Evaluating Mitochondrial Dysfunction and Hepatotoxicity in HUREL Micro Liver Co-cultures via Mito Tox Index and Cell Index

Yoonseok Kam[1], Lisa Winer[1], Cheul H. Cho[2], Xiaoyu Zhang[1], Natalia Romero[1]
[1]Agilent Technologies, Inc., MA, USA, [2]Visikol, Inc., MA, USA

SOT 2024

Agilent Trusted Answers

Introduction

An accurate *in vitro* prediction model for hepatotoxicity screening during early drug discovery is highly desirable to mitigate potential post-market drug withdrawal risks.

The HUREL Micro Liver, which is a self-assembling co-culture of primary hepatocytes and a stromal cell line, has been demonstrated to be an enduring, phenotypically stable, and metabolically competent form of liver cell culture.[1]

Using the Seahorse XF Mito Tox Assay kit in conjunction with the Seahorse XF Pro Analyzer and dedicated software features enables streamlined, sensitive detection and characterization of mitochondrial toxicants. It delivers a standardized quantitative parameter, the Mito Tox Index (MTI), derived from the oxygen consumption rate (OCR) through a customized software tool, Seahorse Analytics.[2,3]

The Cell Index (CI) from Agilent xCELLigence RTCA provides real-time, quantitative information about the status of the cells, including cell number, viability, and morphology. It is a powerful tool for real-time monitoring the effects of drugs on cell health and viability.[4]

This study aimed to optimize the drug safety test workflow using HUREL micro livers on two Agilent cell analysis platforms: Seahorse XF Pro and xCELLigence RTCA Analyzer.

Assay Design

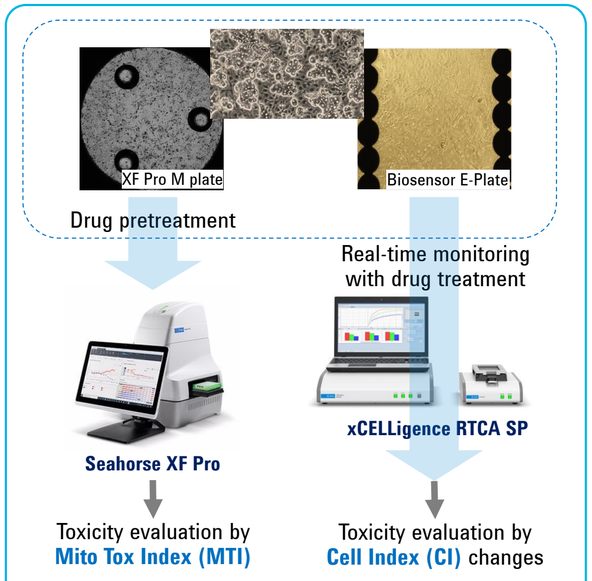

Figure 1. HUREL co-culture on Agilent cell culture plates

HUREL co-culture can be set up on two types of Agilent assay plates, Seahorse XF Pro M plates and xCELLigence E-plates to assess the mitochondrial function and cell viability.

Cells are exposed to test drugs for hour to days and the mitochondria function is measured by Seahorse XF Pro. The mitochondrial toxicity is quantitatively assessed by MTI calculated in Seahorse Analytics.

The CI indicating cell viability can be monitored in real-time manner from HUREL co-culture on E-plate by xCELLigence RTCA analyzer in the presence or absence up to 10 days.

