DEFINING THE LIMITS OF STEM-CELL DERIVED CARDIOMYOCYTES (SC-CMS) TO DETECT CARDIAC PROARRHYTHMIC LIABILITIES

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1 ABSTRACT

Extracellular Field Potential (FP) and impedance recordings of SC-CMs are currently used to detect drug induced cardiac arrhythmia liabilities. Major endpoints include the field potential duration (FPD), the duration of the impedance twitch, and the presence of proarrhythmic markers including early and delayed afterdepolarizations and fibrillations. We used the cardioECR instrument (ACEA Biosciences) and iCell2 cardiomyocytes (Cellular Dynamics International) to 1) identify the FP parameter that best captures the intrinsic frequency-dependence of repolarization and, 2) assess the extent the system can detect the mitigating effect of drugs with multiple ion channels effects (MICE) on proarrhythmic activity. Cardiac repolarization in FP recordings is encapsulated by one positive (T3) and two negative (T1, T2) peaks. We detected a linear relationship with comparable slopes of 0.133 and 0.129 respectively (n=2) between T2 and T3 durations and beat period in the spontaneous frequency range. T1 shows a shallower slope (0.031) suggesting that the parameter does not follow closely the repolarization process and consistent with its limited response to drugs that prolong the FPD. MICE effects were investigated by assessing the effect of different combinations of pure hERG (E-4031) and hCav1.2 (nifedipine) channel blockers designed to simulate distinct IC50 hERG/IC50 hCav1.2 ratios (HCR). The functional relationship between the number of wells showing proarrhythmic markers and log (HCR) was fitted to a logistic function with an inflection point at -0.148 and a HCR of 0.71. Notably, this ratio is comparable to the 0.89 cutoff for torsadogenic drugs in the clinic (Kramer, Obejero-Paz et al., 2013). SC-CMs are useful tools to investigate the cardiac risks of drugs by using T2 and T3 to evaluate changes in cardiac repolarization and HCR values (≤ 0.71) to determine proarrhythmic activity.

2 INTRODUCTION AND RATIONALE

Extracellular Field Potential (FP) and impedance recordings of SC-CMs are currently used to detect drug induced cardiac arrhythmia liabilities. Major endpoints include the field potential duration (FPD), the duration of the impedance twitch, and the presence of proarrhythmic markers including early and delayed afterdepolarizations and fibrillatory activity. The manner these endpoints translate to the clinic is being validated by the industry and regulators. A clear limitation is the fact that SC-CMs display an immature cardiac phenotype showing distinct expression levels of major cardiac currents (i.e., IK1) and spontaneous beating activity.

Given the fact that cardiac repolarization is a major target of drugs showing proarrhythmic potential we aimed to investigate the behavior of the parameters that better define this electrophysiological period. Cardiac repolarization in the CardioECR-iCell system is encapsulated by one positive (T3) and two negative (T1, T2) peaks that span the decay of the impedance twitch (Fig. 1A). Here we tested the notion that the best repolarizing parameter should have a robust frequency dependence and a clear sensitivity to hERG and calcium channel blockers (Figures 1 and 2). We also validated the best method for frequency correction (Fig. 1C).

Finally, we used the impedance signal to investigated the extent SC-CM proarrhythmic markers are predictors of clinical arrhythmias. We have shown that increased calcium channel block decreases the proarrhythmic potential of hERG blockers (Kramer, Obejero-Paz et al., 2013). We showed that the best predictor (Model 5) uses the explanatory variable CavD defined as log(HCR) where HCR is hERG IC_{50}/Cav1.2 IC_{50}. Thus we investigated the effect of distinct HCRs on the inhibition of proarrythmic markers and asked how the functional relationship compares with the clinic (Figure 3).

