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\(^1\).

- 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\(^\circ\) (Thermo Scientific) Combined High Content Screening (HCS)

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

**Number of Replicates**
3 replicates per concentration

**Test Article Requirements**
150 μL of a stock solution to achieve 100x \(C_{\text{max}}\) (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.

---

‘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.’

\(^1\)Naughton CA (2008) Am Fam Physician 78(6): 743-750
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>165</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>0</td>
<td>Phospholipidosis</td>
</tr>
<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>
</tr>
<tr>
<td>Phencicain</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>
<td>-</td>
</tr>
<tr>
<td>Busprone</td>
<td>0.009</td>
<td>No</td>
<td>No response</td>
<td>-</td>
</tr>
<tr>
<td>Piroxicain</td>
<td>12.79</td>
<td>No</td>
<td>No response</td>
<td>-</td>
</tr>
<tr>
<td>Flavoxate</td>
<td>1.788</td>
<td>No</td>
<td>117</td>
<td>Glutathione content</td>
</tr>
<tr>
<td>Flumazenil</td>
<td>1.21</td>
<td>No</td>
<td>No response</td>
<td>-</td>
</tr>
<tr>
<td>Levocarnitine</td>
<td>85.7</td>
<td>No</td>
<td>No response</td>
<td>-</td>
</tr>
<tr>
<td>Mecamylamine</td>
<td>0.142</td>
<td>No</td>
<td>No response</td>
<td>-</td>
</tr>
<tr>
<td>Propanthelien</td>
<td>0.44</td>
<td>No</td>
<td>No response</td>
<td>-</td>
</tr>
</tbody>
</table>

Vehicle (0.1% DMSO)

0.04 µM (S)-(+) Camptothecin

1000 µM Tobramycin

Nuclei

GSH content

PLD

MMP

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

References