Investigating the Effects of Mitochondrial Inhibitors and Uncoupler on hiPSc-CM MEA Assay to Predict Drug-induced Cardiotoxicity

Shuya Wang, Matthew Cato, Patricia A. Valentine, Prasad Purohit, Anthony Bahinski, Khuram W. Chaudhary

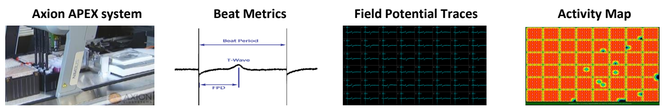
GlaxoSmithKline- Safety Pharmacology, Collegeville, PA, 19426 USA

Introduction

Mitochondria play a significant role in cardiac excitation–contraction coupling by providing the energetics for ATP-dependent processes. Drug-induced mitochondrial toxicity remains an important hurdle in preclinical development. Human inducible pluripotent stem cell-derived cardiomyocytes (hiPSc-CMs) have emerged as a predictive tool to detect proarrhythmic risk, but have not been exploited to interrogate mitotoxicity. We hypothesize that hiPSc-CMs could detect mitotoxicity acutely by changes electrophysiological properties, including beat rate. Methods: In this study, N=4-8 cells per concentration. 3 electron transport chain inhibitors: Rotenone (0.01, 0.037, 0.11, 0.33 and 0.1µM), Antimycin A (0.01, 0.037, 0.11, 0.33 and 0.1µM), Oligomycin A(0.037, 0.11, 0.33, 1 and 3µM); and 1 uncoupler, DNP (0.37, 1.11, 3.33, 10 and 30µM) were tested for potency against hERG, Nav1.5, and Cav1.2 along with evaluation in the hiPSc-CM multi-electrode array assay (Axion BioSystems). MEA assay endpoints included: Total Spike Amplitude (TSA), Field Potential Duration (FPD) and Beat Rate (BR), which are surrogates for QRS interval, QT interval and heart rate, respectively. Results: 0.1% DMSO (Vehicle) produced a time-dependent slowing of beat rate from 1 hour through 72 hours. Rotenone and Antimycin both produced a concentration-dependent slowing of BR at 1 hour of exposure, with no significant change in FPD or TSA. Unlike Antimycin, the effects of Rotenone were additive to time-dependent slowing induced by vehicle. Oligomycin caused a cessation of beating whereas DNP produced little effect on any measured parameter. Ironically, changes in BR caused by Rotenone and Antimycin were not associated with prolongation of FPD; a common property of the human electrocardiogram and QT interval. Conclusion: Our findings indicate that electron transport chain inhibitors may produce acute changes in spontaneous beat rate of hiPSc-CMs. While additional studies are necessary, electrophysiological properties measured on the MEA platform may serve as a surrogate to predict mitotoxicity, as the observation of changes in beat rate was dissociated from field potential duration prolongation.

Methods

hiPSc-CM Multi-electrode array (MEA)



Day 7 - 1 min recording	4 hours serum free media	1 min baseline recording	1 hour compound treatment	1 min recording

- Inducible pluripotent stem cell derived cardiomyocytes (hiPSc-CMs; iCell²; Fuji/CDI).
- Maestro multi-electrode Array System (Axion BioSystems; 48-well plate).
- Cells were maintained in culture medium for 7 days.
- 1 min recording of activity was taken daily starting on Day 3 through Day 7.
- Full media changes were done every 48 hours.
- 50,000 cells/well, N=8/concentration, 5 concentrations, 1 hour compound treatment time.
- 3µM Quinidine as control for hERG and hNaV1.5 block.

Materials

Mitochondrial inhibitors and uncoupler

Complex	I	III	V	Uncoupler
Effected Toxicity	Rotenone	Antimycin A	Oligomycin A	DNP
Concentration range	0.05µM	0.006µM	0.01µM	30µM
*From publifications	Concentration range impacting Mito-function			

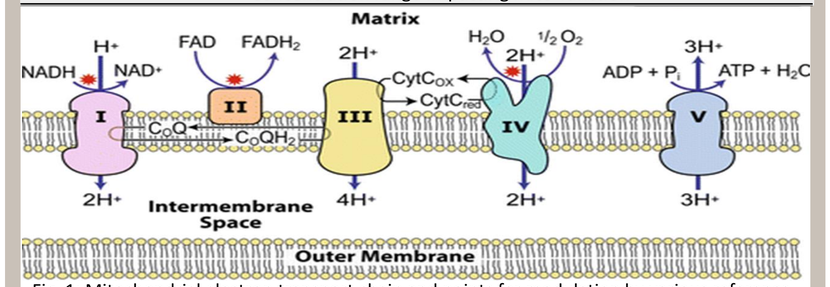


Fig. 1: Mitochondrial electron transport chain and points for modulation by various reference compounds including, Rotenone, Antimycin A, Oligomycin A and DNP

Results

hiPSc-CM MEA assay endpoints: Field Potential Duration (FPD), Total Spike Amplitude (TSA) and Beat Rate (BR)

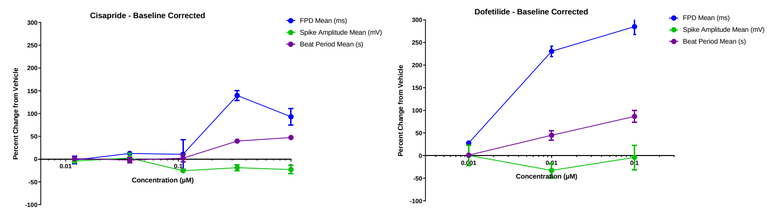
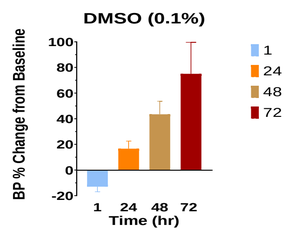


Fig. 2: hERG blockers (Cisapride and Dofetilide) prolong field potential duration with subsequent slowing of spontaneous beat rate.

