Introduction

Cardiotoxicity: Cardiotoxicity and related cardiac impairment remains one of the main reasons for both drug withdrawal [1] and FDA black box warning [2] and are a significant cause of compound attrition in preclinical development.

ATP Demand and Mitochondria: Cardiac tissue requires an uninterrupted supply of respiratory substrates to meet the very high ATP demand imposed by continuous beating. Over 95% of this ATP is generated by oxidative phosphorylation (OxPhos) with the necessary mitochondrial network being up approximately one third of cardiomyocyte cell volume. Energy starvation and mitochondrial dysfunction are therefore significant factors in the progression of cardiotoxicity and, detection of such metabolic dysfunction is an important aspect of cardiotoxicity screening. This is best achieved by monitoring the two main ATP generating processes; OxPhos and Glycolysis (Fig. 1).

Mitochondrial Dysfunction & Contractility: In vivo, the most important respiratory substrates for ATP production are pyruvate and fatty acyl CoA. However, cardiomyocyte metabolism is particularly adaptable and substrates such as amino acids, lactate and ketone bodies can also be used. Examples of the adaptability include HIF mediated metabolic responses to hypoxia and ischemia, and a shift from fatty acid oxidation (FAO) to glucose metabolism that occurs in hypertrophic cardiac tissue. These adaptations highlight the importance of information on substrate preference and oxygenation when designing and interpreting in vitro cardiomyocyte analyses.

Conclusions

- Due to the dual-read TRF measurement approach used, Mitoxpress-Xtra based measurements of O2 consumption and pH-Xtra based measurements of ECA can be performed on xCELLigence E-plates (ACEA) using conventional TRF plate readers.
- This allows contractility and cell metabolism measurements to be performed in sequence on the same test plate.
- The combined use of microplate-based contractility and metabolism measurements have been demonstrated as an mean to generate a more complete picture of cardiomyocyte response to drug treatment and allows the delineation of inter-relationships between cardiomyocyte beating and cell metabolism.
- Complete impairment of OxPhos through treatment with ETC inhibitors did not immediately impair Cor4.4 cardiomyocyte beating. Increased ECA suggests that ATP supply is maintained through increased glycolytic flux allowing beating to continue for >24 h post treatment.
- The β-adrenoceptor agonist isoproterenol increased beating rate and caused a significant increase in O2 consumption but little change in ECA. This suggests that increased ATP demand is being met through OxPhos rather than glycolysis.
- This combined analysis of critical cardiomyocyte functions therefore delivers a more holistic and informative in vitro cardiotoxicity screen in that it related cellular function to the metabolic activity driving that function. In so doing it provides additional mechanistic information as to the cause of observed alterations in cardiomyocyte metabolism or contractility.

Methods

Preparation:

Cor4.4 cells (Axiogenesis) were plated onto fibronectin coated 96 well plates and placed in culture for 2-3 days, performing media changes as per manufacturer instructions. Cells were plated at 4-5x10^4 cells/well for pH-xtra and Mitoxpress-Xtra assay.

Measurement:

Mitoxpress-Xtra (HS method): Fresh media containing Mitoxpress® reagent (Luxcell Biosciences), 150 µl/well was added prior to measurement. Compounds were added directly and all wells were sealed with pre-warmed HS oil. Plates are measured at (37°C) for 2.5-3.5 hour kinetically (Ex580nm, Em550nm and dual-read TRF-F lifetime measurement - FLUOstar Omega, BMG Labtech).

pH-Xtra Glycolysis measurement: The sample plate is placed in CO2 free incubator 3 hours prior to measurement, in order to reduce CO2 depletion around the 3 wells. Respiratory buffer (20 mM phosphate) prepared using the buffer tablet provided. 110 µl of Respiratory buffer containing pH-Xtra® reagent (Luxcell Biosciences) was added to sample wells. Compounds were added directly, and the plate was measured kinetically for 2.5 hours, on a pre-warmed plate reader (37°C). (Ex580nm, Em550nm and dual-read TRF-F lifetime measurement - FLUOstar Omega, BMG Labtech).

Mitoxpress intra-trace measurement: Cells were loaded with Mitoxpress intra-trace (Luxcell Biosciences) overnight (14 hours) in 96-well plate the day prior to measurement. Cells are washed twice and 150 µl of fresh media was added. The plate was measured kinetically at 37°C. (Ex580nm, Em550nm and dual-read TRF-F FLUOstar Omega with ACU, BMG Labtech).

xCELLigence RTCA Cardio measurement: IPS-Cardiomyocytes were plated on 96 well E-Plates and impedance measurements were recorded at set time points (50s sweep at a sampling rate of 77 Hz). Drug treatment was initiated once the signal showed 40-60 synchronous beats/min. The data were normalized to baseline beating rate.

References