REF: HCS-03

HIGH CONTENT HEPATOTOXICITY ASSAY

CELL BASED ASSAYS

Service:	High Content Hepatotoxicity Assay
Available Host Cells:	Human / Rat / Mouse Hepatocytes
Stimulation:	Compounds to be tested

Background

Drug-Induced Liver Injury (DILI) is the most common event causing during the drug withdrawals. But the causes of the hepatic failure provoked by the chemical compounds could be several. Due to this reason, a multiparametric assay is needed to predict toxicities that could be hidden in a simple one parameter assay. Innoprot has developed an *in vitro* High Content Analysis platform to predict the hepatotoxicity. This assay comprises the measurement of the next three parameters involved in the hepatic toxicity.

Outcome Parameters

- Oxidative stress
- Mitochondrial Damage
- Genotoxicity
- Cell Viability

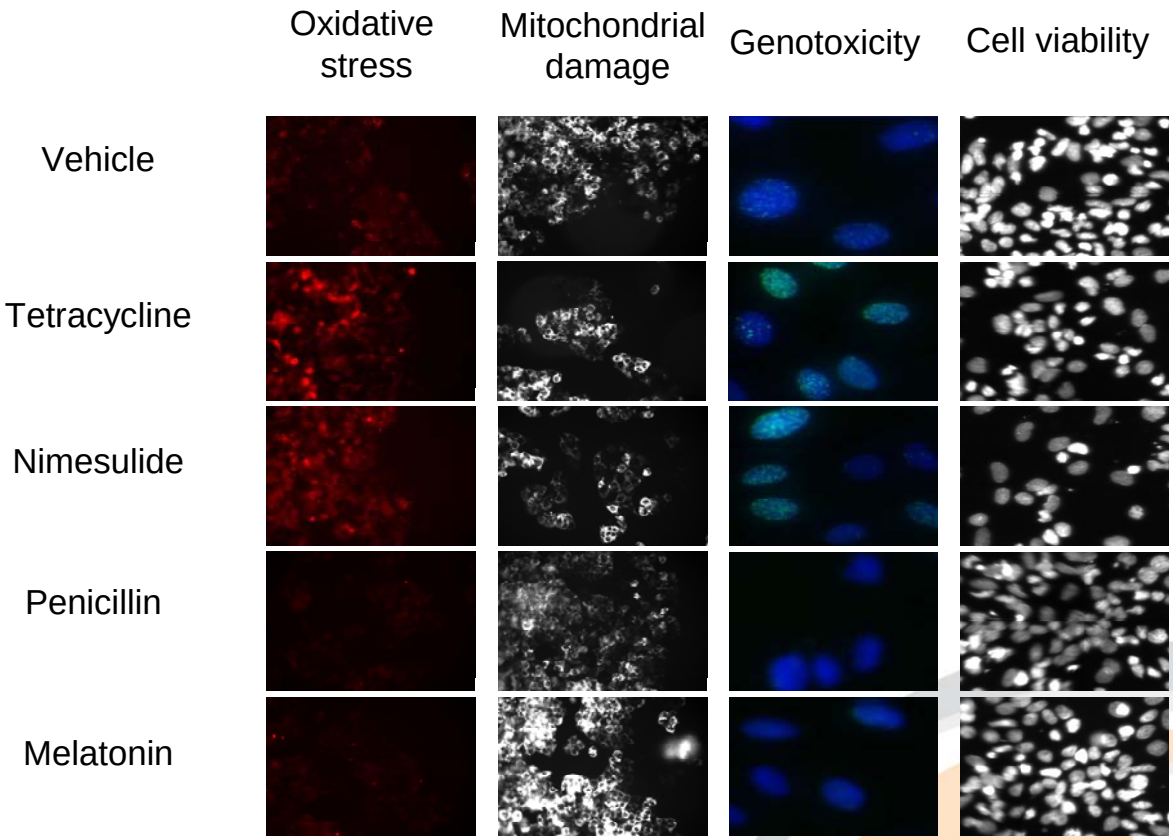
Compound testing

In this assay we predict the hepatotoxicity of the compounds measuring the most important parameters affected by the drugs: Oxidative stress, mitochondrial damage and genotoxicity. Human or animal primary hepatocytes are treated with the drugs at several concentrations and the results are compared with the positive controls of hepatotoxicants for each parameter. We measure the mitochondrial damage with the TMRM 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 CM-H2DCFDA probe. 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 hepatocytes are treated with the compounds during 24 hours at different concentrations and after the treatment two probes are added to measure the oxidative stress and mitochondrial damage. After the probes measurement, the immunoyhistochemistry for HE2AX 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. "Nimesulide" is used like positive control for oxidative stress, "tetraciclyne" for mitochondrial damage & "H_2O_2" for genotoxicity. In the same time, drugs like "penicillin" and "melatonin" which is known that they do not produce liver injury are used like negative controls.



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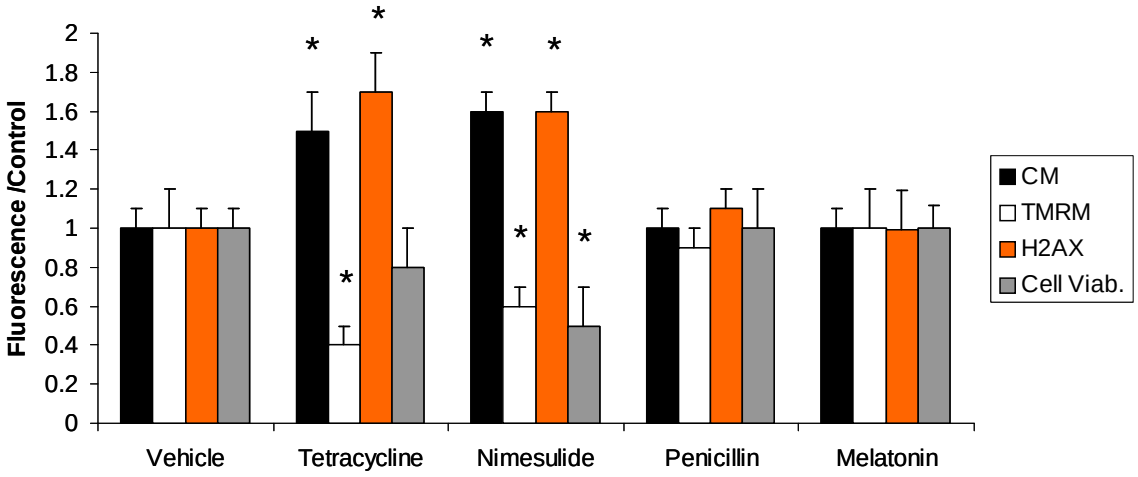

Graphical representation of the image analysis. The data was expressed respect to control and the statistically significant values ($p < 0.5$) was marked with *.