Multimodal Connected Workflow for Cardiotoxicity Assessment Using Human iPSC-Derived Cardiomyocytes

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Introduction

Drug-induced cardiac toxicities, including structural impairment, adverse contractile modulation, and life-threatening polymorphic ventricular tachyarrhythmia remain some of the main reasons for both drug withdrawals and FDA black box warnings. Also, increasing evidence points to the fact that the mitochondria can be a site of off-target effects of drug therapy, resulting in late-stage attrition, black box warnings, and market withdrawals. Cardiomyocytes derived from human induced pluripotent stem cells (iPSC) have been proven extremely versatile for in vitro cardiotoxicity applications. Here, we demonstrate the feasibility of a combined workflow using cell analysis technologies from Agilent Technologies to assess cardiac viability and function using iCell® Cardiomyocytes[2] (FUJIFILM CDI).

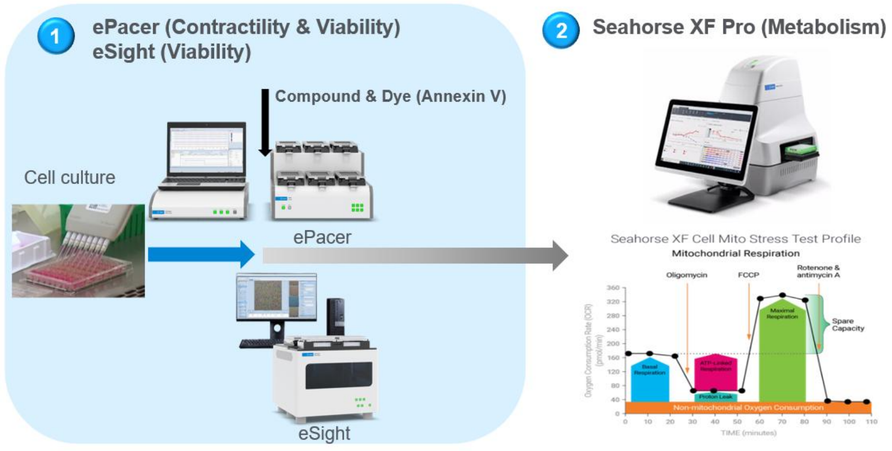
To have a multifaceted interrogation of the cardiac liability of potential drug candidates and increase the effectiveness of cardiac safety screening, a multimodal workflow for cardiac safety and toxicity screening using iCell Cardiomyocytes2 (iCM2) was developed. iCM2 were plated in 96-well Agilent E-Plates and their viability and contractility were assessed in real-time on the Agilent xCELLigence RTCA ePacer (RTCA ePacer) throughout the drug treatment. Concurrently, apoptosis was monitored using a fluorescent marker and live-cell imaging on an Agilent xCELLigence RTCA eSight (RTCA eSight). In parallel, the effect of compounds on mitochondrial function was investigated using an Agilent Seahorse XFe96 analyzer.

Experimental

Cardiomyocytes:

iCell cardiomyocytes[2] (FujiFilm Cellular Dynamic International) are derived from human iPS cells. They are a mixture of spontaneously, electrically active atrial, nodal, and ventricular-like myocytes.

Multimodal workflow for Cardio safety/toxicity assessment



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Results and Discussion

Validation of multimodal workflow using well-known compounds

System	Measurement	Compounds		
		Sofosbuvir (0.1-30 µM)	**Sunitinib** (0.1-10 µM)	**Doxorubicin** (0.1-10 µM)
ePacer	Contractility	No	Yes Decrease BR/increase BP Decrease BAmp (10 µM) Induce arrhythmic beating	Yes Increase BR(>=0.3 µM) Decrease BAmp(>=0.3 µM)
ePacer	Viability (overall CI)	No	Yes Decrease CI	Yes Decrease CI
eSight	Apoptosis	No	Yes	Yes
XF Pro	Mitochondrial Function	N/A	Yes Decrease OCR (10 µM)	Yes Decrease OCR

Table 1. Summary of results on cardiac safety/toxicity assessment using the multimodal workflow. BR: beating rate; BP: beating period; BAmp: beating amplitude.

