In vitro Toxicology

Chronic Exposure Nephrotoxicity Assay

Background Information

- 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.

- 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.

- 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<sub>max</sub> or solubility limit

**Number of Replicates**
3 replicates per concentration

**Test Article Requirements**
150 μL of a stock solution to achieve 100x C<sub>max</sub> (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<sub>50</sub> values with dose response curves for each measured parameter.

*Other options available on request.

To find out more contact enquiries@cyprotex.com
Table 1

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

<table>
<thead>
<tr>
<th>Compound</th>
<th>Human exposure</th>
<th>Known nephrotoxin</th>
<th>Minimum effective concentration; MEC (µM)</th>
<th>Most sensitive feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S)-(+) - Camptothecin</td>
<td>0.083</td>
<td>Yes</td>
<td>0.003</td>
<td>Nuclear size</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td>165.4</td>
<td>Yes</td>
<td>182</td>
<td>Glutathione content</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>2</td>
<td>Yes</td>
<td>0.106</td>
<td>Glutathione content</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>11</td>
<td>Yes</td>
<td>2.104</td>
<td>Phospholipidosis</td>
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<tr>
<td>Diclofenac</td>
<td>10.1</td>
<td>Yes</td>
<td>29</td>
<td>Cellular ATP level</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>13</td>
<td>Yes</td>
<td>367</td>
<td>Mitochondrial membrane potential</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>16</td>
<td>Yes</td>
<td>477</td>
<td>Mitochondrial mass</td>
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<tr>
<td>Phenacetin</td>
<td>12</td>
<td>Yes</td>
<td>337</td>
<td>Mitochondrial mass</td>
</tr>
<tr>
<td>Amikacin</td>
<td>34</td>
<td>Yes</td>
<td>344</td>
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</tr>
<tr>
<td>Buspirone</td>
<td>0.009</td>
<td>No</td>
<td>No response</td>
<td>-</td>
</tr>
<tr>
<td>Piroxicam</td>
<td>12.79</td>
<td>No</td>
<td>No response</td>
<td>-</td>
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<td>Flavoxate</td>
<td>1.788</td>
<td>No</td>
<td>No response</td>
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<td>Flumarin</td>
<td>1.21</td>
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<td>No response</td>
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<td>Levocarnitine</td>
<td>85.7</td>
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<td>No response</td>
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<tr>
<td>Mecamylamine</td>
<td>0.142</td>
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<td>No response</td>
<td>-</td>
</tr>
<tr>
<td>Piroxanthen</td>
<td>0.44</td>
<td>No</td>
<td>No response</td>
<td>-</td>
</tr>
</tbody>
</table>

*Plasma C<sub>max</sub> values were taken from the literature.

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).

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.

References

