Cell-based screening of drug-induced metabolic dysfunction

Introduction

Recent years have seen a growing appreciation for the importance of the mitochondrion as a site for off-target effects of drug therapy [1]. This is particularly relevant in drug-induced liver injury (DILI) and is unsurprising considering both the multitude of sites where mitochondrial function can be perturbed and the deleterious consequences of such perturbation.

There is a need therefore for more physiologically relevant data-rich in vitro approaches that facilitate the early detection of such mitochondrial liabilities. This is driving the continued development of more advanced in vitro models and compatible measurement strategies.

Here we assess the compatibility of such advanced cells models with the MitoXpress Xtra Oxygen Consumption Assay which facilitates direct microplate-based analysis of mitochondrial function measured on a time-resolved fluorescence enabled plate reader.

The assay is successfully applied to both HepaRG cells and to Primary Hepatocytes, while, using the RAFT™ system, mitochondrial function of cells within 3D collagen constructs can be assessed.

To better understand the metabolic consequences of drug treatment, measurements are made under both basal and uncoupled conditions such that any diminution of aerobic metabolic capacity can be better detected.

Metabolism measurements (MitoXpress-Xtra) are also multiplexed with a conventional cell viability assessment (Calcein AM) to determine (i) if a compatible measurement work flow could be developed and (ii) to assess the utility of parallel metabolism and cell viability data in more specifically identifying perturbed mitochondrial function.

The MitoXpress Xtra Oxygen Consumption Assay

The MitoXpress Xtra Assay facilitates the direct microplate-based analysis of mitochondrial function using a time-resolved fluorescence enabled plate reader. Cellular respiration causes a depletion in dissolved oxygen in the test well, which in turn causes an increase in the fluorescence signal of the MitoXpress Xtra reagent. Measuring the rate of signal increase therefore informs on levels of cellular respiration.

Measuring Mitochondrial Function in Advanced Cell Models

Using more advanced cell models can improve the physiological relevance of in vitro assays, by ensuring that the appropriate CYP or transporters are present (HepaRG, 1st Hepatocytes), or providing more in vivo-like 3 dimensional cell-to-cell interactions (RAFT™).

Multiplexing Cell Metabolism and Viability

A combined analysis of cell respiration and viability offers an opportunity to better characterise the impact of drug treatment on cellular function and may help contextualise observed metabolic perturbations subsequent to long term drug treatments.

Multiplexed MitoXpress Xtra and Calcein AM measurements in HepG2 Cells

Figure 3: HepG2 cells were treated with Antimycin A (1 µM) and FCCP (2.5 µM). (A) A MitoXpress assay was carried out on cells loaded ± Calcein AM (5 µM). (B) Calcein AM fluorescence was measured in cells ± MitoXpress Xtra added to wells. (C) A multiplexed experiment was carried out.

Multiplexed measurement of MitoXpress Xtra and Calcein AM did not adversely impact MitoXpress (Figure 3A), or Calcein AM (Figure 3B) assay outputs. Specific mitochondrial insult (Antimycin A) blocks aerobic metabolism but has no immediate effect on cell viability. Non-specific insult (detergent) impacts both cell respiration and viability (Figure 3C).

Mitochondrial toxicity and differential dose response

Figure 4: Multiplexed assay showing mitochondrial perturbation and cytotoxicity. Cells were treated with Antimycin A (A; ~0.5h) and Tamoxifen (B; 24h). MitoXpress Xtra was measured post treatment in Calcein loaded cells. Calcein data was acquired prior to MitoXpress Measurement. Differential dose responses reveal a specific metabolic perturbation for Antimycin treatment (Figure 4A), also evident for longer treatment periods (not shown), while the Tamoxifen response (Figure 4B) is more consistent with a multifactorial or non-specific insult.

Short term and longer term treatments

Figure 5: HepG2 cells were treated Nefazodone (A, C) or Flutamide (B, D) for 1h (A, B) or 48h (C, D). Multiplexed MitoXpress Xtra and Calcein AM measurements were performed post treatment.

Short term (1h) treatments with Nefazodone and Flutamide caused significant mitochondrial impairment without negatively impacting cell viability (Figure 5A/B), while longer term treatments (48h) impacted both parameters (Figure 5C/D). Differential responses are still evident however, particularly for Flutamide, suggesting a significant mitochondrial component to the toxic response. This is consistent with literature reports.

Conclusions

Measuring cell respiration is a more direct and sensitive method for detecting drug-induced mitochondrial dysfunction than conventional cell viability measurements.

Analysis of maximal respiration can reveal drug-induced erosion of mitochondrial capacity not readily apparent when assessing basal respiration alone.

Compatibility with advanced cell models and specific 3D culture methods enables long term treatments and sensitises measurements to CYP or transporter mediated metabolic perturbation.

Measurement of aerobic metabolism (MitoXpress Xtra) and cell viability (Calcein AM) can be combined into a single workflow, generating dual-parameter toxicity data from a single test well.

This approach helps to contextualise observed metabolic perturbations subsequent to long term treatments and can provide additional mechanistic insights.

References


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