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Optogenetic Pacing For Assessment Of Proarrhythmic Potential Of Drugs In Induced Pluripotent Stem Cell Derived Cardiomyocytes

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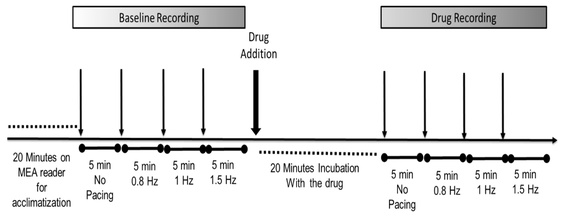
Background

Human induced pluripotent stem cell cardiomyocytes (hiPSC-CM) have been proposed as a model for predicting drug-induced arrhythmias under Comprehensive *In Vitro* Proarrhythmia Assay (CiPA). *In vitro* cultures of iPSC-CMs spontaneously beat in a wide range of frequencies depending on the cell source and culture protocols. The drug effect on hiPSC-CM's action potential duration is often corrected using empirical formulas derived from clinical QT and heart rate correlation, which were not developed or thoroughly validated for iPSC cardiomyocytes. Recent advances in optogenetics allows for control over the beating frequency of cardiomyocytes by expressing an excitatory opsin, channelrhodopsin-2 (ChR2) on hiPSCs cardiomyocytes and exposing them to low intensity (0.05 – 1.5 mW/mm^2) of blue light (470 nM) at a predetermined rate. The objective of the study was to optimize optogenetic pacing protocols for one of the commercially available hiPSC-CM lines and to assess electrophysiological effects of 28 drugs with known proarrhythmic risk in spontaneously beating and optogenetically paced hiPSC-CMs.

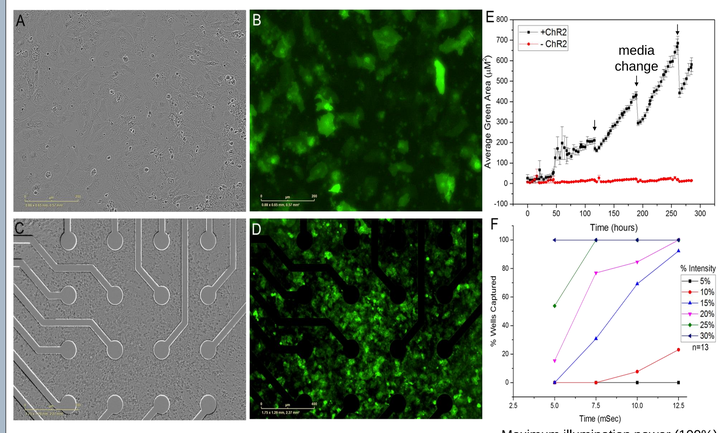
Methods

Using viral vector (rAAV9-CAG-CHR2-GFP, UNC Vector core) we expressed light-sensitive channelrhodopsin-2 (Chr2) in hiPSC-CMs (iCell cardiomyocytes^2, Cellular Dynamics) plated on 48-well Lumos plates (Axion) and used 48-well light delivery device Lumos (Axion) to optically pace cells at three frequencies 0.8, 1, and 1.5 Hz while recording drug-induced field potential (FPD) prolongation and arrhythmia-like events in iPSC-CMs using microelectrode-array system (Maestro, Axion). For the study, we used twice the pacing threshold intensity, 30% (>1mW/mm^2) light for 15 milliseconds to pace hIPSCs at 0.8, 1 and 1.5 Hz before and after addition of the drug. hiPSC-CMs' response to 4 doses of 28 blinded CiPA drugs categorized into low, intermediate and high risk to induce ventricular arrhythmia, Torsade de Pointes, were recorded following the protocol below:



Double delta FPD (ddFPD) change was calculated after correcting drug induced FPD with vehicle as DMSO control and baseline FPD values. Observed arrhythmias were categorized into types A, B or C. Not captured wells were further classified into two types, 'type 1' if the beat period is higher than pacing frequency and, 'type 2', where beat period is lower than pacing frequency. Spontaneous beating data was corrected using Fredericia's correction formula. ddFPD data was excluded from the analysis if more than 50% of wells were arrhythmic. Time dependent changes in hIPSC with and without transfection was imaged using Incucyte S3 live cell imaging system (Essen Biosciences) at 37°C and 5% CO_2 every 4 hours. Data is represented as mean ± 95% confidence interval.

