Molecular initiating events in toxicity pathways using organotypic human in vitro models

Paul Fowler¹, Sharon Scott¹, Sophie Malcomber¹, Paul Walker², Stephanie Ravenscroft², Caroline Bauch² and Paul Russell¹
¹Unilever Safety and Environmental Assurance Centre, Sharnbrook, UK
²Cyprotex, Alderley Edge, Macclesfield UK

Within the new paradigms for toxicology risk assessment, understanding the mechanisms and interactions between chemical exposure and biology is paramount in being able to make pragmatic decisions on safety utilising pathways-based approaches such as AOPs.¹

Key to these approaches is the molecular initiating event (MIE), which has been defined as the initial interaction between a molecule and a biomolecule or biosystem that can be linked to an outcome via a pathway.² Of equal importance is the need to represent biology accurately enough in in vitro models to allow meaningful mechanistic interactions. Such approaches capitalise on technologies that allow the user to interrogate multiple molecular pathways at the transcriptional and translational level.

Cyprotex cardiac model treated with Cyclophosphamide (CPA)

Human iPS cardiac myocytes, fibroblasts and endothelial cells grown in a spheroid which over the course of 5 days matures into a beating cardiac model and survives for up to 58 days post initiation. Cultures dosed with CPA (16 doses from 2 nM to 2000 µM) at day 6 for 72 hours. Protein and RNA samples taken from 8 concentrations across the dose range.

Transcriptional pathways evaluated via Qiagen RT² profiler PCR arrays (84 genes specific to cardiotoxicity).

Proseek Multiplex CVD 1 protein biomarker panel (Olink bioscience, Sweden) used to evaluate 92 proteins attributed to cardiovascular disease.

Cell toxicity and stress markers measured via high content imaging (Cyprotex, UK) including ATP, mitochondrial function and micro-tissue mass.

• MIE for CPA not completely understood but thought to be via generation of ROS.
• In Vitro studies normally need to use 5-9 to see effect of metabolism on CPA toxicity.
• CYP2B6 is inducible in cardiac tissue.²

CPA induced cardiotoxicity is detected at sub toxic levels via gene/protein expression

• Data is from preliminary studies [single experiment, multiple replicates] with a broad spread of doses spanning several orders of magnitude however there are pathways that appear to be altered after treatment with CPA that are linked to toxicity (measured by physical changes in micro-tissue size and mitochondrial mass).
• Changes in gene and protein levels are seen at doses below those where toxicity starts to manifest [reduction in mitochondrial mass] that are consistent with a ROS-mediated mechanism.
• The 3D cardiac model shows enhanced predictivity over 2D equivalents for detection of structural cardiotoxins including CPA, possibly a consequence of more in vivo like biology. (i.e. cardiac specific CYP’s).
• Informatics approaches should help link MIE’s to pathways of toxicological concern. For an MIE to be relevant to human health it needs to be implicated with a biological effect. Interrogating gene and protein level data will enable pathway networks to be constructed that help predict the likely biological effect of chemical interaction with target cells/tissue.

References
4. JM Patel, metabolism and pulmonary toxicity of cyclophosphamide 1999 Pharmac. Ther., 2, 107-146
5. Stocker et al. Cardiac changes with Cyclophosphamide 1991 Pediatric blood and cancer 8 417-423

AOP Framework

16 proteins associated with CVD

Change in mitochondrial mass and micro-tissue size from cell tox marker panel

TOXICITY MEASURED BY REDUCTION IN MICRO TISSUE SIZE OR MITOCHONDRIAL MASS (REDUCTION OF MICRO TISSUE SIZE SHOWN ABOVE)

Changes to the expression of 10 genes associated with cardiotoxicity

Molecular Initiating Event
Organotypic Molecular Assembly Effects
Cellular Effects
Tissue Effects
Organ Effects
Organ Systems Effects
Individual Effects
Population Effects
Community Effects
Tissue
Exposure
Environmental Contamination
Source
SAFETY & ENVIRONMENTAL ASSURANCE CENTRE

SAFETY SCIENCE IN THE 21ST CENTURY
For more information visit www.hse1.org

CYP2B6 is inducible in cardiac tissue.