3 MATERIAL AND METHODS

We used the xCELLigence RTCA CardioECR instrument (ACEA Biosciences) to record impedance and extracellular field potentials. SC-CMs (iCell cardiomyocytes2) were from Cellular Dynamics International/FUJIFILM. Twitch activity was recorded for spontaneously beating cells or field potential stimulated cells at frequencies allowed by the intrinsic spontaneous activity. Analysis was performed using Origin, Matlab and macros written in VBA. The output of the instrument is the Cell Index, a measure of the electrode impedance relative to the background reading. During the analysis values were transformed to ohms according to Z=CI x 15Ω. Field potential and impedance twitches in each well were detected, time shifted relative to the negative peak of the sodium spike signal (t=0) and averaged.

T1-T3 repolarizing peaks were used to define corresponding FPDs relative to the negative peak of the sodium signal. Frequency correction methods: 1) Linear correction [FPDc=FPD- (slope*(Period-1000))], 2) Bazett [FPDc=FPD/(Period/1000)^(1/2)], 2) Fridericia [FPDc=FPD/(Period/1000)^(1/3)], Framingham [FPDc=FPD+0.154*(1000-Period)] and Hodges [FPDc=FPD+1.75*((60000/Period)-60)], where FPD and Period are in ms. The slope factor used in the linear correction was calculated based on the functional relationship between FPD and Periods from spontaneous and paced wells exposed to vehicle. The performance of the method was evaluated by calculating the slope of the corrected FPD as a function of beat period; an efficient method will have slope values not different from zero.

To investigate the extent different levels of calcium channel block modulate the response of SC-CMs to hERG blockers we exposed individual wells to single concentrations or combination of E-4031 and nifedipine. At least five wells were used for each condition. We used the impedance signal to detect proarrythmic markers including impedance irregularity during repolarization and triggered activity. We defined P(Proarrhythmia) as the fraction of wells showing proarrhythmic activity. To compare the data with the clinic we designed the experiments to obtain specific CavD values based on the IC50s for hERG and Cav1.2 block (E-4031: hERG IC50 0.012 μM (h= 0.90), Cav1.2 IC50 30 μM (h=1.00); nifedipine: hERG IC50 44 μM (h=0.80), Cav1.2 IC50 0.012 (h=1.02)). IC50s were obtained using the Q-Patch platform (Sophion). Clinical and SC-CM data was fitted to the following logistic function using JMP software: P(Proarrhythmia)=(1+exp(-growth rate*(CavD-Inflection point)))$^{-1}$.

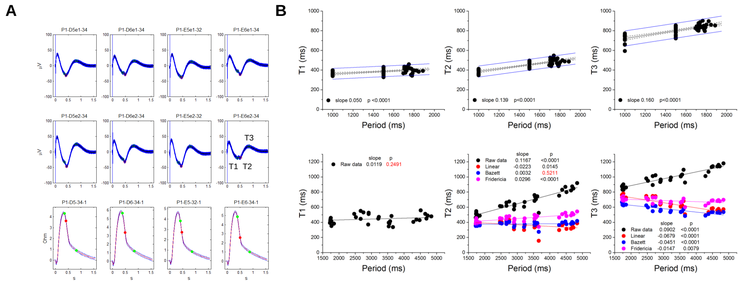
5 CONCLUSIONS

1) The time lapsed between the negative peak of the sodium spike and the peak at T2 (FPD@T2) is a valuable surrogate of the action potential duration. FPD@T2 shows the most robust frequency dependence and responds to hERG and Cav1.2 channel blockers in the predicted way. FPD@T2 is best corrected using the Bazett's equation.

2) SC-CMs accurately predict MICE and clinical cardiac risk and confirm the ion channel CavD model in a more physiologically relevant system.

