Assessment of drug-induced structural toxicity in human iPSC-derived cardiomyocytes, an image-based approach to complement traditional in vitro safety studies

Ncardia
Stem cell experts

SP092

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BACKGROUND AND PURPOSE OF THE STUDY

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are novel alternatives to classic models in the field of safety pharmacology. Significant progress has already been made towards validating the use of hiPSC-CM electrophysiology assays to predict pro-arrhythmic potential. Yet, for other clinically relevant parameters such as structural toxicity – which could facilitate more accurate in vitro safety assessments – further optimization is still warranted.

This study aimed to optimize a cost-effective, scalable method to detect compound-induced morphological changes using high content imaging in combination with MEA recordings and RNAseq to facilitate more accurate *in vitro* safety assessment.

1 METHODS

Experiment design

Multiple lines of hiPSC-CMs (e.g., Ncyte CMs, NCRM-5), manufactured using Ncardia's proprietary protocols, were cultured according to manufacturer's instructions in chemically defined, serum-free medium. hiPSC-CMs were treated for a period of 24 hours with 7 concentrations of each drug, in a range comparable to the clinical maximum plasma concentrations (c_{max}) followed by functional, mechanistic and morphological assays.

Parameters assessed

- Morphology of subcellular target structures
- Functional changes via multi-electrode array (MEA) field potential recordings
- Gene expression analysis (RNAseq)

Methods

- High-magnification (40x) fluorescence image stacks were acquired using an ImageXpress Micro Confocal microscope. Features such as fluorescence intensity, detected signal area and number of detected objects per cell, were extracted via analysis of 2D image projections using MetaXpress software v6.6
- MEA recordings of electrophysiological alterations including changes in field potential duration (FPD), beat rate (BR) and beat rate irregularity (BRI)
- Mechanism of action of each drug was also investigated via gene expression analysis (RNAseq)

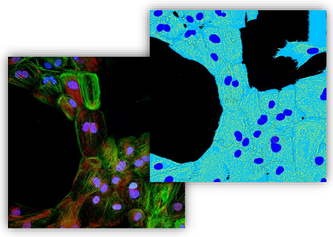


Image analysis. Fluorescent image (right) and corresponding analysis mask (left) for feature extraction.

Expected toxicity	Compound	Main mechanism of action
High (A)	Doxorubicin	DNA Intercalator
	Cisplatin	DNA synthesis inhibitor
	Ponatinib	Tyrosine Kinase inhibitor
	Dasatinib	Tyrosine Kinase inhibitor
	Lapatinib	Tyrosine Kinase inhibitor
	5-Fluoro-uracil	Antimetabolite
	Methotrexate	DNA synthesis inhibitor
Intermediate (B)	Omecamtiv Mecarbil	Cardiac myosin activator
	Propofol	GABAa receptor modulator
	Bupivacaine	Sodium channel inhibitor
	Amiodarone	Class III antiarrhythmic
	Dofetilide	Class III antiarrhythmic
	Digoxin	Na/K ATPase inhibitor
	Chlorpromazine	D2 (dopamine) receptor antagonist
Low (C)	Erlotinib	Tyrosine Kinase inhibitor
	ASA	COX1/2 inhibitor
	Empagliflozin	SGLT2 inhibitor

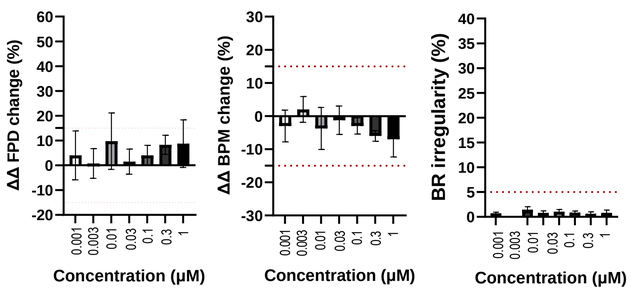
Table 1. Compound library

Target structures	Fluorescent probes
Mitochondria	MitoTracker CMX Ros
DNA damage	Anti-γH2.AX antibody
Golgi	Wheat Germ Agglutinin
Endoplasmic reticulus (ER)	Concanavalin A
Gap junctions (connexin 43)	Anti-Cxc43 antibody
Lysosomes	LysoTracker Red
Peroxisomes	Anti-PMP70 antibody
Nucleoli	Anti-fibrillarin antibody
Nuclei	DAPI

