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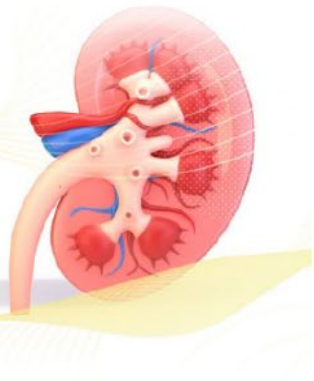


KIDNEY

Nephrotoxicity studies

Overview

Newcells Biotech has developed and validated an in vitro pre-clinical assay for measurement of drug and xenobiotic nephrotoxicity in kidney proximal tubule cells.

The assay has been used to assess nephrotoxicity across a wide range of chemical structures including small molecules, aminoglycosides and oligonucleotides.

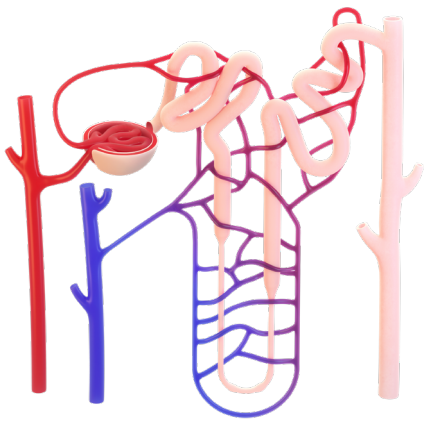
Using cells that express all the major transporters gives a more represented assessment of nephrotoxicity compared to cell lines that contain only a limited number of transporters.

The assay is available in human, rat, mouse and NHP in a 96-well format and measures a range of parameters including the generation of kidney injury biomarkers.

Services and products

Newcells offer a full service for assessment of customers compounds carried out by our fully experienced scientists under the guidance of our Director of ADMET. We work with customers to design a study protocol that provides key data to support pre-clinical decisions.

In addition, we have developed the option for collaboration projects to ship plates to customers for in-house use.



Applications

Kidney toxicity accounts for 2% of drug attrition during preclinical studies and as high as 18-27% of all incidents of acute kidney injury in clinical studies. This high prevalence is despite the fact that pre-clinical animal studies lead to more than 30% attrition due to kidney toxicity, highlighting the poor predictability of these studies to toxicity in humans.

The kidney proximal tubule epithelia cells are a major target for nephrotoxicants due to their roles in glomerular filtrate concentration and drug transport and metabolism. By using primary cells, the assay provides a close representation of the in vivo kidney physiology to give improved predictability.

The assay measures a number of parameters associated with kidney toxicity including ATP and LDH levels as well as regulator recommended biomarkers such as KIM-1, NGAL and Clusterin.

Using a 96-well format enables the experiments to be efficiently and rapidly carried out on 10-100's of compounds to determine absolute nephrotoxicity as well as comparison between candidate series using measures suggest as IC50.

Assay Format

- Primary isolated kidney proximal tubule epithelial cells cultured on a 96-well Transwell® plates

Species Available

- Human, rat, dog, NHP

Measurements

- KIM-1, NGAL, Clusterin and others as required (Meso Scale Discovery)
- ATP, LDH
- Trans-epithelial electrical resistance (TEER)

Time points and replicates

- 0, 24h up to 7 days multiple dosing
- Triplicates per concentration
- Multiple kidney repeats on request

Test article requirements

- Volumes added 0.1 ml (apical) and 0.2 ml (basolateral) per well
- Six dose concentrations

