Assessment of mitochondrial activity in 3D Human Liver Microtissues as a tool for mechanistic investigations of adverse drug effects

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Abstract
Mitochondrial dysfunction is a major mechanism of drug-induced liver injury, which can be induced through a parent drug or reactive metabolites generated through cytochrome P450 activity. Until now, most existing cell-based models utilized 2D-cultured hepatocytes, which exhibit only little metabolic competence over long-term culture and thus are only suitable for short-term drug exposures. Here, we investigated whether 3D human liver microtissues would be a useful tool for assessment of mitochondrial activity, as these tissues exhibit preserved metabolic functions, enabling repeat-dose, long-term toxicity testing. We utilized the Agilent XP96 platform to assess the mitochondrial oxygen consumption rate (OCR). We found that 3D human liver microtissues exhibited an increased spare respiratory capacity (SRC) in comparison to 2D culture. Moreover, a panel of potent mitotoxic drugs, including Amiodarone, Diclofenac, and Troglitazone, and non-mitotoxic drugs including Bosentan, Enacapone, and Kelmatsam, was tested. Mitotoxic drugs showed a dose-dependent decrease in SRC, before the cellular viability was affected, whereas for non-mitotoxic drugs this was not observed. This suggests that measurement of mitochondrial activity is a valuable tool to detect drugs with potential mitochondrial liabilities and is therefore a powerful tool for mechanistic investigations of adverse drug effects.

Mitochondrial Bioenergetics 2D vs. 3D cultured PHH

Figure 1: 3D InSight™ Mitochondrial Toxicity Assay Workflow. The two-step microscopy assay workflow consists of drug exposure (typically 2 days, up to 14 days possible) in GravityTRAP™ Spheroid Microplates followed by a discrete transfer of microtissues to the Agilent analysis platform (XFe96 Analyzer). After normalization to the baseline, an approximately 4-fold greater OCR was obtained compared to 2D cultured PHH after 2 days of treatment with a 100 µM concentration of Ximelagatran.

Table 1: Summary of compounds analyzed and classified using the 3D InSight™ Mitochondrial Toxicity Assay. 3D InSight™ Human Liver Microtissues were exposed to different drugs of various drug classes for 48 h. After determination of the viability (CellTiter-Glo® Assay) and the IC50SRC values (XFe96 Analyzer), the classification scheme (see Figure 5) for identifying mitochondrial liability was applied.

Conclusions
Investigating mitochondrial impairment in a physiologically relevant environment requires in vitro liver models that reflect in vivo function, long-term metabolic activity and functionality, and compatibility with state-of-the-art analytical instruments. The 3D InSight™ Mitochondrial Toxicity Assay described herein combines 3D human liver microtissues with OCR analysis to provide a novel platform for assessment of mitochondrial liabilities.

The assessment of viability and spare respiratory capacity after compound treatment allowed detection of 7 out of 8 compounds (88% sensitivity), which were known to be associated with mitochondrial impairment. The assay correctly predicted 5 out of 5 non-mitotoxic compounds (100% specificity).

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References