REF: HCS-05

HIGH CONTENT NEPHROTOXICITY ASSAY

CELL BASED ASSAYS

Service: High Content Nephrotoxicity Assay
Available Cell Line: HK-2 cell line
Available primary cells: Human / Rat / Mouse proximal tubule epithelial cells
Stimulation: Compounds to be tested

Background

Proximal tubules of the kidneys are one of the most common targets of nephrotoxic drugs and chemicals. Screens to predict nephrotoxic potential of compounds with insights to mechanisms of toxicity facilitate lead optimization, guide structure-activity relationships, minimize risks of clinical nephrotoxicity and therefore are valuable in the process of drug discovery. Innoprot has developed an *in vitro* High Content Analysis platform to predict the nephrototoxicity. This assay comprises the measurement of the next parameters involved in the renal toxicity.

Outcome Parameters

- Cell Viability
- Oxidative stress
- Mitochondrial Damage
- Genotoxicity (Nuclear Damage)
- Caspase 3-7 Activation
- LDH Release

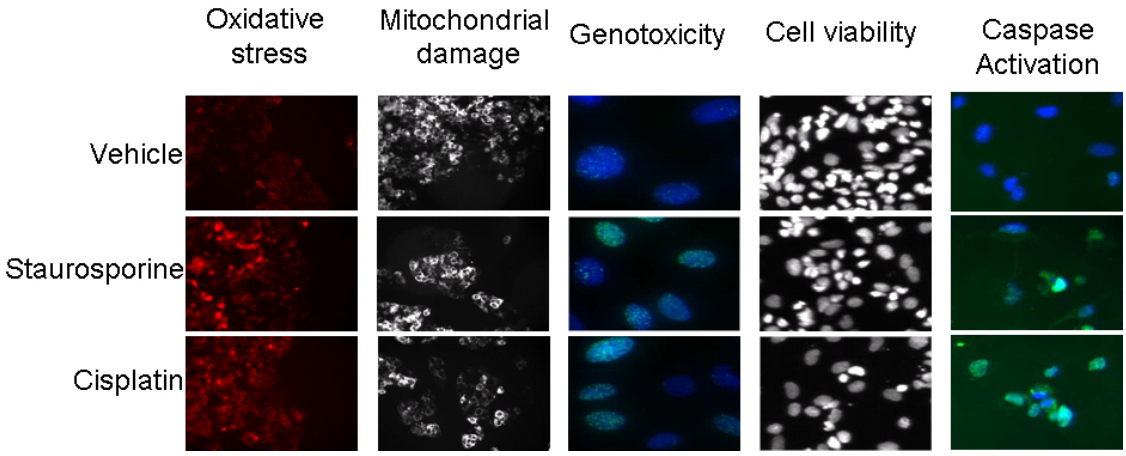
Compound testing

In this assay we predict the nephrotoxicity of the compounds measuring the most important parameters affected by the drugs: Oxidative stress, caspase activiation, mitochondrial damage and genotoxicity. Human or animal renal proximal tubule epithelial cells are treated with the drugs at several concentrations and the results are compared with the positive controls of neprhotoxics for each parameter. We measure the mitochondrial damage with the CM-H2DCFDA probe. This probe measure mitochondrial membrane potential and the results are correlated with the mitochondrial integrity. The oxidative stress is measured quantifying the reactive oxygen strains using the TMRM. Meanwhile, we use an antibody against phosphorilated H2AX protein to measure DNA damage. The H2AX is a member of the histone H2A family which phosphorilate when the double strand of DNA is broken. The presence of phosphorilated H2AX is correlated with DNA damage & subsequently with genotoxic agents presence.

INNOVATIVE TECHNOLOGIES IN BIOLOGICAL SYSTEMS, S.L.
Parque Tecnológico Bizkaia, Edifício 502, 1ª Planta | 48160 | Derio | Bizkaia
Tel.: +34 944005355 | Fax: +34 946579925
innoprot@innoprot.com | www.innoprot.com

Sample Assay

Human or animal renal proximal tubule epithelial cells are treated with the compounds during 24 hours at different concentrations and after the treatment, probes are added to measure the oxidative stress, caspase 3-7 activation, and mitochondrial damage. After the probes measurement, the immunoyhistochemistry for H2AX phosphorilation is perfomed. Results from the different parameters are compared with negative controls for each parameter and the drug will be refused in the case that the differences for one parameter could be statistically significant in comparison with the negative controls. The experiment has been tested with the compounds that are used like references for the different paramenters. "Staurosporine" is used as an apoptosis inductor and cisplatin as a nephrotoxic reference compound. In the same time, drugs like "penicillin" or "melatonin" which is known that they do not produce liver injury are used like negative controls.



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