Time dependent changes of GFP expression in hiPSCs after transfection of ChR2



A) Bright field representative image of hiPSC-CMs monolayer at DIV7 after transfection on glass bottom plates

B) GFP expression of monolayers depicted in figure A

C) Bright field representative image of hiPSC-CMs monolayer at DIV7 after transfection on optical MEA plates with 16 electrodes

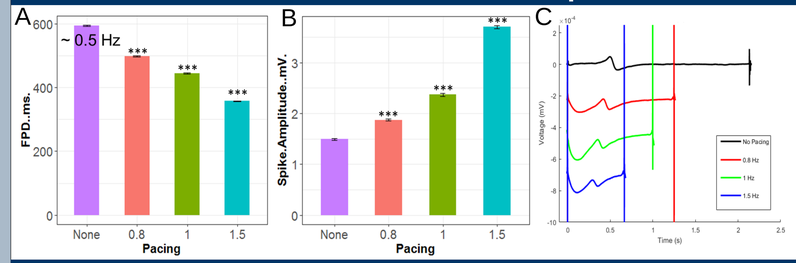
D) Green fluorescence of cells as shown in C, suggesting ChR2 expression

E) Time-dependent reduction in expression of ChR2 (Average green area, µM^2) with and without viral transfection.

F) Percentage of wells captured at different intensities of light and duration of light stimulation.

Maximum illumination power (100%) corresponds to >3.5 mW/mm^2

Baseline characteristics of paced iCell cardiomyocytes^2 on MEA



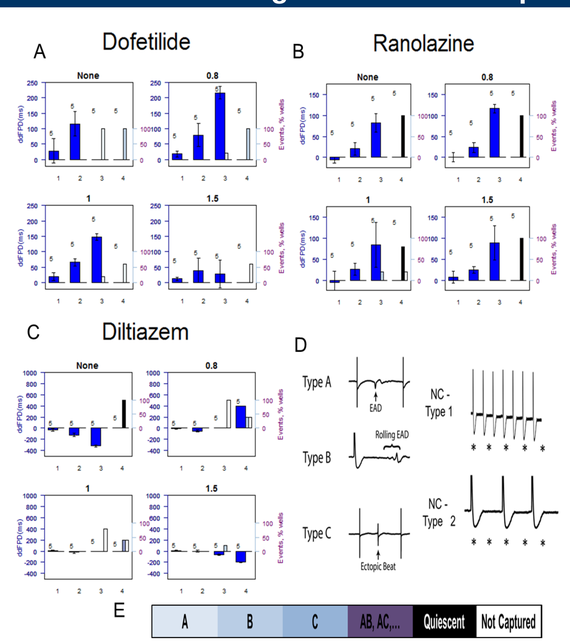
A) iPSC-CM FPD was inversely proportional to the beating frequency

B) iPSC-CM field potential spike amplitude increased with pacing frequency.

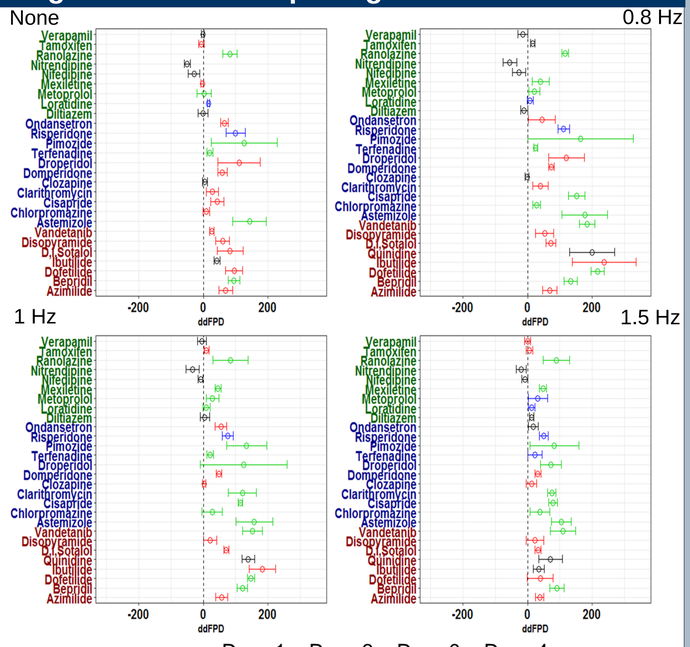
C) Representative baseline MEA traces at each pacing frequency.

p-value < 0.0001 compared to 'None' group.