Vehicle-dependent effects on MEA parameters



Summary

	Conditions (hrs.)			
	1	24	48	72
BP	no effect	17%	44%	81%
FPD	no effect	no effect	no effect	no effect

Fig. 3: 0.1% DMSO (vehicle) exposed controls produced a time-dependent slowing of beat rate without prolongation of field potential duration.

Results

Effects of mito-toxicants on MEA parameters

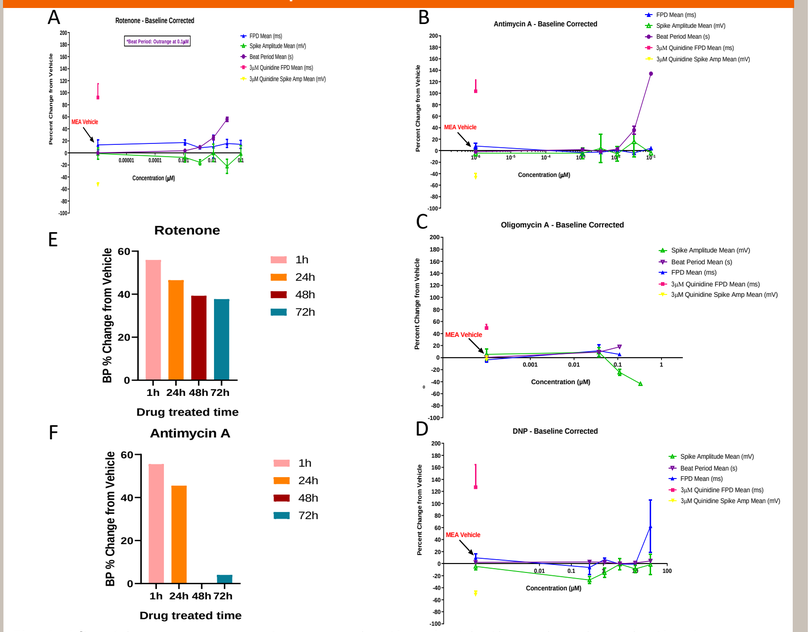


Fig. 4: After 1 hour exposure, rotenone and antimycin A both produced a reduction in spontaneous beat rate that was dissociated from prolongation of FPD (A-D). Oligomycin A produced a cessation of beating at mitotoxic concentrations, while DNP produced no effect. Rotenone produced a sustained chronic slowing of beat rate, while Antimycin A-dependent slowing was normalized to vehicle over time (E-F).

Summary

Mitochondria	Name	Concentrations (µM)	FPD Mean (ms)	Beat Period Mean (s)
Complex I	Rotenone	0.1, 0.33, 0.11, 0.037, 0.011		Increased at ≥ 0.01µM
Complex III	Antimycin A	0.1, 0.33, 0.11, 0.037, 0.011	Cessation of beating at ≥ 0.1µM	Increased at > 0.01µM with cessation of beating at ≥ 0.1µM
Complex V	Oligomycin A	3, 1, 0.33, 0.11, 0.037	Cessation of beating at ≥ 0.3µM	Cessation of beating at ≥ 0.3µM
Uncoupler	2,4 Dinitrophenol	30, 10, 3.33, 1.11, 0.37	Prolongation at ≥ 30 µM	

Conclusion

- hERG blockers prolong hiPSc-CM field potential duration followed by slowing of beat rate.
- Exposure to vehicle (0.1%DMSO) produced a time-dependent slowing of beat rate.
- Rotenone and Antimicin A produce a concentration-dependent slowing of beat rate independent of field potential prolongation.
- Spontaneous beat rate of hiPSc-CMs may be dependent on energy production and modulation of electron transport chain by mitochondrial toxicity may cause a slowing of firing, independent of field potential prolongation.

References

1.Lee S, Lee HA, Choi SW, Kim SJ, Kim KS. Evaluation of nefazodone-induced cardiotoxicity in human induced pluripotent stem cell-derived cardiomyocytes. Toxicology and Applied Pharmacology. 296:42-53 2016

2.Geoff Williams, Gary R. Mirams. A web portal for in –silico action potential predictions. Journal of Pharmacological and Toxicological Methods 75 (2015) 10-16

3.Yosuke Sanuki, Tetsuro Araki, Osamu Nakazono. Kazuyuki Tsurui. A rapid mitochondria toxicity assay utilizing rapidly changing cell energy metabolis. The Journal of Toxicological Sciences 42(3), 349-358 2017

Acknowledgements

The authors would like to thank GSK Investigative Safety for their support