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In vitro **Toxicology**

Chronic Exposure Nephrotoxicity Assay

Background Information

'Drugs cause approximately 20 percent of community- and hospital-acquired episodes of acute renal failure. Among older adults, the incidence of drug-induced nephrotoxicity may be as high as 66 percent.'

[2]Naughton CA (2008) *Am Fam Physician* **78(6)**; 743-750

- Drug-induced nephrotoxicity (DIN) is a leading cause of renal failure in the clinic; creating a major concern within drug discovery programs.
- Being a highly structured filtration network, with a rich blood flow, the kidney is often exposed to high concentrations of drugs and/or metabolites creating vulnerability to drug-induced toxicity[1].
- Renal proximal tubule epithelial cells (RPTEC) are the predominant cell type in the kidney proximal tubule and one of the main sites for re-absorption and drug accumulation often resulting in tubular damage by interfering with mitochondrial function, impairing tubular transport, increasing oxidative stress or forming free radicals[1,2,3].
- A combined high content screening (HCS) approach allows a measure of multiple cell health markers including glutathione content (GSH), phospholipidosis (PLD), mitochondrial mass (mito mass) and mitochondrial membrane potential (MMP) alongside cellular ATP levels in a human kidney relevant *in vitro* cell model in order to better predict drug induced nephrotoxicity (DIN).

Protocol

Cell Type
Renal proximal tubule epithelial cells (RPTEC)

Analysis Platform and Method
Cellomics ArrayScan® (Thermo Scientific) Combined High Content Screening (HCS)

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

Number of Replicates*
3 replicates per concentration

Test Article Requirements
150 µL of a stock solution to achieve 100x C_{max} (1000x top concentration to maintain 0.1% DMSO) or equivalent amount in solid compound.

Time Points*
9 days (216 hr)

Toxicity Markers*
Cell loss
Nuclear size
DNA structure
Mitochondrial mass
Mitochondrial membrane potential
Phospholipidosis
Glutathione content
Cellular ATP

Quality Controls*
Negative control: 0.1% DMSO (vehicle)
Positive controls: Sertraline and L-buthionine-sulfoximine

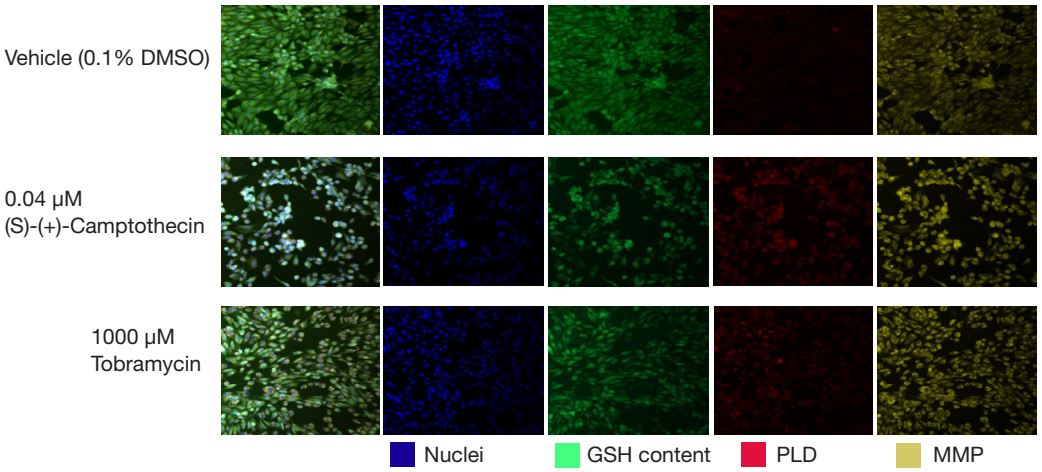
Data Delivery
Minimum effective concentration (MEC) and AC_{50} values with dose response curves for each measured parameter.

*Other options available on request.

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

Figure 1

Representative high content screening (HCS) images of (a) (S)-(+)-camptothecin and (b) tobramycin in RPTECs labelled with Syto11 (blue) to detect DNA structure, monochlorobimane (mBCl) (green) to detect GSH content, LipidTOX™ Red (red) to detect phospholipidosis (PLD) and MitoTracker® Deep Red (yellow) to detect mitochondrial membrane potential (MMP).



