Hepatotoxicity: Measuring drug-induced mitochondrial toxicity in HepaRG™ cells using the Mito-ID® Extracellular O_2 Sensor Kit.

Mito-ID® Extracellular O_2 Sensor Kit (High Sensitivity) ENZ-51045

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INTRODUCTION

Cell type choice is a critical parameter in the design of an in vitro toxicological assessment. For hepatotoxicity, the benchmark is primary human hepatocytes; however, these cells are associated with the obvious limitations of access, lot-to-lot variability and cost. Cell lines such as HepG2, THLE and Fa2N-4 are therefore commonly used as a cheaper, more accessible alternative but these too are associated with significant limitations as they lack critical enzyme function such as CYP and transporter activity.

The **HepaRG™** cell type (www.heparg.com) is an appealing alternative as it addresses a number of these limitations. It is a bi-potent hepatic progenitor cell line derived from an hepatocholangiocarcinoma (1) and differentiates into hepatocyte-like and biliary-like cells (2-3). The cells are received in cryopreserved format as a terminally differentiated co-culture and maintain many key primary human hepatocyte characteristics including CYP, MAO and specific transporter activity (4-5).

Oxygen consumption can be assessed with HepaRG™ cells using the **Mito-ID® Extracellular O_2 Sensor Kit (High Sensitivity)** (ENZ-51045, Enzo Life Sciences). Analysis is conducted on a standard 96-well plate format and facilitates a detailed analysis of the mitochondrial function. These cells are a superior model for the assessment of **drug-induced mitochondrial dysfunction** as they offer the capability to assess CYP and transporter mediated toxicity which is not possible with the standard alternative cell lines.

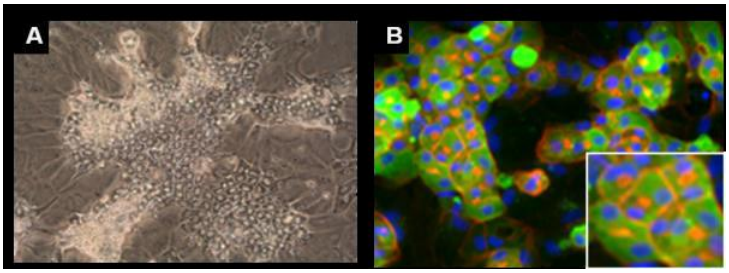

Figure 1: A) Phase contrast image of terminally differentiated after 15 days of culture and B) Immunofluorescence of HepaRG™ cells showing F- actin and CYP3A4 co-localization.

MATERIALS AND METHODS

HepaRG™ cells

The differentiated HepaRG® cells (www.heparg.com) are provided frozen in cryo vials at ≥ $8x10^6$ viable cells per vial and are applied to collagen coated 96-well microtiter plates as per manufacturer's instructions. Specific supplemented media (www.heparg.com) is required for different aspects of culturing

these cells which are provided: basal hepatic cell medium with either (i) the Thaw, Seed, and General Purpose supplement added or (ii) with Maintenance and Metabolism supplement added. These cells can be measured both in suspension immediately post thaw or as a monolayer either 4 hours or 4 days post thaw. The cells are prepared and added to the plate in the same way for both measurement types intended.

Plate preparation

- Use the provided 96-well microtiter plates which are pre-coated with collagen I (www.heparg.com).
- Thaw and count the viable differentiated HepaRG™ cells as per manufacturer instructions, adjusting to ~4.8x10^5 cells/ml and adding 150µl to each well.

Plate Measurement

Follow manufacturer's protocol for probe preparation, addition and measurement. The following protocol is applicable for the measurement of cell suspensions or for cell monolayers 4 hours post seeding.

- Prepare a Mito-ID® Sensor Probe stock in 1ml of pre-warmed Thaw, Seed, and General Purpose Medium 670.
- Add 10µl of this solution to each well containing 150µl of cells and include a 'no-cell' control containing only media.
- Add 1µl of compound stock (150X) to each well and ensure to include untreated samples.
- Seal the plate by overlaying with pre-warmed HS mineral oil, 100µl per well. This is best done using a repeater pipette.
- Measure 96-well plate kinetically for 90-120mins with ~2 minute interval (Ex/Em 380nm/650nm).

RESULTS

Oxygen profiles are presented in Figure 2 for HepaRG™ cells measured in suspension on a FLUOstar Omega plate reader (BMG Labtech) as outlined above. Untreated cells show a steady signal increase during measurement reflecting the depletion of oxygen caused by the activity of the electron transport chain (ETC).

When ETC activity is inhibited through treatment with complex III inhibitor Antimycin, no signal increase is observed. When ETC is uncoupled via FCCP treatment, a more rapid rate of signal change is observed.

Rates of signal change were calculated over the linear portion of the curve for each sample (~5-30mins), and can be used for the assessment of replicate statistics or the generation of dose response data. Antimycin A, Nefazodone dose responses are presented in Fig 2B and 2C.

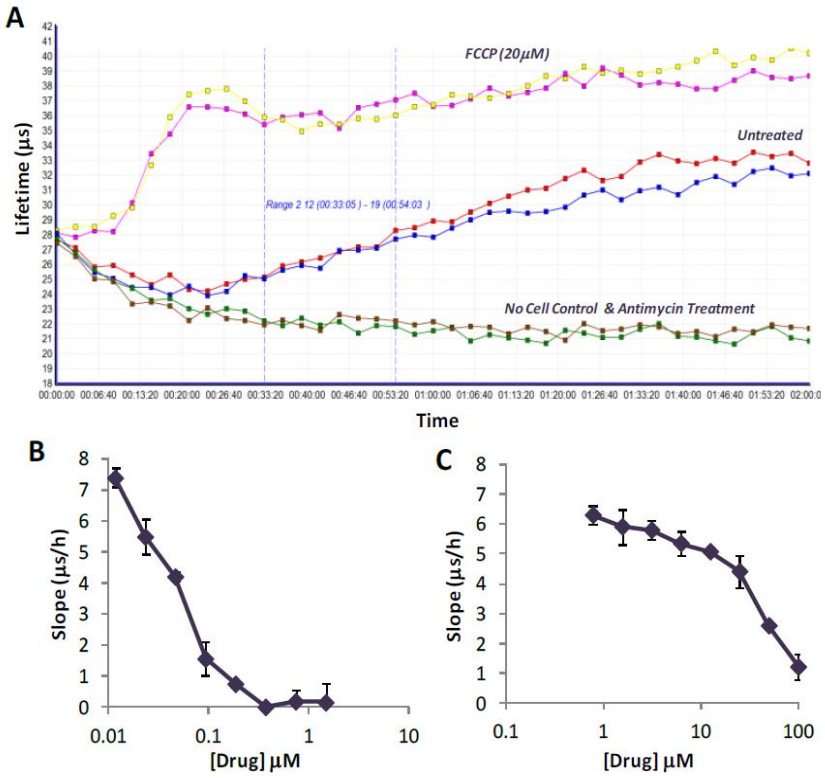

Figure 2: Kinetic Profiles of 0h culture HepaRG™ cells treated with , FCCP and Antimycin A (A) and dose response analysis of the ETC inhibitor Antimycin (B) and the piperazine Nefazodone (C). Measurement performed immediately post treatment.

CONCLUSION

The data demonstrates the capability of Mito-ID® Extracellular O_2 Sensor Kit to detect perturbed mitochondrial function in differentiated HepaRG™ cells.

References

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