Table 2. Validated immunofluorescence assays

2 A NON-TOXIC COMPOUND – EMPAGLIFLOZIN

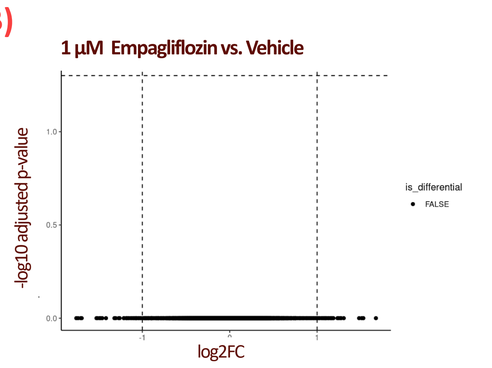
A)



Mean ± SEM, %ΔΔ normalized to mean of vehicle controls and baseline (N=1, n=4)

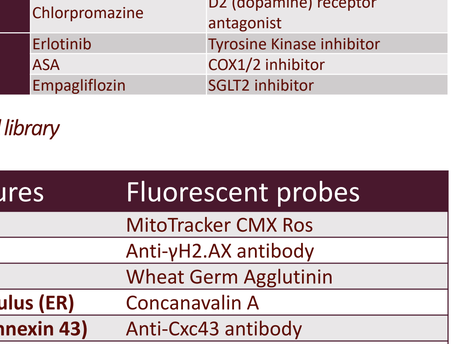
Changes ≥ 15% in ΔΔFPD and ΔΔBR were considered a compound induced effect, BR irregularity ≥ 5% was labeled arrhythmic

B)



C)

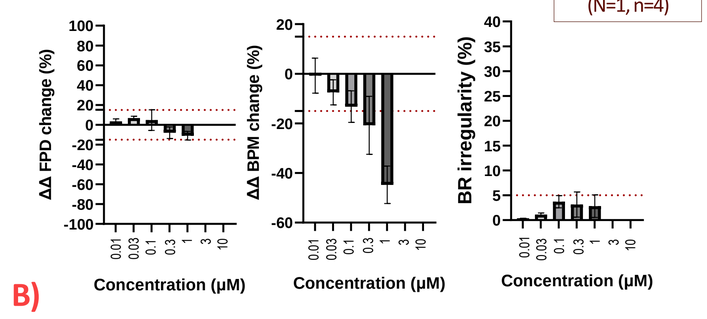
DAPI, WGA, Concanavalin A, PMP70



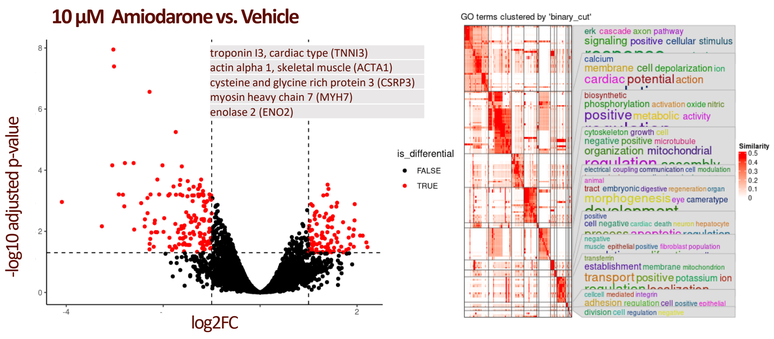
- **A. No functional changes considered as drug effects were detected**
- **B. RNAseq did not show any significantly differentially expressed genes**
- **C. No prominent morphological phenotype was observed**