Cardiac safety/toxicity evaluation of Remdesivir using multimodal workflow

1. Remdesivir's negative impacts on cell viability and contractility observed by RTCA ePacer

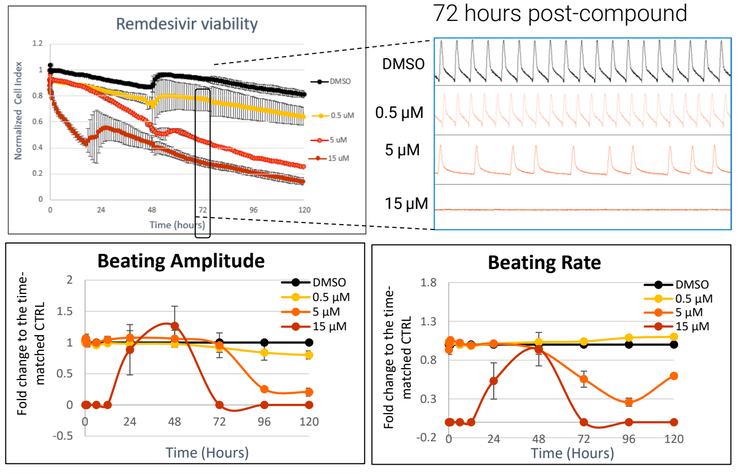

Figure 1. Cardiac safety/toxicity assessment of remdesivir using the Agilent xCELLigence RTCA ePacer. (A) The kinetic changes of overall CI during remdesivir treatment. (B) Thirty seconds of impedance waveforms were obtained at 72 hours after remdesivir exposure. C) The time course of contractile responses of hiPSC-CMs to the drug, including the fold change of beating amplitude and beating rate to the time-matched control (CTRL), and BRI. Except for BRI, the data were represented by mean ±SD, N ≥3. Q: cell quiescence.

Results and Discussion

2. A reduction in cell viability caused by Remdesivir was also detected using RTCA eSight

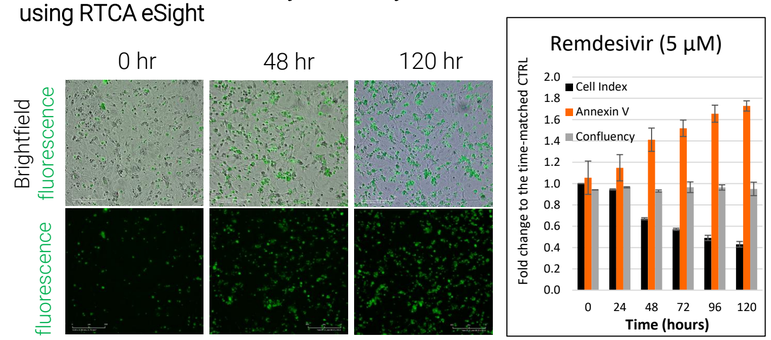

Figure 2. Kinetic assessment of cell viability and apoptosis after remdesivir addition using the Agilent xCELLigence RTCA eSight. (A) Image panels demonstrate the progression of apoptosis 48 and 120 hours after treating hiPSC-CMs with 5 µM of remdesivir. (B) Tracking of apoptosis induced by 5 µM of remdesivir using the fold change of the number of annexin V-positive events, cell confluency, and CI to the DMSO control (CTRL).

3. 24-hour pretreatment of Remdesivir reduced mitochondrial respiration

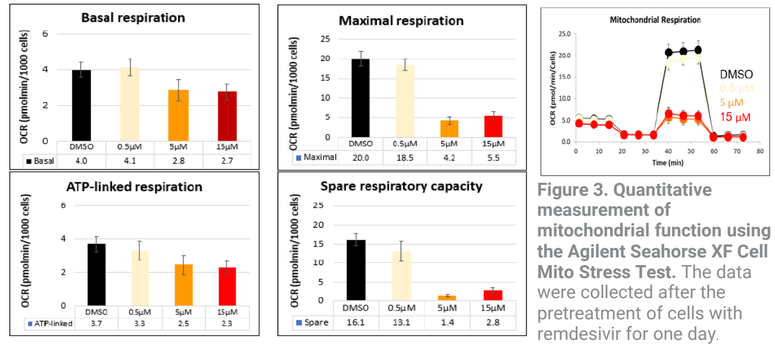

Figure 3. Quantitative measurement of mitochondrial function using the Agilent Seahorse XF Cell Mito Stress Test. The data were collected after the pretreatment of cells with remdesivir for one day.

Conclusions

1. Sofosbuvir, a cardiac-safe drug, displayed low cardiac risk.
2. Sunitinib and doxorubicin displayed the expected mitochondrial and contractility dysfunction, and cellular toxicity.
3. Remdesivir induced progressive and dose-dependent decline in overall Cell Index and disruption in beating rhythm and amplitude with effects observed after 48 to 80 hours.
4. Remdesivir's inhibition on mitochondrial respiration was observed at 24 hours posttreatment.
5. The multiparametric workflow allows for evaluating contractility, viability, and metabolic function using validated iCell Cardiomyocytes2 to better understand the mechanisms associated with drug-induced cardiotoxicity.