Drug induced ddFPD prolongation at various pacing rates



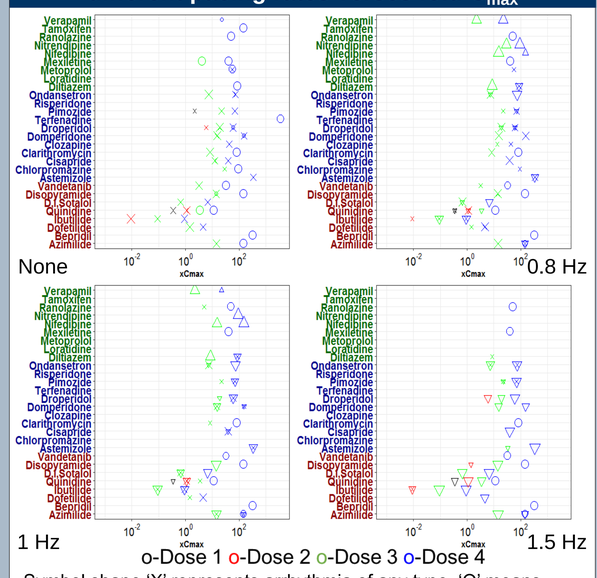
A) Dofetilide, B) Ranolazine, C) Diltiazem, induced ddFPD prolongation at different doses and pacing rates.
D) Traces show various forms of drug induced arrhythmia types.



o-Dose 1 o-Dose 2 o-Dose 3 o-Dose 4

Maximum ddFPD prolongation at any dose by 28 drugs at different pacing rates. Drug names in red, blue and green color are high, intermediate and low Tdp risk, respectively.

Drug induced arrhythmia and quiescence at different pacing rates verses fold C_{max}



o-Dose 1 o-Dose 2 o-Dose 3 o-Dose 4

- Symbol shape 'X' represents arrhythmia of any type, 'O' means quiescence, '△' represent 'type 1' and '▽' represent 'type 2', potentially arrhythmic non-captured wells.
- Symbol size is proportional to the arrhythmia percent.(n=5, for each)

Results

High TdP risk – All the drugs, showed significant ddFPD prolongation at all pacing rates, except in disopyramide and quinidine, where no effect was observed due to arrhythmia at all doses. No arrhythmias were observed in bepridil, while, azimilide, ibutilide and quinidine showed arrhythmias at all pacing rates. Vandetanib induced arrhythmias in no pacing and 0.8 Hz while these arrhythmias were resolved at 1 and 1.5 Hz. **Intermediate TdP risk** - No arrhythmias at any pacing rates were recorded in terfenadine and risperidone, however, domperidone, droperidol, pimozide and ondansetron showed arrhythmia at the highest dose in all pacing rates. Arrhythmias were noted at the highest doses in astemizole, chlorpromazine, cisapride, clarithromycin and clozapine at 0.8 Hz pacing rate and in astemizole, cisapride, domperidone and droperidol at 1 Hz pacing rate. Significant prolongation of ddFPD was observed for all the drugs in spontaneously beating wells except chlorpromazine and clozapine. **Low TdP risk** - No arrhythmias were observed in any of these drugs at any pacing rate except for metoprolol (at no pacing and 0.8 Hz), ranolazine (1 Hz ,1/5 wells) and diltiazem (0.8 and 1 Hz). At all pacing frequencies, loratidine, tamoxifen, metoprolol, mexiletine did not induce significant changes in ddFPD. We also observed significant ranolazine induced ddFPD prolongation with and without pacing.

Conclusion

- Pacing significantly reduced arrhythmic events from 166 events in no pacing to 147,127 and 121 events in 0.8 Hz, 1 Hz, and 1.5 Hz respectively. This enabled us to collect ddFPD data at a higher drug dose (fold C_{max}) using pacing as compared to non-paced experiments.
- Pacing eliminates the need for rate-correction formulas, which have not been thoroughly validated for hIPSC-CMs.
- Optical pacing is a simple, reliable and reproducible technique to study the frequency dependent screening of drugs in iPSC-CMs beating in a physiological range.

References

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