3 AMIODARONE – A POTENT ANTIARRHYTHMIC WITH SAFETY RISKS

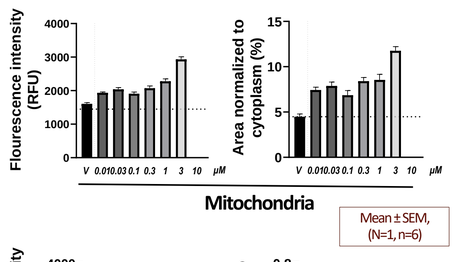
A)

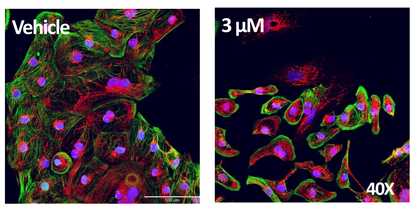


B)

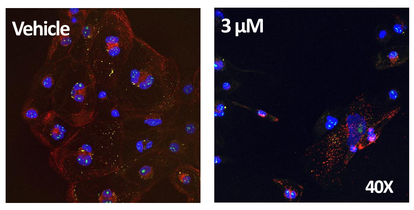


C) Distinct morphological phenotype





DAPI, MitoTracker, γH2.AX antibody, cTNT antibody

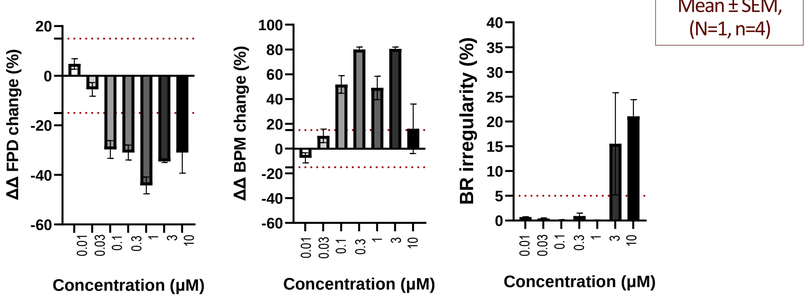


DAPI, Cxc43 antibody, Fibrillarin antibody, LysoTracker

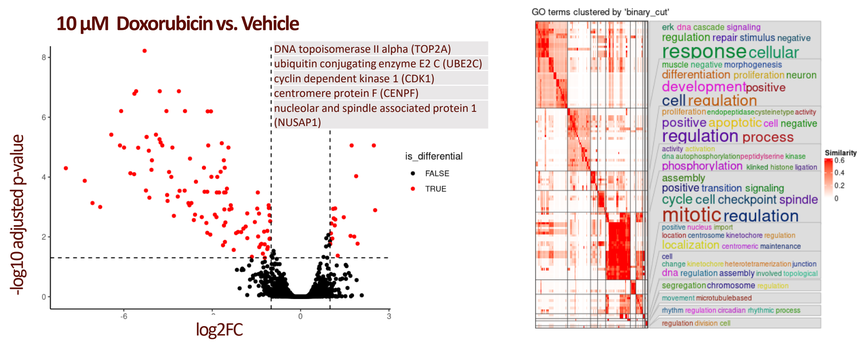
- **A. Treatment led to BR ↓, quiescence, cell detachment at high concentrations**
- **B. Differential expression of genes regulating membrane potential, mitochondria, cytoskeleton, etc. corresponded to morphological and functional data**
- **C. Morphology was affected in a dose-dependent manner;**
  - **mitochondrial fluorescence intensity, detected area ↑**
  - **lysosomal fluorescence intensity, detected area ↓**