Controls

- Cisplatin as positive control
- TA vehicle as negative control

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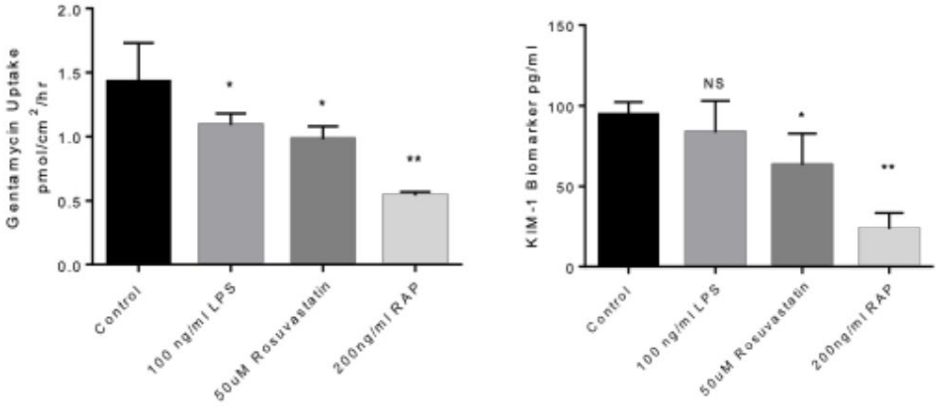
KIDNEY

Examples and Supporting Data

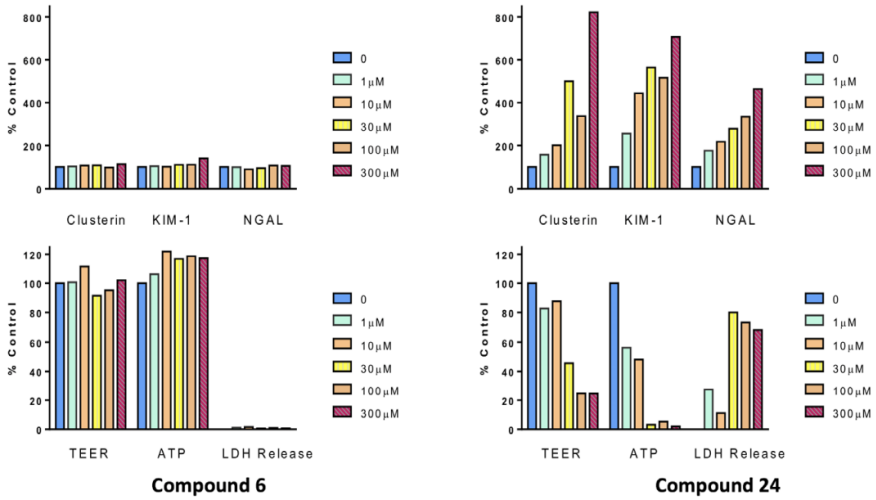
Modelling of gentamycin nephrotoxicity

Gentamycin is a well-known and studied nephrotoxicant that causes cytotoxic effect primarily in the proximal tubule epithelia cells. In order for this aminoglycoside to exert its effect it needs to be accumulated into the cells and this occurs primarily via the megalin and cubilin complex. As shown in the data below the addition of compounds that are substrates for the megalin and cubilin transporter competitively reduce the uptake of gentamycin from the apical (urine) side of the monolayer of human kidney proximal tubule cells. The reduction in uptake correlates with a reduction in the expression on the KIM-1 biomarker



Screening of drug candidate series

Using the 96-well format it is possible to screen candidate series of compounds in a cost effective approach to screen for nephrotoxicity. In this example a series of 30 compounds was tested in human proximal tubule cells to determine relative toxicity by measuring a range of biomarkers. The two example compounds shown here exhibit markedly differing toxicity: compound 6 shows no toxic responses at a range of concentrations while compound 24 shows a dose dependent increase in the release of NGAL, Clusterin and KIM-1 and a decrease in measures of cell health.



Compound 6 **Compound 24**

References

Brown CDA, Sayer R, Windass AS, Haslam IS, De Broe ME, D'Haese PC, Verhulst A. Characterisation of human tubular cell monolayers as a model of proximal tubular xenobiotic handling. Toxicology and Applied Pharmacology 2008, 233(3), 428-438.

https://www.fda.gov/Drugs/DevelopmentApprovalProcess/ DrugDevelopmentToolsQualificationProgram/ BiomarkerQualificationProgram/ucm535383.html

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