4 RESULTS

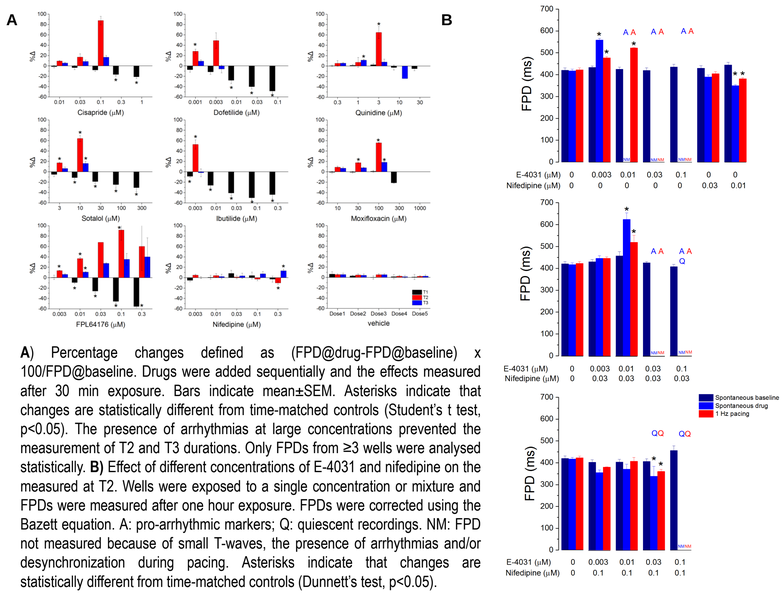
1- SC-CM REPOLARIZATION IS DEFINED BY THREE PEAKS WITH DISTINCT FREQUENCY DEPENDENCE



Parameter	Experiment	Raw data	linear	Bazett	Fridericia	Framingham	Hodges
T1	170518	0.0067 (p=0.294)	-0.0075 (p=0.241)	-0.0309 (p<0.001)	-0.0231 (p<0.001)	-0.1473 (p<0.001)	-0.0048 (p=0.462)
	170913	0.0119 (p=0.185)	-0.0377 (p<0.001)	-0.0369 (p<0.001)	-0.0264 (p<0.001)	-0.1421 (p<0.001)	-0.0009 (p=0.929)
	180112	0.0149 (p=0.021)	-0.0231 (p<0.001)	-0.0421 (p<0.001)	-0.0285 (p<0.001)	-0.1391 (p<0.001)	-0.0038 (p=0.537)
T2	170518	0.1077 (p<0.001)	-0.0206 (p=0.119)	-0.0022 (p=0.763)	0.0233 (p<0.015)	-0.0463 (p<0.003)	0.095 (p<0.001)
	170913	0.1163 (p<0.001)	-0.0223 (p<0.015)	0.0032 (p=0.521)	0.0296 (p<0.001)	-0.0377 (p<0.001)	0.1036 (p<0.001)
	180112	0.1208 (p<0.001)	0.0568 (p<0.001)	0.0006 (p=0.975)	0.0304 (p<0.001)	-0.0312 (p=0.006)	0.1021 (p<0.001)
T3	170518	0.0677 (p<0.001)	-0.0324 (p<0.022)	-0.0453 (p<0.001)	-0.0206 (p=0.019)	-0.0863 (p<0.001)	0.0562 (p<0.001)
	170913	0.0902 (p<0.001)	-0.0679 (p<0.001)	-0.0451 (p<0.001)	-0.0147 (p=0.008)	-0.0638 (p<0.001)	0.0774 (p<0.001)
	180112	0.0751 (p<0.001)	0.0168 (p=0.027)	-0.0704 (p<0.001)	-0.0353 (p<0.001)	-0.0789 (p<0.001)	0.0564 (p<0.001)

