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In vitro **Toxicology**

3D Neurotoxicity Assay:

Human brain microtissues.

Background Information

'HCA can be used to precisely distinguish between neuron-specific toxicity and general cytotoxicity while simultaneously enabling inclusion of other parameters to detect novel neurotoxic effects of chemicals.'

[1] Wilson MS *et al*., (2014) *NeuroToxicology* **42**; 33-48

- The prevalence of adverse neurotoxic reactions of the brain in response to drugs or environmental hazards continues to prompt the development of novel cell-based assays for accurate neurotoxicity prediction[1].
- *In vitro* three-dimentional (3D) cell cultures allow better recapitulation of the complex *in vivo* microenvironment than traditional 2D monolayer models[2].
- 3D models also permit long-term compound exposures allowing a closer replication of clinical dosing strategies[3].
- Mitochondrial dysfunction and calcium homeostasis[4] are commonly observed responses to toxic compounds and are implicated in neurotoxicity.
- Confocal high content imaging (HCI) allows the simultaneous detection of multiple cell health parameters within a 3D microtissue structure in combination with a measure of cellular ATP content.

Protocol

Microtissue
Included pluripotent stem cell (iPSC) derived neurocytes and astrocytes.

Analysis Platform
Confocal Cellomics ArrayScan® XTI or CX7 (Thermo Scientific).

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

Compound Requirements
Maximum (dependent upon number of repeat doses) 150 µL of a DMSO* solution to achieve 200x top concentration maintained at 0.5% DMSO or equivalent amount in solid compound.

Time Points
Any time point up to 14 days.

Quality Controls
Negative control: 0.5% DMSO (vehicle)
Positive controls: chloroquine and colchicine

Data Delivery
Minimum effective concentration (MEC) and AC_{50} value for each measured parameter; microtissue count, microtissue size, DNA structure (DNA), calcium homeostasis (Ca^{2+}) mitochondrial mass (Mito Mass), mitochondrial membrane potential (MMP) and cellular ATP content (ATP)*.

*Other options available on request

Figure 1

Representative 3D confocal high content screening (HCS) images of brain microtissues labelled with Hoechst (Blue; DNA structure and microtissue size), Fluo-4 AM (Green; calcium homeostasis) and TMRE (Red; mitochondrial function) following exposure to either control DMSO or effective concentrations of known neurotoxins chloroquine, colchicine and lead acetate.

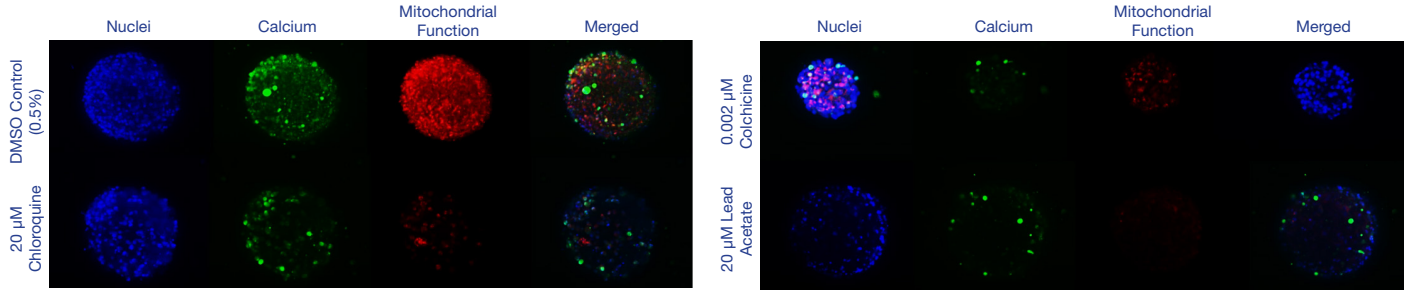


Table 1

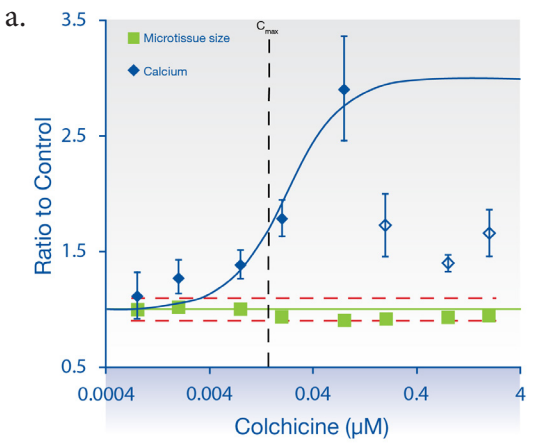
Neurotoxicity prediction of 10 reference compounds categorised according to literature data and normalized to total plasma C_{max} in immature and matured brain microtissues.