Results and Discussion

Optimization of Seahorse XF Mito Tox assay for HUREL micro liver model

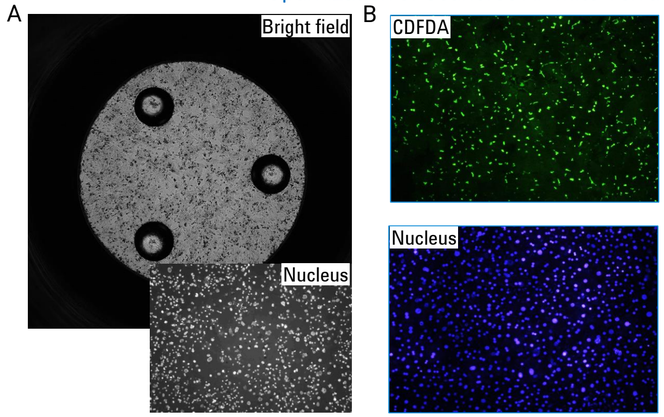

Figure 2. HUREL co-culture on Agilent cell culture plates

The cell density of HUREL co-culture optimized for the Seahorse XF analysis and for the functional bile canaliculi formation.

A. A representative image of HUREL co-culture supporting the optimal oxygen consumption rate measurements.

B. A representative image displaying the uniform formation of bile canaliculi (CDFDA) on a Seahorse XF Pro M plate.

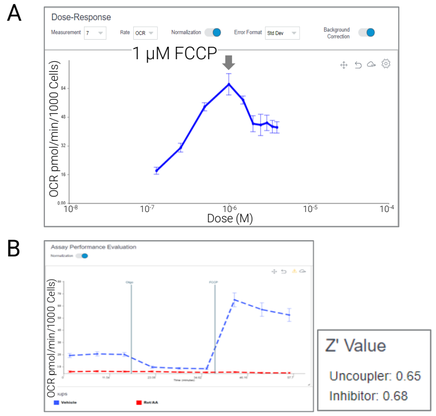

Figure 3. Seahorse XF Mito Tox assay optimization and evaluation

A. The optimal FCCP concentration for the XF Mito Tox assay was identified as 1 µM using HUREL co-culture.

B. XF Mito Tox assay using the HUREL co-culture was validated as a high-throughput assay (Z' > 0.5) in screening both inhibitor and uncoupler.

XF Mito Tox Assay Data

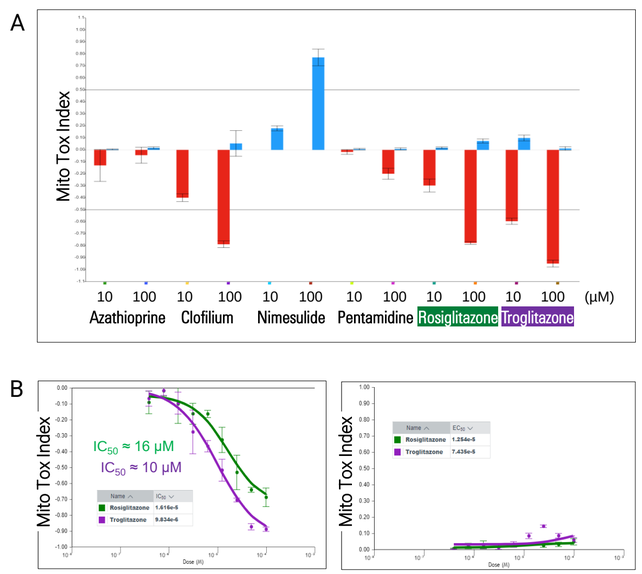

Figure 4. Mitochondrial toxicity test

HUREL co-culture were exposed to 6 model drugs for 1 hour at two different doses, 10 µM and 100 µM.

A. Clofilium, Rosiglitazone and Troglitazone were identified as potent mitochondrial inhibitors and Nimesulide was as a uncoupler.

B. The dose-dependency assay enables the comparison of quantitative toxicity by IC_{50}. Troglitazone is a more potent inhibitor than Rosiglitazone. No significant uncoupling was detected from both drugs.