4 HIGHLY TOXIC ANTHRACYCLINE - DOXORUBICIN

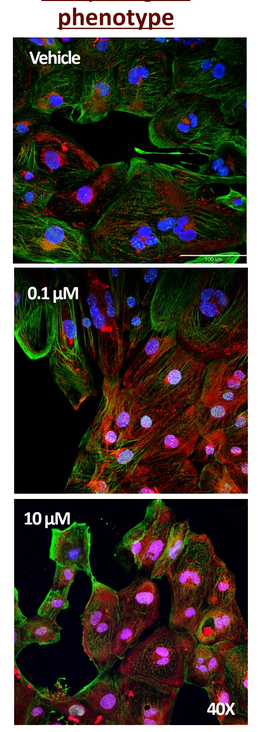
A) Doxorubicin increases BR and irregularity



B) Genes effected play crucial role in the cell cycle

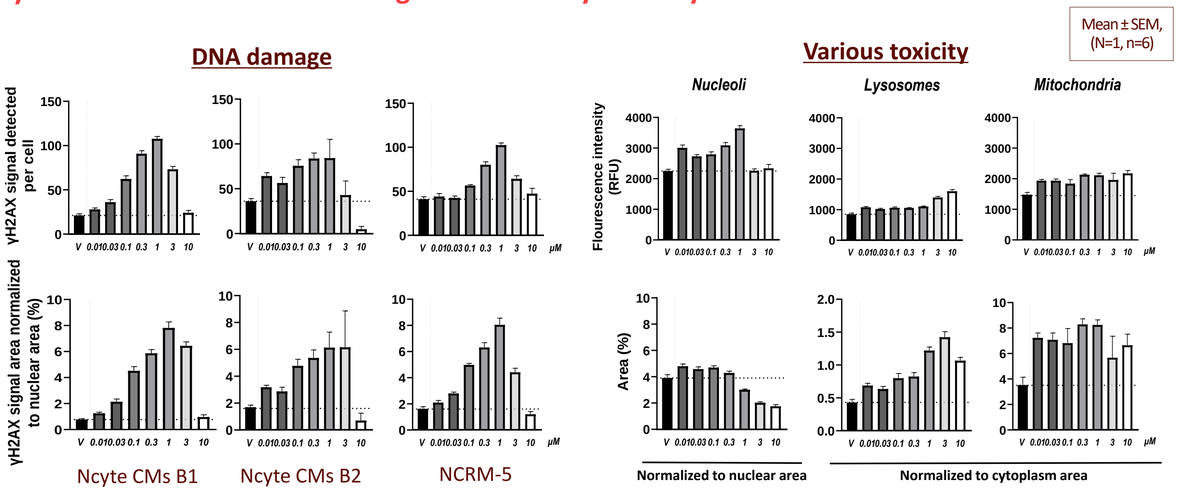


Morpholigical phenotype



DAPI, MitoTracker, γH2.AX antibody (nuclei), cTNT antibody (cytoplasm)

C) Doxorubicin induces DNA damage and diverse cytotoxicity



- **A. Treatment led to FPD ↓, BR ↓ and quiescence**
- **B. Most differentially expressed genes play crucial role in the cell cycle**
- **C. Dose-dependent formation of γH2AX foci, an early cellular response to the induction of DNA double-strand breaks were detected as well as differences in sensitivity in the hiPSC-CM lines**

Other cytotoxic effects were also revealed in sub-cellular organelles

CONCLUSIONS

- **Human iPSC-CM derived models are scalable and cost-effective tools for safety assessments**
- **We were able to optimize staining protocols for a selection of dyes targeting subcellular organelles and membranes, utilizing high content imaging methods, which complement already standardized methods for safety assessment in vitro using hiPSC-CMs**
- **Using multiple hiPSC-CM lines, our results show the potential of high-throughput image-based structural analysis as a tool in gaining a deeper understanding of morphological changes as a mechanism for drug-induced cardiotoxicity**

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