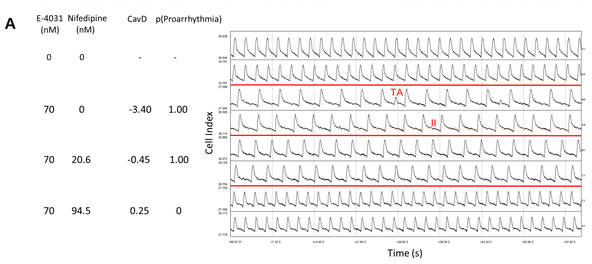
A) FP and impedance twitches from four wells. Lines are mean (black) and SD (blue) of the number of FP and twitches indicated in the top. Empty and filled dots indicate the position of the T1, T2 and T3 peaks. **B**) Frequency dependence of FPDs at T1-T3. Top panels show T1-T3 durations from spontaneous beating and stimulated cells at 1 Hz and 0.667 Hz. Lines show the 95% CI and the predicted regions. The slope of the fitted lines were used for the linear correction below. Bottom panels show T1-T3 durations from cells exposed to 0.1, 0.3 and 1 μM If inhibitor ZD7288 and vehicle control (black symbols) and the effect of corrections with different methods. **C**) Performance of different frequency correcting methods in removing the frequency dependence of FPDs at T1-T3 in three independent experiments. Probabilities shown in red indicate no frequency dependence (p>0.05).

2- REPOLARIZING PEAK TIMES ARE AFFECTED BY HERG AND CALCIUM CHANNEL BLOCKERS IN A MANNER CONSISTENT WITH MULTIPLE ION CHANNEL EFFECTS

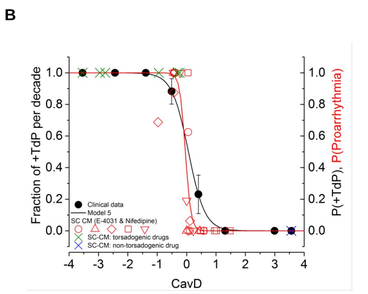


A) Percentage changes defined as (FPD@drug-FPD@baseline) x 100/FPD@baseline. Drugs were added sequentially and the effects measured after 30 min exposure. Bars indicate mean±SEM. Asterisks indicate that changes are statistically different from time-matched controls (Student's t test, p<0.05). The presence of arrhythmias at large concentrations prevented the measurement of T2 and T3 durations. Only FPDs from ≥3 wells were analysed statistically. **B**) Effect of different concentrations of E-4031 and nifedipine on the measured at T2. Wells were exposed to a single concentration or mixture and FPDs were measured after one hour exposure. FPDs were corrected using the Bazett equation. A: pro-arrhythmic markers; Q: quiescent recordings. NM: FPD not measured because of small T-waves, the presence of arrhythmias and/or desynchronization during pacing. Asterisks indicate that changes are statistically different from time-matched controls (Dunnett's test, p<0.05).

3- MITIGATION OF HERG ASSOCIATED PROARRHYTHMIA BY CALCIUM CHANNEL BLOCK IN SC-CMS SHOWS COMPARABLE INFLECTION POINT WITH THE CLINIC BUT STEEPER CAVD DEPENDENCE



A) Effect of different combinations of E-4031 and nifedipine designed to obtain CavD values at both sides of the inflection point of the clinical data. Recordings are from two wells in each condition. TA: triggered activity. II: impedance irregularity.



B) Black symbols: functional relationship between the fraction of drugs showing torsadogenic liability and CavD. The clinical data is an update, based on CredibleMeds (https://crediblemeds.org), of the training set reported previously (Kramer, Obejero-Paz et al., 2013). The data was fitted to a logistic function with growth rate and inflection point parameters of -3.566 and 0.06 respectively indicating an HCR of 1.15.
Red symbols: functional relationship between the fraction of wells showing proarrhythmic markers and CavD. SC-CM data is from five independent experiments identified by different shapes. The fitted growth rate and inflection point parameters of the SC-CM data was -10.5 and -0.045 respectively with an HCR of 0.90.
Green symbols: torsadogenic drugs including dofetilide, cisapride, quinidine, moxifloxacin, sotalol and ibutilide. The P(Proarrhythmia)= 1 was based to the fact that cells exposed to concentrations close to the hERG IC50 the compounds induced proarrhythmic markers.
Blue symbols: nifedipine.