xCELLigence RTCA Data

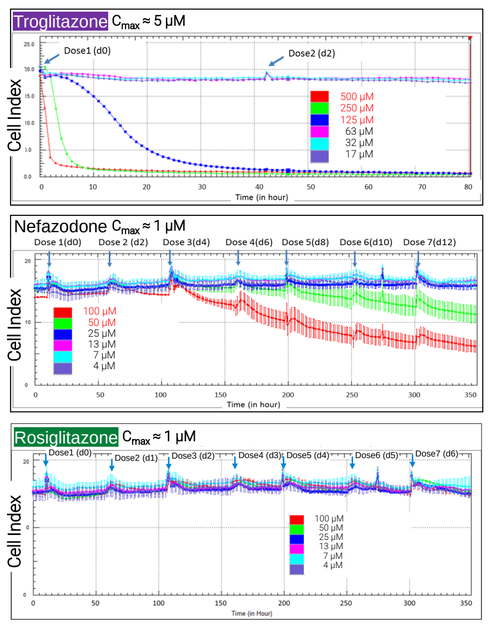

Figure 5. Differential cytotoxicity induced by mitochondrial drugs.

The cytotoxicity of 3 drugs, known to cause mitochondrial dysfunction, was evaluated. In contrast to Troglitazone, which showed cytotoxicity within 24 hours at 25x C_{max} (125 µM) or higher, Rosiglitazone did not exhibit any *in vitro* hepatotoxicity for 7 days, even at 100x C_{max} (100 µM). Nefazodone demonstrated moderate cytotoxicity at 50x C_{max} (50 µM) or higher, but only after 3 days of drug treatment.

Summary

The Agilent Seahorse XF Pro and xCELLigence RTCA platforms have been confirmed to work effectively with the HUREL micro liver co-culture model for evaluating mitochondrial toxicity and cytotoxicity. The Seahorse XF Mito Tox assay, which uses an optimal seeding density and FCCP concentration, makes high throughput screening possible.

The XF Mito Tox assay was able to identify drugs that cause mitochondrial dysfunction in the HUREL micro liver, including two anti-diabetic thiazolidinediones, Troglitazone and Rosiglitazone. Troglitazone was removed from the US market due to hepatotoxicity, while Rosiglitazone was withdrawn from the EU market due to cardiotoxicity.[5,6]

The xCELLigence RTCA platform's multi-day live cell analysis workflow allows for the assessment of temporal cytotoxicity. Of the three drugs identified to be mitochondrially toxic, Troglitazone showed significant hepatotoxicity at 25x C_{max} (125 µM, 12.5x MTI IC_{50}) or higher while Rosiglitazone did not show any hepatotoxicity up to 100x C_{max} (100 µM, 6x MTI IC_{50}).

Even though both Troglitazone and Rosiglitazone can cause significant mitochondrial dysfunction in the HUREL co-culture at 100 µM, no significant cytotoxicity was detected up to 6x MTI IC_{50}, 60 µM (12.5x Cmax) for Troglitazone and 96 µM (100x Cmax) for Rosiglitazone.

In conclusion, the Agilent Seahorse XF Pro and xCELLigence RTCA platforms have proven to be effective tools for providing precise and quantitative data on in vitro mitochondrial toxicity and hepatotoxicity using the HUREL micro liver system. In this study, Troglitazone was identified as a potent drug inducing significant hepatotoxicity and strong mitochondrial toxicity. On the other hand, while Rosiglitazone was found to cause mitochondrial dysfunction, its risk of hepatotoxicity was determined to be low.

References

1. https://visikol.com/blog/tag/hepatoxicity/
2. Rogers *et al.* Principle of Mitochondrial Toxicity Assessment Using Agilent Seahorse XF Solution Agilent Technologies white paper, publication number 5994-4732EN, 2022
3. Kam *et al.* A Customized XF Workflow for Detection and Characterization of Mitochondrial Toxicity. Agilent Technologies, application note, publication number 5994-4778EN, 2022
4. https://www.agilent.com/en/solutions/cell-analysis/cell-health-viability/real-time-cytotoxicity-assays
5. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Troglitazone. [Updated 2018 Jun 6]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK548142/
6. LiverTox: Clinical and Research Information on Drug-Induced Liver Injury [Internet]. Bethesda (MD): National Institute of Diabetes and Digestive and Kidney Diseases; 2012-. Rosiglitazone. [Updated 2018 Jun 6]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK548114/