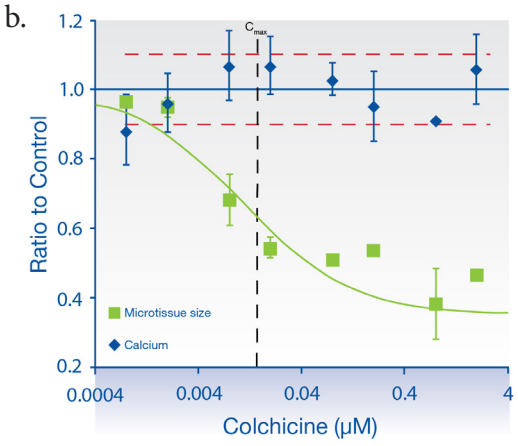
				72 hour (AC_{50})		336 hour (AC_{50})	
Name	**Expected outcome**	**Top conc (µM)**	**C_{max} (µM)**	**Immature brain MT**	**Matured brain MT**	**Immature brain MT**	**Matured brain MT**
Amoxicillin	Non-toxic	100	0.87	43.6	NR	NR	NR
Acetaminophen	Non-neurotoxic	15000	130	8790	13800	6450	3230
Acrylamide	Neurotoxic	10	0.029	4.26	NR	0.235	NR
Chloroquine disphosphate	Neurotoxic	200	1.62	52.5	28.2	13.1	13.1
Colchicine	Neurotoxic	2	0.015	0.0138	0.00954	0.00748	0.00488
Lead acetate	Neurotoxic	200	1.3	NR	NR	NR	23.1
Lidocaine	Neurotoxic	3000	25.6	NR	NR	1650	2120
Paclitaxel	Neurotoxic	200	2	45.4	16.7	11.8	<0.08
Tamoxifen	Neurotoxic	10	0.083	NR	9.45	6.37	5.45
Vinblastine sulfate	Neurotoxic	20	0.24	0.0429	<0.008	<0.008	<0.008
Correct prediction within 100x C_{max} or solubility limit				**50%**	**60%**	**80%**	**80%**

Immature (3 day old) or matured (14 day old) brain microtissues were exposed to test compound for 72 or 336 hrs. During the 336 hr period re-dosing occurred on 3 occasions (72, 168 and 210 hr). At either 72 or 336 hr the cell model was analysed using the confocal mode of Cellomics ArrayScan® XTI or CX7 (Thermo Scientific) following incorporation of fluorescent dyes. Cellular ATP content was subsequently measured using CellTiterGlo® (Promega).

Figure 2

Graphical representation of early calcium dyshomeostasis followed by a decrease in microtissue size in response to colchicine following (a) 72 hours and (b) 336 hours in brain microtissues.





Utilising the 3D neurotoxicity assay approach 80% of reference compound toxicities were correctly predicted within a 100x C_{max} cut off with a 336 hour exposure period in both immature and matured brain microtissues. Following the acute time point of 72 hour compound exposure, only 50% and 60% of compounds were correctly predicted within a 100x C_{max} cut off in the immature and matured brain microtissues, respectively.

An *in vitro* 3D brain microtissue model with improved longevity and better recapitulation of *in vivo* cellular physiology in combination with an automated multiparametric HCI and a cytotoxicity assay presents a viable screening strategy for the accurate *in vivo* relevant detection of novel therapeutics with neurotoxicity potential early in drug development.

References

[1] Wilson MS *et al.*, (2014) Multiparametric high content analysis for assessment of neurotoxicity in differentiated neuronal cell lines and human embryonic stem cell-derived neurons. *Neurotoxicology* **42;** 33-48

[2] Anderl JL *et al.*, (2009) A neuronal and astrocyte co-culture assay for high content analysis of neurotoxicity. *J Vis Exp* **27;** e1173

[3] Pamies D *et al.*, (2017) A human brain microphysiological system derived from induced pluripotent stem cells to study neurological diseases and toxicity. *ALTEX* **34**(3); 362-376

[4] Guo GW & Liang YX (2001) Aluminium-induced apoptosis in cultured astrocytes and its effect on calcium homeostasis. *Brain Res* **888;** 221-226