass (Mito Mass), mitochondrial membrane potential (MMP), glutathione content (GSH), oxidative stress (ROS) and cellular ATP content (ATP)*

*other options available on request

To find out more contact **enquiries@cyprotex.com**

Table 1

Hepatotoxicity prediction of 20 reference compounds categorised according to literature data.

Drug	Human exposure C_{max} (µM)	*In vivo* DILI category (P/N)	3D HepaRG DILI prediction: Multi-endpoint assay (MEC; µM)	3D HepaRG DILI prediction: ATP alone (MEC; µM)	Most sensitive mechanism (MSM)
Amiodarone	5.3	P	2.41	5.12	DNA
Trovafloxacin	19.7	P	7.27	7.27	ATP
Diclofenac	10.1	P	30.5	38.8	DNA
Flutamide	5.4	P	7.43	7.75	SIZE
Lapatinib	19.2	P	0.77	1.21	GSH
Nitrofurantoin	21	P	4.89	9.27	SIZE
Sunitinib	0.25	P	0.28	1.1	GSH
Troglitazone	6.29	P	1.69	25	DNA
Fialuridine	1	P	1.41	1.41	ATP
Perhexiline	2.16	P	1.69	1.76	DNA
Tolcapone	21.96	P	18.2	20.5	MMP
Acetaminophen	165.4	P	240	342	SIZE
Bosentan	4.7	P	10.4	35.2	DNA
Ticlopidine	8.1	P	34	36.2	MitoMass
Azathioprine	2.22	P	0.28	0.28	ATP
Chlorpromazine	0.94	P	1.07	3.48	SIZE
Tamoxifen	1.18	P	3.52	10.8	SIZE
Buspirone	0.01	N	NR	NR	-
Entacapone	3.28	N	45.4	45.5	GSH
Metformin	7.74	N	NR	NR	-

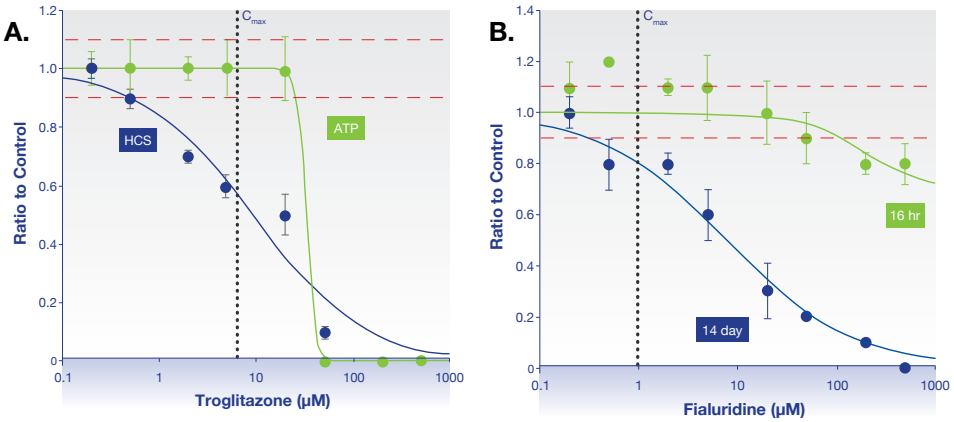
HepaRG spheroids were exposed to test compound for 14 days. During the 14 day period re-dosing occurs on 4 occasions with the final re-dose occurring 16 hours prior to the assay. The cell models were analysed using the confocal mode of Cellomics ArrayScan® XTI (Thermo Scientific) following which cellular ATP content was measured using CellTiterGlo® (Promega). MEC = Minimum effective concentration. NR = No response. DILI = Drug induced liver injury. P = Positive, N = Negative. Red ≤ 5x C_{max}, Green ≥ 5x C_{max}.

≤ 5x C_{max}

≥ 5x C_{max}

Figure 2

Graphical representation of (a) DNA structure (HCS) and cellular ATP response to troglitazone and (b) cellular ATP response to acute (16 hr) and chronic (14 day) fialuridine exposure in HepaRG spheroids.



All reference compound toxicities were correctly predicted by the HepaRG spheroid chronic exposure model using the 3D liver toxicity assay with a 5x C_{max} cut off (table 1). Bosentan and tamoxifen are categorised as false negative responders with ATP alone (table 1) highlighting the enhanced sensitivity of a combined assay. Troglitazone response is one example of the improved assay sensitivity with HCS (ATP MEC 25 µM; DNA MEC 1.69 µM) (figure 2a), while fialuridine highlights the need for long term exposures *in vitro* (14 day MEC 1.41 µM; 16 hr MEC 451 µM) (figure 2b).

The combination of an *in vitro* 3D model that better recapitulates the *in vivo* cellular physiology of hepatic tissue with a multiparametric HCS and cytotoxicity assay presents a viable screening strategy for the accurate *in vivo* relevant detection of novel therapeutics that cause drug induced liver injury early in drug development.

References

[1] Persson M *et al*., (2014). High-content analysis/screening for predictive toxicology: Application to hepatotoxicity and genotoxicity. *Basic & Clin Pharma & Tox* **115(1)**; 18-23.

[2] Hornberg JJ *et al*., (2014). Exploratory toxicology as an integrated part of drug discovery. Part II: Screening strategies. *Drug Discovery Today* **19(8)**; 1137-1144.

[3] Sakatis MZ *et al*., (2012). Preclinical strategy to reduce clinical hepatotoxicity using *in vitro* bioactivation data for >200 compounds. *Chem Res Toxicol* **25(10)**; 2067-82.

[4] Thompson RA *et al*., (2012). *In vitro* approach to assess the potential for risk of idiosyncratic adverse reactions caused by candidate drugs. *Chem Res Toxicol* **25(8)**; 1616-32.