Compound	Human exposure C_{max} (µM)*	Known nephrotoxin	Minimum effective concentration; MEC (µM)	Most sensitive feature
(S)-(+)-Camptothecin	0.083	Yes	0.003	Nuclear size
Acetaminophen	165.4	Yes	182	Glutathione content
Cisplatin	2	Yes	0.106	Glutathione content
Cyclosporin A	11	Yes	0.709	Phospholipidosis
Diclofenac	10.1	Yes	29	Cellular ATP level
Gentamycin	13	Yes	367	Mitochondrial membrane potential
Tobramycin	16	Yes	477	Mitochondrial mass
Phenacetin	12	Yes	397	Mitochondrial mass
Amikacin	34	Yes	344	-
Buspirone	0.009	No	No response	-
Piroxicam	12.79	No	No response	-
Flavoxate	1.788	No	117	Glutathione content
Flumazenil	1.21	No	No response	-
Levocarnitine	85.7	No	No response	-
Mecamylamine	0.142	No	No response	-
Propanthelien	0.44	No	No response	-

< 1x C_{max} < 10x C_{max} < 30x C_{max} > 50x C_{max}

Table 1

Nephrotoxicity prediction of 16 reference compounds categorised according to literature data.

Utilising the RPTEC chronic exposure HCS assay all reference compound toxicities were correctly predicted with 100% accuracy, sensitivity and specificity within a 30x C_{max} cut off (table 1). Multi-parametric high content screening allows detection of nephrotoxicity below therapeutic levels (C_{max}) for cisplatin (MEC 0.106 µM; C_{max} 2 µM) and cyclosporin A (MEC 0.709 µM; C_{max} 11µM), highlighting the sensitivity of the assay.

The combination of an *in vitro* human relevant cell model with chronic compound exposures and multi-parametric endpoint assessment presents a viable screening strategy for the accurate *in vivo* relevant detection of novel therapeutics that cause nephrotoxicity early in drug development.

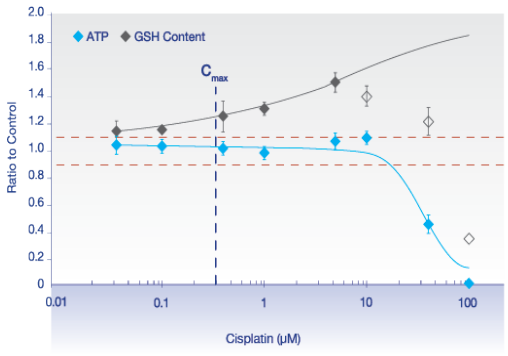
*Plasma C_{max} values were taken from the literature.

Figure 2

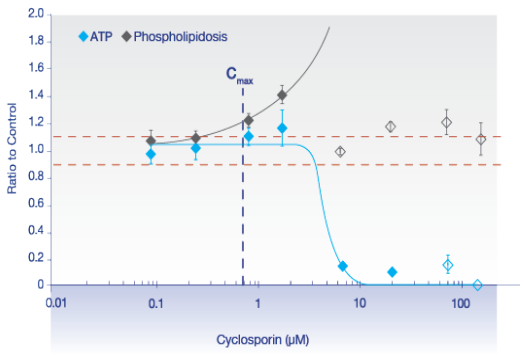
Graphical representation of (a) cellular ATP content and GSH content response following 216 hr of cisplatin exposure and (b) cellular ATP content and phospholipidosis response following 216 hr of cyclosporin A exposure in RPTECs.

RPTECs were exposed to test compound for 216 hours, re-dosing occurred on 3 occasions over this period. At 216 hours the cell model was analysed using a Cellomics ArrayScan® (Thermo Scientific) following incorporation of fluorescent dyes for cell health parameters including DNA structure (Syto11), GSH content (mBCl), phospholipidosis (HCS LipidTOX™ Red), mitochondrial dysfunction (MitoTracker® Deep Red). Subsequently cellular ATP content (CellTiter-Glo®, Promega) was determined.

(a).



(b).



References

[1] Pazhayattil GS & Shirali AC (2014). Drug-induced impairment of renal function. *Int J Nephrol Renovascular Dis* **7**; 457-468.

[2] Naughton CA (2008). Drug-induced nephrotoxicity. *Am Fam Physician* **78(6)**: 743-750.

[3] Ozer JS *et al.* (2010). A panel of urinary biomarkers to monitor reversibility of renal injury and a serum marker with improved potential to assess renal function. *Nat Biotechnol*. **25(5)**: 486-494.